ANNUAL RESEARCH PROGRESS REPORT

ICMR-NATIONAL INSTITUTE OF VIROLOGY, PUNE

(2020-2021)

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ANIMAL HOUSE GROUP

BACTERIOLOGY

(i) Project 1(a): Seroepidemiology, maternal immune status and missed diagnosis of pertussis among young infants in India: a multicentric study

(ii) Project 1(b): To explore the aetiology of prolonged cough in young infants from an urban pediatric centre in western India, focusing on Bordetella pertussis, other species of Bordetella and respiratory viruses (Preliminary data from Pune site)

(iii) Diagnostic Services for Bacteriology

BIOINFORMATICS & DATA MANAGEMENT GROUP

(i) Core facility services

(ii) Project 1: Structure-based design and evaluation of the antiviral activity of potential lead compounds against the Chikungunya virus

(iii) Project 2: Repurposing of drugs towards anti-dengue and chikungunya viruses using systems biology approach

(iv) Project 3: Bioinformatics characterization of Chandipura virus proteins

(v) Project 4: Development of forecasting models for dengue and chikungunya in the Pune region

DENGUE - CHIKUNGUNYA GROUP

(i) Project 1: Apex referral laboratory activity for National Vector Borne Disease Control Program

(ii) Project 2: Development and evaluation of an in-house multiplex real time RT-PCR assay for simultaneous detection of dengue, chikungunya and Zika viruses in clinical samples

(iii) Project 3: Antiviral activity of approved drugs and natural peptides against dengue and chikungunya virus

(iv) Project 4: Use of lipid nanoparticles for effective delivery of siRNA in Chikungunya virus

(v) Project 5: Structure-based design and evaluation of lead compounds targeting Chikungunya virus

DIAGNOSTIC REAGENT FACILITY

(i) Project 1: Scale up of facilities for production of Diagnostic kits/ Reagents for detection of JE, DEN & CHIK IgM antibodies

ELECTRON MICROSCOPY & HISTOPATHOLOGY GROUP

(i) Core Service Project for emerging and re-emerging viruses in India.

ENCEPHALITIS GROUP

(i) Project 1: Investigations (diagnosis) of referred human clinical specimens during encephalitis outbreaks from different parts of India.
(ii) Project 2: Role of Histidine residues of envelope protein in membrane fusion of Japanese encephalitis virus.
(iii) Project 3: To study the mechanism of endosomal membrane fusion process in Japanese encephalitis GI strain using DiD labelled virus
(iv) Project 4: Expression of Japanese encephalitis virus genotype-1 envelope and non-structural proteins to explore in early diagnosis.
(v) Project 5: To determine antiviral activity of viral RNA dependent RNA polymerase inhibitors against Chandipura virus infection
(vi) Project 6: Development of an indirect ELISA assay for surveillance of Japanese encephalitis.
(vii) Project 7: Establishing Rabies diagnostics and research capabilities.

ENTERIC VIRUS GROUP

(i) Project 1: Diversity of non-rota enteric viruses in patients with acute gastroenteritis.
(ii) Project 2: Hospital based surveillance of rotavirus strains in children with acute gastroenteritis
(iii) Project 3: Identification and molecular characterization of group C rotaviruses in infected humans and animals from Western India: A retrospective study
(iv) Project 4: Identification and Molecular Characterization of non-rota enteric viruses in Neonates admitted at Neonatal Intensive Care Unit (NICU).
(v) Project 5: Study of cross neutralization of neonatal G12P[11] strain against convalescent phase sera from children vaccinated against current Rotavirus vaccines.
(vi) Project 6: Detection and molecular characterization of potentially zoonotic enteric viruses in animals
(vii) Project 7: Hospital based surveillance of enteric viruses/strains in children with acute gastroenteritis

EPIDEMIOLOGY GROUP

(i) Project 1: Japanese encephalitis epidemiology in Central part of India: enhanced sentinel surveillance for etiological contribution and burden following vaccination in Maharashtra and Telangana
(ii) Project 2: Mobile Application for Immunization Data in India (MAIDI)

(iii) Project 3: Resource Center under Health Technology Assessment in Research Project under the scheme (3103) human resource and capacity development
(iv) Cost-analysis of diagnostic tests for COVID-19 at National Reference Laboratory in India.

ENTOMOLOGY GROUP

(i) Project 1: Metagenomics analysis of viromes of Aedes mosquitoes in India
(ii) Project 2: Dual infection studies of dengue and chikungunya viruses in Aedes aegypti mosquitoes using molecular approach.
(iii) Project 3: Vector competence of Anopheles mosquitoes to Chittoor virus, an Indian variant of Batai virus
(iv) Project 4: Studies on sand fly fauna of Pune city: revisited after 20 years
(v) Project 5: Surveillance of dengue and chikungunya viruses in *Aedes aegypti* populations in Pune district

MAXIMUM CONTAINMENT FACILITY GROUP

(i) Project 1: Establishment of a facility for production of standard virus positive controls for diagnostic PCRs and RT-PCRs tests for the important public health viral diseases
(ii) Project 2: Prospective investigation of transmission of Crimean Congo Haemorrhagic Fever (CCHF) amongst close contacts of confirmed CCHF cases
(iii) Project 3: Development of serodiagnostic assays for Nipah virus
(iv) Project 4: Identification and characterization of novel viral isolates using Next-generation sequencing platform
(v) Project 5: Preparation of reagents for highly infectious diseases
(vi) Project 6: Providing diagnostic support for referred samples of viral hemorrhagic fever and other unknown etiology and outbreak investigation

HEPATITIS GROUP

(i) Project 1: Computational drug repurposing approach for the development of therapeutics against Hepatitis E Virus
(ii) Project 2: Hepatitis E virus replication and cellular autophagy
(iii) Project 3: Assessment of risk factors for the development of Cardiovascular disease (CVD) in patients with active HCV infection

INFLUENZA GROUP

(i) Core Service Project: National Influenza Center activities
(ii) Project 2: Surveillance of respiratory viruses in Pune city
(iii) Project 3: Strengthening/promoting evidence-based advocacy for influenza prevention and control in India
(iv) Project 4: Tracking community mortality due to respiratory syncytial virus in collaboration with University of Colorado and MAHAN Melghat

DIAGNOSTIC VIROLOGY GROUP

(i) Core service project: Resource Centre for Virus Diagnostic research Laboratories (RCVRDL) at NIV, Pune

(ii) Project 1: Surveillance for Zika virus infections in India
(iii) Project 2: Cohorts for Zika Epidemiology in India
(iv) Project 3: Impact of measles and rubella vaccination campaign on population immunity in India (IMRVI study)
(v) Project 4: Congenital Rubella Syndrome (CRS) Surveillance in India
(vi) Project 5: Development of serodiagnostic assays for Nipah Virus

POLIO VIROLOGY GROUP

(i) Core service Project: Operation and maintenance of High Containment Laboratory
(ii) Project 1: Development of Polio Essential Facility in line with the Global Action Plan III at ICMR-NIV, Pune to support work on Polio
(iii) Project 2: Gene pool analysis of highly pathogenic H5N1 and low pathogenic H9N2 avian influenza viruses isolated from India

VIRUS REGISTRY AND VIRUS REPOSITORY

(i) Service project: Outbreak Investigations/ Diagnostic services
(ii) Genetic and antigenic characterization of measles, mumps and rubella virus isolates
(iii) Project 2: Measurement of virus specific IgM, IgG and neutralizing antibody levels in suspected Measles and Rubella cases

BENGALURU UNIT

(i) Project 1: AFP Surveillance (WHO-SEAR Polio Lab Network in the WHO's Global Eradication of Poliomyelitis Program
(ii) Project 2: Environmental surveillance of sewage samples from Bangalore city as a part of WHO-SEAR Polio Lab Network in the WHO.
(iii) Project 3: Surveillance of Measles in Karnataka and Kerala States as part of WHO-SEAR Measles Laboratory Network in the WHO's Global Measles Elimination Programme.
(iv) Project 4: National Vector Borne Disease Control Program (NVBDCP) for human and mosquito samples
(v) Project 5: Congenital Rubella Syndrome (CRS) Surveillance
(vi) Project 6: Laboratory investigation of severe acute respiratory infection (SARI) cases

NIV KERALA UNIT

(i) Project 1: Establishment of a network of laboratories for managing epidemics and natural calamities
(ii) Project 2: Development and Efficiency evaluation of tick repellent from plant extract: A scientific and social intervention on tribal health against ticks and tick-borne diseases in Kyasanur forest disease endemic area of Kerala.
(iii) Project 3: Clinical and epidemiological study of Lyme disease: a multi-centric taskforce study in India.
(iv) Project 4: Response of urban health service systems to road traffic injuries
(v) Project 5: Public trust in vaccine: A qualitative study on the determinants of acceptance and hesitancy towards JE vaccines in various Blocks in Alappuzha District.

NIV MUMBAI UNIT

(i) Service project: National Polio Surveillance Project (NPSP), India
(ii) Service Project: Measles and Rubella Surveillance
(iii) Project 1: Strengthening laboratory quality management systems including EQA of DHR / ICMR VRDL laboratories for confirmation of outbreaks including measles and rubella
(iv) Project 2: In-vivo potency evaluation for the hexavalent and IPV trivalent vaccine formulation containing IPV type 1, 2 and 3 viruses
(v) Project 3: Study on Polio and Non-Polio enterovirus infections in children with Primary Immunodeficiency at multiple medical institutes across India
(vi) Project 4: Seroprevalence of Enterovirus 71 antibody among Indian children
(vii) Project 5: RT-LAMP Assay for detection of human β -Actin housekeeping gene
(viii) Project 6: A Point of Care Device, Method and Kit involving Club Cell protein 16 as a marker for Silicosis/ Silico-Tuberculosis
(ix) Project 7: Assay for detection of epidemiologically important SARS-CoV-2 genetic variants
(x) Project 8: CD155/PVR knockout cell strains from human rhabdomyosarcoma cell line (RD) for use in polio and non-poliovirus diagnostics and research.
(xi) Project 9: Investigation of host genetic susceptibility markers to Enterovirus A71 infection in Indian population
(xii) Project 10. Outbreak investigation of highly pathogenic avian influenza (HPAI) H5N1 virus in India

Other activities

(i) ACADEMIC CELL
(ii) LIBRARY & INFORMATION SERVICES
(iii) ENGINEERING SUPPORT GROUP
(iv) ICMR- NIV ADMINISTRATION
(v) RAJYA BHASHA REPORT

From the Director's desk

It is indeed my pleasure and privilege to present the Annual Report of ICMR-National Institute of Virology, Pune, for the year 2020-21. As the Director, I feel proud to announce the tremendous contribution the Institute has made as team NIV to make the country proud. The journey was challenging, but ICMR-NIV was in the forefront and shouldered the responsibility straight from sample testing to the development of an indigenous efficacious vaccine. The first indigenous vaccine, the COVAXIN®, was developed and evaluated at ICMR-NIV, Pune. The vaccine has now got the emergency use listing (EUL) approval by WHO. The 'COVID KAVACH', the anti-IgG SARS-CoV-2 kit to detect IgG antibodies was another feather in the cap of ICMR-NIV that has found application for sero-surveillance throughout the country. ICMR-NIV also played a pivotal role in the procurement of reagents, formulation into kit format and distribution to all the COVID-19 testing laboratories in India. The Institute also played an important role in the validation of reagents, test kits, and equipment as part of the "Atmanirbhar Bharat" program for COVID-19 diagnostics. ICMR-NIV has reached new heights and received many laurels for the exemplary services rendered in the fightback against COVID-19. It is really a proud moment for me and my dedicated team.

Despite the COVID-19 challenges, my colleagues carried out excellent research in their respective domains and produced quality work output. Timely diagnostic support has been provided for testing samples referred from different parts of the country. ICMR-NIV was always in the forefront when it came to outbreaks and supported the state governments whether it was for Zika or Nipah. NIV scientists have been part of different central teams investigating outbreaks and providing active support to many states as an apex resource centre for the Virus Research & Diagnostic Laboratories (VRDLs). The three peripheral units of ICMR-NIV, *i.e.,* NIV Bengaluru, Alappuzha, and Mumbai units continued to provide their respective State governments with diagnostic support including COVID testing and human resource development.

ICMR-NIV continues to be a WHO collaborating center (CC) on emerging viruses and the Institute has provided a significant number of diagnostic kits for dengue, chikungunya and Japanese encephalitis to national programs. The National Influenza Center (NIC) proved its mettle by meeting the challenges of SARS-CoV-2 testing as well as providing diagnosis for other respiratory viruses.

Despite the hectic schedule, basic research continued to flourish as is evident from the high quality publications in reputed national and international journals. This year we have achieved an average impact factor above four. ICMR-NIV always supported academic programs, and the M. Sc. Virology and Ph. D courses have also shown excellent progress.

Of course such phenomenal activities cannot be possible without the active support from the technical and administrative teams including the engineering core. I would like to congratulate each and every one of the ICMR-NIV family who have supported me to execute my responsibilities as Director. My sincere gratitude to Prof. Balram Bhargava, DG ICMR for his support, to the Chairman SAC for his continued guidance, and team ICMR for the continuous support extended to all NIV research and diagnostic activities. I am confident that the Institute shall in every way meet any future challenge posed and live up to the expectations of the nation.

Prof. Priya Abraham
Director

Composition of committees

Scientific Advisory Committee

Sr No.	Name of member
1.	Lt Gen. (Dr) Velu Nair (Retd), Group Head-Medical Seevices & Chief Consultant –hemato-oncology & borne marrow transplant, Comprehensive blood & cancer centre Res: 632, C-1, Ansals Palam Vihar, Carterpuri, Gurgaon, Haryana-122 017.
2	Dr Ashok Kumar, ADG, Animal Health, Indian Council of Agricultural Research, 405, Krishi Bhavan, New Delhi, India
3	Dr DA Gadkari, Former, Director, ICMR-NIV, Pune.
4	Prof. Amita Jain Professor & Head, Department of Microbiology Incharge, Intermediate reference tuberculosis lab Incharge, Virus research and diagnostic lab, King George University, Lucknow, UP, India 226003
5	Dr. Manoj Kumar Bhat, Ph.D. Director, National Centre for Cell Science, Pune NCCS Complex, Savitribai Phule Pune University Campus Pune - 411007
6	Dr Shubhasish Kamal Guha , MSVP, School of Tropical Medicine, 108, Chittaranjan Avenue, Kolkata – 700073.
7	Professor Vijaya Satchidanandam Room SA07, Biology Building, Department of Microbiology and Cell Biology Indian Institute of Science Bangalore 560012, INDIA
8	Dr. Samiran Panda Cheif-ECD, ICMR, New Delhi & Director, NARI, Pune
9	Prof. Priya Abraham Director, ICMR-NIV, Pune
10	Dr JP Muliyil, Epidemiologist could not attend the meeting

Institutional Bio-Safety Committee (IBSC)

Sr No.	Name & affiliation	Role
1	Dr Priya Abraham, Director, ICMR-NIV, Pune	Chair man/Chair person
2	Dr Kavita Lole, Scientist F, ICMR-NIV, Pune	Member Secretary
3	Dr Arvind Sahu, Scientist G, National Centre for Cell Science, Pune - Maharashtra	DBT Nominee
4	Dr Rima Sahay, Scientist B, ICMR-NIV, Pune	Biosafety Officer
5	Dr Vikram Ghole, Retired Professor, SPPU (Pune University), Pune	External Expert
6	Dr Anita Shete, Scientist D, ICMR-NIV, Pune	Internal expert
7	Dr Tejaswini Deshmukh, Scientist D, ICMR-NIV, Pune	Internal expert

Institutional Human Ethics Committee

S.No	Name & Affiliation	Role
1.	Dr. Amitav Banerjee, MD Professor & Head, Community Medicine Dr D Y Patil Medical College, Pune 411 018	**Chairperson** (External)
2	Dr. Rajesh Kulkarni, MD Associate Professor (Ped) B J Medical College, Pune 411 001 B J Medical College, Pune 411001	Clinician (External)
3.	Dr Sheila Godbole, MD Scientist F ICMR-National AIDS Research Institute, Pune 411026	Clinician (External)
4.	Dr. Vikram Padbidri, MD Consultant Microbiology & Infection Control, Jehangir Hospital, Pune 411001	Basic Medical Scientist (External)
5.	Dr Abhijit Kadam Scientist C, ICMR-National AIDS Research Institute, Pune 411026	Basic Medical Scientist (External)
6.	Dr. Aarti Nagarkar, PhD Assistant Professor Interdisciplinary School of Health Sciences, Savitribai Phule Pune University,, Pune 411 007	Social Scientist (External)
7.	Dr. Jyoti S Bhakare, LLM, PhD, Dept. of Law, Savitribai Phule University, Pune 411 007	Legal Expert (External)
8.	Mr. Joseph Cherian, BA, Dip.Health, Community Medicine Dept. Dr. D.Y. Patil Medical College, Pune-411018	Person from Community (External)
9.	Dr. Anuradha Tripathy, PhD Scientist E, ICMR-National Institute of Virology,Pashan, Pune 21	Basic Medical Scientist (Internal)
10.	Dr K Alagarasu, PhD Scientist D, Dengue/ Chikungunya Group, ICMR-National Institute of Virology, Pune 411 001	Basic Medical Scientist (Internal)
11.	Mr Atul M Walimbe, M.Sc BDM Group,ICMR-National Institute of Virology, ,Pune 411 001	Statistician (Internal)
12.	Mr Santosh M Jadhav, M.Sc BDM Group, ICMR-National Institute of Virology, Pune 411 001	Statistician (Internal)
13.	Dr. Rajlakshmi Viswanathan, MD Scientist D, Bacteriology Group, ICMR-National Institute of Virology, Pashan, Pune 21	Member Secretary (Internal) & Scientist D, ICMR-NIV, Pune

Institutional Animal Ethics Committee

Sr No.	Name, Designation & Address	Role
1	Dr. Jayati Mullick Scientist F & Group Leader, Polio Virus Group (former Avian Influenza), ICMR-National Institute of Virology, 130/1, Sus Road, Pashan, PUNE- 411021	Chairperson
2	Dr. Mangesh Shamrao Kamble C-901, Aarohi Project, Sr. No. 123, Susgaon, Tal. Mulshi, Dist-PUNE - 411021	Main Nominee
3	Dr. Balasaheb Siraskar Principal, SVHNT's College of Pharmacy, Rahuri Factory, Pin: 413706, Dist. Ahmednagar	Link Nominee
4	Dr. Ramanamurthy Boppana Scientist G & In charge, Animal House, National Centre for Cell Science, Pune University Campus, Ganeshkhind, PUNE – 411007	External Expert
5	Shri. Ravindra P. Kulkarni B-5, Building C, Anjira Sankul Vidyanagar, Karad-Masur Road, Karad Tal. , Dist. SATARA- 415124	Socially Aware Nominee
6	Dr. Dilip Rewa Patil Scientist D & Group Leader, Animal House Group, ICMR-National Institute of Virology, 20-A, Dr. Ambedkar Road, PUNE- 411001	Member Secretary & In charge, Animal House Facility, ICMR-NIV, Pune
7	Dr Paresh Sumatilal Shah Scientist E & Group Leader, Diagnostic Reagent Facility, ICMR-National Institute of Virology, 20-A, Dr. Ambedkar Road, PUNE- 411001	Scientist from different biological discipline
8	Dr. Sreelekshmy Mohandas Scientist B, Maximum Containment Laboratory, ICMR-National Institute of Virology, 130/1, Sus Road, Pashan, PUNE- 411021	Veterinary scientist
9	Mr. Virendra Kumar Meena Scientist B, Electron Microscopy Group, ICMR-National Institute of Virology, 20-A, Dr. Ambedkar Road, PUNE-411001	Scientist from different biological discipline

Specimens tested for the reporting period

COVID 19 samples

Tested:	5, 41, 447
Positives:	59, 056

Other viruses*

Tested:	20, 922

*Japanese encephalitis, dengue, chikungunya, hepatitis, enteric viruses, Chandipura, Influenza, respiratory syncitial virus, rhinovirus, adenovirus, measles, rubella, Zika, Nipah, CCHF, KFD etc

Academics

***M. Sc. Virology*:** Introduced in 2005, the 2 year specialized M. Sc course in Virology, affiliated to Savitribhai Phule Pune University, remains one of the most prestigious and coveted programs of the university, is run entirely by ICMR-National Institute of Virology, Pune. The intake has increased to 33 students since 2020 from 21 due to heavy demand for the course. Students who clear one of the toughest competitive examinations were given admission to the course. ICMR-NIV provides hostel accommodation to student in the office campus situated at Pashan, which gives access to the students to core facilities even after office hours and holidays. It is one of the unique courses where students get the opportunity to interact with scientists directly and also have hands-on experience with most advanced laboratory equipments. The six month project work (fourth semester) under experienced faculties intiates the students to the field of research that helps them to get overseas scholarships to pursue Ph. D. degree.

Ph.D. Program: As a premier research institute, ICMR-NIV continues to support aspirants to pursue Ph. D under various disciplines, *i.e*., Biochemistry, Biotechnology, Basic Medical Science Microbiology, Zoology, etc. ICMR-NIV is affiliated to Savitribhai Phule Pune University and has 15 well experienced faculties recognized guide students for Ph. D. During the year, four students from ICMR-NIV were awarded Ph. D. degree by the university.

Section 1: Report on COVID activities

Research and development activities on COVID-19

1. Research on vaccines, diagnostics and therapeutics for SARS-CoV-2

Development and evaluation of a whole virus inactivated vaccine

Assessment of immunogenicity and safety evaluation of whole virion inactivated SARS-CoV-2 vaccine candidate BB152 in small animal models

Investigators: BBIL team & Yadav P, Sapkal G, Shete A, Mohandas S, Deshpande G, Kumar S, Patil D, Abraham P, Gupta N, Gangakhedkar R, Bhargava B.

Funding agency: Bharat Biotech International Limited, Hyderabad & ICMR-NIV, Pune

Project Duration: 2020-2021

Emergence of SARS-CoV-2 in a pandemic form causing high morbidity and mortality has warranted an urgent need to develop effective vaccines against the virus to safeguard humankind across the world. Govt. of India also did not want to lag behind and wanted to have its own vaccine. ICMR-NIV contributed in isolating the virus, developing vaccine candidates, designing and execution of vaccine efficacy studies in laboratory animals and played a key role in studying the efficacy and immunogenicity of BBV152 vaccine among humans by conducting SARS-CoV-2 live virus neutralization tests and anti-SARS-CoV-2 recombinant ELISAs (N and S-RBD).

Using a well-characterized SARS-CoV-2 strain isolated at ICMR-NIV, Pune, three inactivated vaccine candidates were developed. Appropriate production strategies were adopted to ensure purification and complete inactivation to obtain high-quality antigen. BBV152, one of the candidates, was evaluated at two antigen concentrations (3 & 6µg) with two adjuvants, with a target two-dose schedule administered intramuscularly.
Immunogenicity and safety evaluation of the candidate vaccine at three antigen concentrations (3, 6, and 9µg) and two adjuvants was carried out in three animal models, *i.e*., mice, rats, and rabbits. Vaccine-induced disease enhancement was observed in preclinical studies in animal models due to Th2-like immunity, posing a concern. To circumvent the Th-2 bias and to develop a safe vaccine, a new adjuvant that contains an imidaquizoquinoline class TLR7/8 agonist adsorbed to Algel was used. TLR7/8 agonists induce strong type I interferon responses from dendritic cells and monocyte-macrophages that facilitate the development of Th1 biased immunity instead of a pathogenic Th2-biased immunity. Studies have shown that the vaccine formulation induced significantly elevated titers of antigen binding and neutralizing antibodies in all the animal models with minimum safety concerns. Vaccine formulated with Algel-adsorbed TLR7/8 agonist-induced Th1 biased immunity with significantly elevated SARS-CoV-2 specific IFNI+ CD4 T cell response showing protective and durable neutralizing antibody and T cell responses.

Safety and immunogenicity trial of SARS-CoV-2 vaccine (BB152) in humans

A double-blind, multi-centric, randomised, controlled phase 1 trial to assess the safety and immunogenicity of BBV152 at 11 hospitals across India was conducted. Participants were randomly assigned to receive either one of three vaccine formulations (3 μg with Algel-IMDG, 6 μg with Algel-IMDG, or 6 μg with Algel) or an Algel only control vaccine group. Two intramuscular doses of vaccines were administered on day 0 (the day of randomisation) and day 14. The trial was registered at ClinicalTrials.gov (NCT04471519). Reactogenicity was found to be rare and mild in majority of the participants and resolved without any serious setbacks, barring one, but found unrelated to the vaccination. All the three vaccine formulations resulted in robust immune response comparable to a panel of convalescent serum. Neutralizing responses to homologous and heterologous SARS-CoV-2 strains were detected in all vaccinated individuals. Cell-mediated responses were biased to a Th-1 phenotype. BBV152 is the first inactivated SARS-CoV-2 vaccine that has induced Th1-biased response as binding and neutralising antibody responses were detected. Vaccine induced neutralizing antibody titers were also reported with two divergent SARS-CoV-2 strains. BBV152 can be stored between 2-8°C, which is compatible with the national immunization program cold chain requirements.

Clinical Trial Phase-1: As part of the double-blinded, multicentric, randomised, controlled phase 1 trial to assess the safety and immunogenicity of BBV152 vaccine, a sub set of study participants' (N=375) samples were tested for immune response by antibody neutralization test at ICMR-NIV, Pune. Seroconversion rates were 87·9, 91·9, and 82·8 in the 3μg with Algel-IMDG, 6μg with Algel-IMDG, and 6μg with Algel groups, respectively.

- *Clinical Trial Phase-2*: A sub set of the study participants' samples (phase 1 follow-up) were evaluated for SARS-CoV-2 neutralizing antibody at ICMR-NIV, Pune. The $PRNT_{50}$ sero-conversion rates of neutralizing antibodies on day 56 were 92·9% (88·2, 96·2) and 98·3% (95·1, 99·6) in the 3μg and 6μg with Algel-IMDG groups, respectively. Higher neutralizing titres (2-fold) were observed in the phase 2 study than in the phase 1 study ($p<0.05$). After two doses, the proportions (95% CI) of local and systemic adverse reactions were 9.7% (6.9, 13.2) and 10.3% (7.4, 13.8) in the 3μg and 6μg with Algel-IMDG groups, respectively. No serious adverse events were reported in the study. Phase 1 follow-up samples at day 104 showed sero-conversion in 73.5% (63.6-81.9), 81.1% (71.4-88.1), and 73.1% (62.9-81.8) of individuals in the 3μg and 6μg Algel-IMDG, and 6μg with Algel groups respectively.
- *Clinical Trial Phase-3*: This trial was conducted during November 16, 2020 and January 7, 2021 involving 25,798 participants and concluded that BBV152 vaccine was immunogenic and highly efficacious against symptomatic and asymptomatic COVID-19 variant associated disease, particularly against severe disease in adults. Vaccination was well tolerated with an overall incidence of adverse events observed over a median of 146 days that was lower than that observed with other COVID-19 vaccines. Both vaccine groups elicited more Th1 cytokines than Th2 cytokines. Phase III clinical trial of COVAXIN support was also provided in

evaluating samples from participants from NIZAM Institute (TS/NS), Hyderabad (n=4068) and GMERS, Ahmedabad (385) and reports were submitted timely.

Evaluation of protective efficacy of ZyCoV-D DNA vaccine against SARS-CoV-2 virus challenge in rhesus macaques

Investigators (from ICMR-NIV): Yadav PD, Mohandas S, Patil D, Mathapati B, Shete A, Sapkal G, Patil DY, Kaushal H, Nyayanit D, Sahay R
Investigators (from Zydus-Cadila): Jain M, Maithal K, Giri S, Dey A, Chandra S, Rajanathan C, Raju HP, Patel S, Shah N, Dwivedi P, Singh D

Protective efficacy of ZyCoV-D, a DNA vaccine, expressing the spike protein (S) of SARS-CoV-2 virus was evaluated in rhesus monkeys. The S protein includes the receptor binding domain (RBD), responsible for binding to human angiotensin converting enzyme (ACE)-2 receptor that mediates entry of the virus into the cell. The DNA vaccine construct expressing the spike protein of SARS-CoV-2 was transformed into prokaryotic cells for large scale production of DNA vaccine candidate. The proof-of-concept was established by the immunogenicity and safety studies in animal models.
ZyCoV-D DNA vaccine candidate administered at 1 or 2mg/animal by intradermal route (by needle/needle free injection system) to each treatment group of animals (n=4) on day 1, 28 and 56 at Zydus Research centre, Ahmedabad and the animals were transferred to ICMR-NIV, Pune for virus challenge. Control animals without any treatment were also included for the challenge study. After 4 weeks post last dose, animals were challenged with SARS-CoV-2 virus by intratracheal and intranasal routes and monitored for body temperature, body weight, food intake and clinical signs for 14 days. Swab, bronchoalveolar lavage and blood samples were collected every alternate days. The placebo group developed high viral load and lung disease following virus challenge. However, the vaccine candidate at 2mg/animal dose administered by needle free injection system induced potent cellular response, significant enhancement in neutralizing antibody levels and lesser viral loads. The study demonstrated immunogenicity and protective efficacy of ZyCoV-D vaccine candidate in rhesus macaques.

Evaluation of a SARS CoV-2 RBD-VLP display vaccine candidate in preclinical model

Investigators (ICMR-NIV): Yadav PD, Mohandas S, Patil D, Shete A
Investigators (SIIPL): Rao H, Gautam M, Ghuma PD, Shengule S

A VLP based vaccine candidate developed by Serum Institute of India (SIIPL) was evaluated by ICMR-NIV, Pune. The vaccine candidate was based on the well-established

Hepatitis B surface antigen (HBsAg) fused to the SpyCatcher protein, so that antigen of interest *viz*., receptor binding domain (RBD), linked to the SpyTag peptide, can be easily displayed on it. The receptor-binding domain in SARS-CoV-2 S protein binds strongly to human angiotensin-converting enzyme 2 receptors and, hence prevents attachment of the virus to host cells. The monovalent and combination vaccines including HBSAg VLP is proven to be safe, well tolerated and highly immunogenic.

Six formulations of recombinant SIIPL RBD-VLP display COVID-19 vaccine were used for the study in combination with either alum or CpG adjuvant in Syrian hamsters. The candidates in two dose or single dose intramuscular vaccination regimens could not elicit anti-RBD IgG response or neutralizing antibodies in 90% of hamsters immunized two weeks post second dose. The vaccine candidates in different concentrations and in combination with alum/CpG adjuvant could not prevent body weight loss or pneumonia and decrease in the viral load in the target organs compared to adjuvant control one week post challenge.

Development of an indigenous ELISA for detection of SARS-CoV-2 IgG

Investigators: Yadav PD, Shete AM, Sapkal G, Jain R, Deshpande G

With the isolation of SARS-CoV-2 isolate at ICMR-NIV, an anti- SARS CoV-2 human IgG ELISA was developed and evaluated. Complete inactivation of SARS-CoV-2 was achieved at gamma radiation doses of 6 kGy and the concentrated antigen was used to develop the assay which can be handled even at BSL-2 level laboratory settings. The assay was validated and found to be 92.37% sensitive, 97.9% specific, robust and reproducible with positive and negative predictive values of 94.44 and 98.14% respectively. The ELISA was named as 'COVID KAVACH' and external validation was performed at two National laboratories and found 99.3 to 100% concordant with ICMR-NIV results. The technology has been transferred to seven commercial companies for scale up under public-private partnership.

Therapeutic studies on SARS-CoV-2 using convalescent plasma

Investigators (ICMR New Delhi): Agarwal A, Mukherjee A

Investigators (ICMR-NIV) Yadav PD, Sapkal GN, Kaushal H

Convalescent plasma is a source of antiviral neutralizing antibodies. Other immune pathways, such as antibody dependent cellular cytotoxicity, complement activation, or phagocytosis are putative mechanisms through which convalescent plasma might exert its therapeutic effect in COVID-19 patients. Here, we investigated the therapeutic efficacy of convalescent plasma among moderate COVID-19 adult patients in India.

A total of 464 adults (≥18 years) admitted to hospitals during 22 April to 14 July 2020 with confirmed moderate COVID-19 (partial pressure of oxygen in arterial blood/ fraction of inspired oxygen (PaO2 /FiO2) ratio between 200 mm Hg and 300 mm Hg or a respiratory rate of more than 24/min with oxygen saturation 93% or less on room air) were identified for the study. Of 464, 235 were assigned to convalescent plasma with best standard of care (intervention arm) and 229 were assigned to only best standard of care (control arm). Participants in the intervention arm received two doses of 200 ml convalescent plasma, transfused 24 hours apart. Presence and levels of neutralizing antibodies were not measured a priori; stored samples were assayed at the end of the study. Progression to severe disease or mortality at 28 days after enrolment occurred in 44 (19%) participants in the intervention arm and 41 (18%) in the control arm (risk difference 0.008 (95% confidence interval −0.062 to 0.078); risk ratio 1.04, 95% confidence interval 0.71 to 1.54). The study found the non effectiveness of convalescent plasma in reduction of disease progression to severe COVID-19 and mortality. A priori measurement of neutralising antibody titres in donors and participants might further clarify the role of convalescent plasma in the management of COVID-19.

Evaluation of therapeutic efficacy of purified F(ab')2 fragments of SARS-CoV-2 antiserum immunoglobulins in a Syrian hamster model

Investigators (ICMR NIV): Yadav PD, Mohandas S, Shete A, Sapkal G
Investigators (Biological E): Yadav A, Paradkar V

Immunoglobulins are well-known for its therapeutic property by inhibiting viruses at an entry stage, *i.e.,* membrane fusion, viral attachment etc. Since obtaining plasma from recovered patients is difficult, a SARS-CoV-2 antiserum immunoglobulin (Purified F (ab') 2 fragments) developed by Biological E Ltd., has been evaluated as a therapeutic agent against SARS-CoV-2 in Syrian hamster model. The animals were intra-nasally inoculated with SARS-CoV-2 virus and administered the antiserum at 10,000 neutralization titre (NT/ml) at an interval of 24, 12, and 6 hr post inoculation by intra-peritoneal route. Control group of hamsters were given placebo buffer following virus inoculation. The animals were observed for clinical signs or mortality for 10 days. In both the treated (24 /12 / 6 hours) and the non treated groups, considerable body weight loss was observed while no reduction in virus load in organ was observed in the former in comparison to the placebo group. Histopathological findings were also similar in both the groups. Anti-sera treatment following SARS CoV-2 challenge in Syrian hamster model was found to be not protective against SARS-CoV-2 virus.

Evaluation of therapeutic efficacy of equine antisera to SARS-CoV-2 in a Syrian hamster model

Investigators (ICMR-NIV): Yadav PD, Mohandas S, Shete A, Sapkal G, Deshpande G
Investigators (Serum Institute of India): Shaligram U

Antibody treatment with blood or plasma of convalescent patients has shown promise for many diseases. We evaluated the therapeutic efficacy of SARS-CoV-2 antiserum raised in equine model (provided by Serum Institute of India Pvt. Ltd) in Syrian hamsters. Hamsters infected with SARS-CoV-2 via intranasal route were challenged with COVID-19 antisera at doses of 100000, 50000 or 25000 $PRNT_{50}$ via intra-peritoneal route on day 2 post infection. The animals were monitored for clinical signs and weight loss; necropsy was done on days 4 and 6 and throat swab, nasal wash, lungs and nasal turbinates were harvested. The equine antiserum with $PRNT_{50}$ titre of 100000, 50000 and 25000 neither prevent body weight loss nor viral load in target organs of the treated hamsters. Progression of lung pathological changes were observed in all the antisera treated groups showing the ineffectiveness of the anti-sera to prevent disease progression.

Evaluation of neutralization potential of inactivated COVID-19 vaccine BB152/COVAXIN against B.1.1.7 variant

Investigators: Yadav PD, Mohandas S, Shete A, Sahay R, Sapkal G, Sarkale P, Baradkar S, Deshpande G

The rapid surge of SARS-CoV-2 cases due to Variant of Concern (VOC) 202012/01, (lineage B.1.1.7 or 20B/501Y.V1), in the United Kingdom (UK) in December 2020 raised concerns in several countries including India due to its high transmissibility.

We determined the NAb titers ($PRNT_{50}$) of sera collected (four-weeks after the second dose) from 38 vaccine recipients, who received BBV152 vaccine-candidate in phase-II trial to underline the immunogenicity of BBV152 vaccine candidate against SARS-CoV-2 UK-variant with (VOC) 202012/01 hallmarks belonging to GR clade and strain hCoV-19/India/2020770 belonging to G clade. The results showed that vaccinee sera could neutralize the new UK-variant and heterologous strains with equal efficiency, discounting the uncertainty of possible neutralization escape.

2. Basic research and immunological studies on SARS CoV-2

Evaluation of the susceptibility of mice and hamsters to SARS-CoV-2 infection

Investigators: Mohandas S, Yadav PD, Shete A, Jain R

Funding: Intramural

Laboratory mice and hamsters are advantageous due to their low cost, small size and availability for experimental studies. During the beginning of COVID-19 pandemic, data on susceptibility of animal models to SARS-CoV-2 was rare except for the transgenic mice with hACE2. Therefore, studies were initiated to test the susceptibility of rodent models *viz*., BALB/c mice, C57BL/6 mice and golden Syrian hamsters to the

virus. All experiments were performed with the approval of Institutional Animal Ethics Committee and Institutional Biosafety Committee, ICMR-NIV, Pune. SARS-CoV-2 having $TCID_{50}$ titre of $10^{6.6}$/ml was used in this study. Sixteen mice (BALB/c and C56BL/7) and 18 hamsters were inoculated by intranasal route with $5\times10^{4.5}$ and $1\times10^{5.5}$ $TCID_{50}$ of SARS-CoV-2, respectively, under brief isoflurane anaesthesia. No apparent clinical signs or mortality was observed in BALB/c mice, C57BL/6 mice and the Syrian hamsters. No viral RNA could be detected in the respiratory tract samples of mice on day one post exposure (PE). In Syrian hamsters, viral RNA could be detected in nasal turbinates, trachea, lungs, spleen and kidney on day 3 PE. This is in agreement to a report on human COVID-19 cases which indicated virus transmission in the early course of infection. Serum samples of hamsters showed neutralizing antibody from day 5 onwards with progressive titres till day 21. Syrian hamsters showed high viral loads in the upper and lower respiratory tracts; virus shedding through the nasal cavity and mounting of humoral immune response by the first week, similar to humans. Our findings are suggestive of the susceptibility of hamsters to SARS-CoV-2 infection and its effective use as an animal model.

Comparison of the pathogenicity and virus shedding pattern of SARS-CoV-2 VOC 202012/01 and DG614 variants in a hamster model

Investigators: Yadav P, Mohandas S, Shete A, Sahay R, Kadam M, Kumar A, Jain R

Emergence of SARS-CoV-2 variants has posed a serious challenge to public health system and vaccination programs across the globe and hence initiated the study. We compared the pathogenicity and virus shedding pattern of SARS CoV-2 VOC 202012/01 and D614G variants in hamster model. Studies in Syrian hamsters have shown VOC 202012/01 could produce disease in hamsters characterized by body weight loss and respiratory tract tropism but with mild lung pathology. Higher load of VOC 202012/01 in the nasal wash specimens was observed during the first week of infection comparing D614G variant. Neutralizing antibodies developed against VOC 202012/01 could equally neutralize D614G variant. The findings suggest increased fitness of VOC 202012/01 to the upper respiratory tract could lead to higher transmission of virus.

Propagation of new SARS-CoV-2 variant and characterization in cell culture and in animal models

Investigators: Yadav PD, Mohandas S, Potdar V, Shete A, Sapkal G, Sahay R, Sarkale P, Baradkar S.

In the mid December 2020, United Kingdom (UK) and Ireland reported a new SARS-CoV-2 variant, referred to as SARS-CoV-2 VOC 202012/01 which has 40% to 70% transmissibility than the original strain. The emergence of B.1.1.7 lineage *(a.k.a. 20B/501Y.V1 variant of concern [VOC] 202012/01) and* B.1.351 lineage *(a.k.a. 20C/501Y.V2)* has been reported from UK and South Africa, respectively and has been identified in several countries including India subsequently.

ICMR-NIV has isolated and characterized the *(VOC) 202012/01* from five UK returnees who tested positive by real-time RT PCR. Four sequences from three cases had all the hallmarks for the (VOC) 202012/01, whereas the fifth sequence varied. According to Global Initiative on Sharing All Influenza Data (GISAID) nomenclature, the four sequences having (VOC) 202 012/01 hallmarks belonged to GR clade and the fifth belonged to G clade. The percentage nucleotide difference between the hCoV-19/India/NIV_P1_20203524/2020 and the GR clade SARS-CoV-2 sequences in this study was observed to be 0.05%, indicating variation in the isolate sequences.

Humoral immune response among the COVAXIN® and Covishield vacinee sera against different SARS-CoV-2 variants

Investigators (ICMR-NIV): Spakal GN, Yadav PD, Deshpande D, Patil D, Mohandas S, Shete A, Sahay R, Abraham P
Investigators (ICMR, New Delhi): Gupta N, Rajni Kant, Panda S, Bharghava B
Investigators (BBIL): Vadrevu KM, Ganneru B, Raches Ella, Prasad SD, Harsh Jogdand.

Emerging variants of SARS CoV-2 strains with increased infectivity and transmissibility had raised concern about neutralization escape mutants and reduced protection by existing vaccines (Covaxin and Covischield) available in India. Studies were undertaken to understand the *in-vitro* neutralization ability of vaccinee sera and convalescent sera from COVID-19 recovered patients. In this study we determined the neutralization potential of Covaxin and Covishield vaccines against multiple variants of SARS CoV-2 (Alpha, Beta, Kappa, Delta, Delta Plus etc).

Covaxin and Covishield vaccinee sera of the following category were used for neutralization studies using well characterized SARS-CoV-2 strains isolated at ICMR-NIV, Pune.

1) Sera collected 28 days after 1st and 2nd dose of vaccination
2) Sera collected from individual recovered and vaccinated
3) Sera collected from breakthrough cases (Infection after two doses of vaccination)

(i) <u>Neutralization of UK-variant VUI-202012/01 with BBV152 vaccinated human serum:</u> ICMR-NIV tested BBV152 vaccinated human serum (n=26) against hCoV-

19/India/20203522 (UK-variant) and hCoV27 19/India/2020Q111 (heterologous strain) and obtained a comparable neutralization activity by the vaccinee sera with UK-variant and the heterologous strain.

(ii)Neutralization of variant under investigation B.1.617 with sera of BBV152 vaccinees: The result of B.1.1.7 variant neutralization with BBV152 vaccine sera and findings of B.1.617 emphasize that this vaccine is robust against emerging mutation and maintains the efficacy of the vaccine.

(iii)Neutralization of B.1.1.28 P2 variant with sera of natural SARS-CoV-2 infection and recipients of BBV152 vaccine: The neutralization efficacy of the convalescent sera of individuals with natural infection and BBV152 vaccination of B.1.1.28.P2 variant was evaluated. The vaccine induced significantly high titers of IgG and neutralizing antibody against both B.1.1.28.2 and D614G variants as compared to natural infection. The study demonstrated 1.92 and 1.09 fold reductions in the neutralizing titer against B.1.1.28.2 and prototype D614G variant with sera of vaccine recipients and natural infection respectively.

(iv)Neutralization potential of Covishield vaccinee sera against B.1.617.1: Although we observed a reduction in the neutralizing titer against B.1.617.1 variant, Covishield vaccine-induced immunity may still limit the severity of disease and mortality in the vaccinated individuals. Also, COVID-19 recovered individuals with immunization can maintain protective antibody titer for longer periods.

(v)Neutralization against B.1.351 and B.1.617.2 with sera of COVID-19 recovered cases and vaccinees of BBV152: The study demonstrated a reduction in neutralization titers with sera of COVID-19 recovered cases (3.3-fold and 4.6-fold) and BBV152 vaccinees (3. 0 and 2.7 fold) against B.1.351 and B.1.617.2 respectively. Although, there is reduction in neutralization titer, BBV152 vaccine demonstrated protective response against VOC B.1351 and B.1.617.2.

(vi)Serendipitous COVID-19 vaccine-mix in Uttar Pradesh, India: Safety and immunogenicity assessment of a heterologous regime: Immunization with a combination of Covishield followed by Covaxin was not only safe but also elicited better immunogenicity.

Use of SARS-CoV-2 spike and nucleocapsid proteins in the assessment of antibody response in infected/recovered individuals

Investigators: Lole KS, Sapkal GN, Gurav YK

Funding: Intramural

Humoral response to SARS-CoV-2 is primarily directed towards highly immunogenic viral proteins *viz*., nucleocapsid (N) and spike (S). S protein determines the viral host range and its infectivity and consists of two subunits S1 and S2. The S1 subunit harbors a receptor binding domain (RBD) that binds to cell surface receptor ACE2 and helps virus entry, while S2 is involved in membrane fusion. RBD is an immunodominant region and majority of virus neutralizing antibodies bind to this region. N protein is closely associated with viral RNA during virus assembly; plays a crucial role during viral transcription and expressed abundantly during virus infection. N protein is highly immunogenic, induces IgG, IgA and IgM class of antibodies which are detectable in confirmed COVID-19 patient's serum samples. Hence detection of anti-N IgG antibodies is useful in assessing prior exposure to SARS-CoV-2. N-protein is used in serological assays, either alone or in combination with S protein for detecting antibodies against SARS-CoV-2.

The main objectives of the present study were to clone and express the S1 region of the SARS CoV-2 receptor binding domain and the N protein using a baculovirus expression system. Such expressed proteins would then be used to develop an ELISA based surrogate virus neutralization assay using hACE2 and RBD proteins for evaluation of neutralizing proteins.

N-protein expression was induced with 1m*M* IPTG and purified from *E. coli* cell pellets in native condition using cation exchange chromatography followed by size exclusion chromatography. The purified protein was used to develop indirect ELISA for detecting anti-N antibodies in SARS-CoV-2 infected individuals. The assay was optimized using a set of serum samples collected during pre-COVID period and from confirmed RT-PCR positive patients. Optimum coating of N protein was estimated to be 150ng/well and anti-human IgG HRP conjugate dilution to be 1:15000 by checker board titration. After ROC analysis, the area under the curve was found to be 0.986. Cut off for ELISA was set at 0.561, which gave sensitivity of 92.9% and specificity of 92.1% (Fig 1). Several attempts to clone S1 and RBD regions of spike protein were unsuccessful and found to be highly unstable.

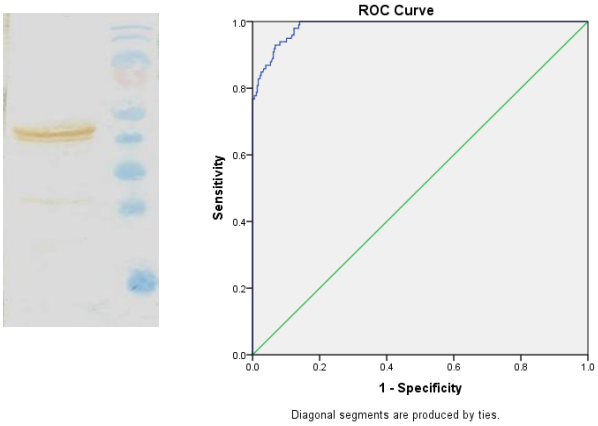

Fig 1: N-protein based ELISA: A) Western blot (lane 1: N protein, lane 2: protein marker), B) Receiver Operating Characteristic (ROC) curve

Durability of antibodies, SARS-CoV-2 specific immune cells, cytokines and T-cell response post SARS-CoV-2 infection: longitudinal analysis upto one year

Investigators: Tripathy AS, Gurav YK, Trimbake D, Singh D, Babar P, Dange V, Abraham P
Funding: Intramural

COVID-19 recovered individuals develop both humoral and T cell responses against SARS-CoV-2. However, there have been conflicting reports on the durability and protective role of antibodies and T cell response post SARS-CoV-2 infection. Neutralizing antibodies have been used as the correlates of protection in COVID-19 vaccine trial studies. It has been reported that neutralizing antibodies and antibodies against S1 protein go hand in hand. Putative roles of long lived plasma cells and T cell response following infection have also been suggested. Here, we have assessed the antibodies, specific immune cell profiles, specific cytokine profiles and CTL response (IFN-γ release) in a set of recovered individuals at 1 month, 6-8 months and 1 year post recovery from SARS-CoV-2 infection.
At one month post recovery, 21 of 34 (62%) recovered individuals were positive for IgG antibodies against SARS-CoV-2, while in 20/34 (58%) T cell response was detected. It is interesting to note that in 10/21 (47%) antibody positives, SARS-CoV-2 specific T cell response was detected, while same was also detected in 10/13 antibody negatives (77%), which is an important observation. At 8-9 month post recovery, only 6/39 (15%) had detectable T cell response while 21 were antibody positive and 5 (24%)

had T cell response, while 1/18 was antibody negative but had detectable T cell response.

Fourteen recovered individuals were followed up to 8-9 months post recovery. SARS-CoV-2 S1 antigen stimulated cytokine assay was carried out for estimation of cytokine/ chemokines on the Bio-plex Multiplex Immunoassay System (Bio-Rad, Hercules, CA, USA) using a Bio-plexPro^{TM} Human Cytokine 27-plex assay kit. The results are:

Recovered individuals 1-2 months post recovery vs control group: Levels of SARS CoV-2 specific pro-inflammatory cytokines IL-1β, IL-17, chemokines CCL-2, CCL-3, and basic fibroblast growth factor (FGF) were significantly low in the recovered group compared to control group. Importantly, the level of chemokine CXCL-10 was significantly high in the recovered group. Levels of anti-inflammatory cytokines, Th1 cytokines and other Growth factors were comparable among the recovered individuals and uninfected controls.

Recovered individuals 8-9 months post recovery vs control group: Levels of SARS CoV2 specific pro-inflammatory cytokines IL-1β, IL-15, and basic FGF were significantly low in the recovered group. Levels of anti-inflammatory cytokines, Th1 cytokines, chemokines and other growth factors were comparable.

Recovered individuals post 1-2 months of recovery vs Recovered individuals 8-9 months post recovery: Levels of SARS CoV-2 specific pro-inflammatory cytokines IL-17 and Th1 cytokine IL-12,chemokine CCL5 were significantly low in recovered group post 1-2 months of recovery compared to recovered group post 8-9 months. Importantly, the level of chemokine CXCL-10 was significantly high in the recovered group post 1-2 months of recovery. Levels of remaining anti-inflammatory cytokines, Th1 cytokines, chemokines and Growth factors were comparable.

Complete transcriptome analysis of cells in throat/nasal swabs of COVID-19 patients

Investigators: Lole KS, Potdar V, Gurav YK, Tomar S, Cherian SS
Funding: Intramural

SARS-CoV-2 is a cytopathic virus, induces cell apoptosis which in turn triggers inflammatory response. Increased secretions of pro-inflammatory cytokines and chemokines were observed in COVID-19 patients. We propose to do profiling of complete transcriptome of oropharyngeal/ nasopharygeal cells collected from different categories of COVID-19 patients to understand host response and viral pathogenesis. The generated transcriptome data will be used for undertaking computational drug repurposing.

The main objectives of the study were (i) transcriptome profiling of oropharyngeal/nasopharyngeal cells (from swab samples) of COVID-19 patients

manifesting mild, moderate and severe disease and (ii) use the data for drug repurposing.

Ninety-six swab samples were collected from mild (n=23), moderate (n=29) and severe cases (n=34) of COVID-19 patients after taking written consent from the patients/relatives between 7 to 10 days post onset days. For comparison, samples were collected from 10 healthy controls. Total RNA extraction was optimized using conventional trizol method with modifications. The mRNA sequencing libraries were prepared for six representative RNA samples to check RNA extraction method protocol being employed for development of library using low quantities of RNA. Remaining samples were processed for RNA extraction. Considering low RNA yield from individual swab samples, pooling of two similar samples were done and processed for transcriptome analysis. Further work is in progress.

Possible role of accessory proteins in the viral replication for the 201/501Y.V1 (B.1.1.7) SARS-CoV-2 variant

Investigators: Yadav PD, Nynait D, Shete A, Kumar A, Patil S, majumder T, Baradkar S, Gawande P, Sarkale P

This study investigated the replication cycle and transcriptional pattern of the B.1.1.7 variant. It was observed that B.1.1.7 variant required a longer maturation time. The transcriptional response demonstrated higher expression of ORF6 and ORF8 compared to nucleocapsid transcript till the eclipse period which might influence higher viral replication. The number of infectious viruses/ titer is higher in B.1.1.7, despite a lesser copy number than B.1, indicating higher infectivity.

Natural selection plays an important role in shaping the codon usage of structural genes of viruses belonging to Coronaviridae family

Investigators: Yadav PD, Nyayanit D, Kharde R, Cherian S

Each gene in the *Coronaviridae* family plays an essential role in viral replication, survival and infection, due to which it becomes essential to analyze the evolutionary factors involved in determining its choice for the codon bias. In this study, we analyzed the synonymous CUB of representative *Coronaviridae* sequences available in the GenBank database as well as the role of mutational pressure and natural selection on the evolution of codon usage of different genes, as observed in the *Coronaviridae* family. Comprehensive analysis of the CUB for different genes encoded by members of *Coronaviridae* family was carried out in the present study.

Base composition and RSCU analysis demonstrated presence of A-ended and U-ended codons being preferred in the 3rd codon position and are suggestive of mutational selection. The lesser ENc value for the spike 'S' gene suggests a higher bias in the codon usage of this gene compared to other structural genes. Parity plot 2 and neutrality plot analyses demonstrate the role and the extent of mutational and natural selection towards codon usage pattern. It was observed that structural genes of *Coronaviridae* family analyzed in this study were at least under 84% influence of natural selection, implying a major role of the natural selection in shaping the codon usage.

Performance evaluation of TrueNat ™ Beta CoV test on TrueLab ™ workstation

Investigators: Yadav PD, Shete AM, VRDL team Bengaluru NIV

Funding: Intramural

The TrueNat Beta CoV E-gene screening assay and TrueNat SARS-CoV-2 RdRp gene-confirmatory assay (Molbio Diagnostics, India) were earlier validated as a two-step test. The assays were deployed for COVID-19 testing in various parts of India between April and June, 2020. A multiplex assay combining E-gene screening and Orf1a-gene confirmatory assay has also been validated recently. Technical guidance for planning and validation of the assay was provided to team at BMCRI, Bengaluru. Performance evaluation test of TrueNat™ BETA CoV test on Truelab™ workstation was carried out. All three assays exhibited 100% sensitivity and specificity, and positive and negative predictive value when compared to the gold-standard Real time RT-PCR assay. A total of 2530 Truelab workstations are currently operational at 1008 sites in 530 districts of India. Of the total 70.7 million COVID-19 tests done in India up to 25 September 2020, 2.7 million (3·8%) have been run on Truelab workstations. This innovative technology-driven COVID-19 testing platform has been a game changer for testing in under-served areas and quick testing in emergency departments of health-care facilities in India.

Studies on the effect of full length SARS-CoV-2 envelope spike glycoprotein on physiology of cultured vascular endothelial cells in-vitro

Investigators: Basu A, Jain P, Meena VK, Prasad SS

Funding: Intramural

A significant body of evidence from both *in-vivo* and *in-vitro* studies have emerged to suggest that endothelial injury due to direct or indirect disease pathology of SARS-CoV-2 may constitute a fundamental event in the pathogenesis of COVID-19 associated vascular dysfunction. Several recent *in-vitro* studies have suggested that the SARS-CoV-2 spike glycoprotein (GP) by itself may also cause perturbations in endothelial cell functions. In the present study we examined the effect of exogenous exposure of the full length SARS-CoV-2 spike glycoprotein to cultured human umbilical vein endothelial cells.

The preliminary results suggests that exogenous exposure of cultured endothelial cells to SARS-CoV-2 GP results in a dose independent morphological change and associated alterations in the actin cytoskeleton distribution (Fig 2). This observation strongly implicates the possibility of nanotube formation that is under investigation. The viral protein exposed cells also show alterations in IL6, IL8, vWF, VCAM and ACE2 suggesting engagement of deeper vascular pathophysiology that are being studied (Fig 2).

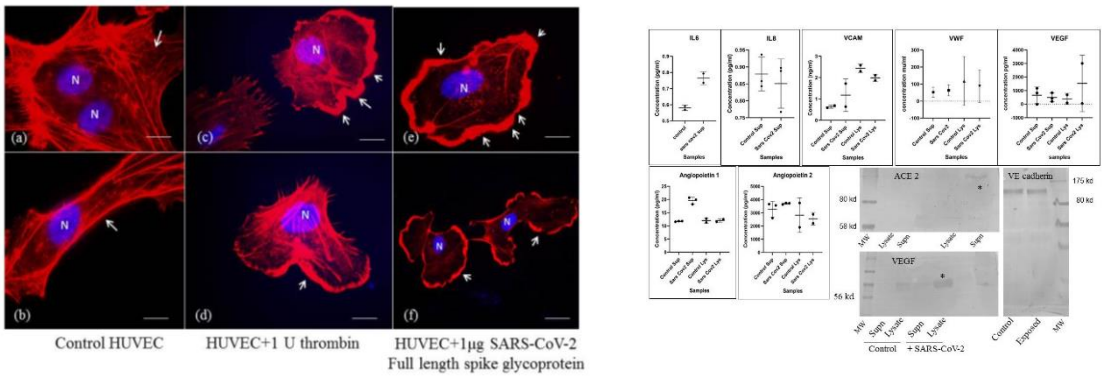

Fig 2: Changes in actin distribution imaged by phalloidin labeling and expression of several key vasoactive molecules from cultured endothelial cells following exogenous exposure to SARS CoV-2 GP *in-vitro*.

Immunological studies II

II (i) Assessment of the role of host immune response in COVID-19 infection: Pro-inflamatory CXCL-10, TNF-α, IL-1β and IL-6: biomarkers of SARS-CoV-2 infection

Investigators: Tripathy SS, Gurav YK, Potdar VA, Mokashi ND, Patsute SD, Chaudhury ML, Abraham P

Funding: Intramural

Differential clinical manifestations and outcomes in SARS-CoV-2 infection could be attributed to factors like virus replication, infiltration of inflammatory cells and altered cytokines. Virus induced aberrant and excessive cytokine production has been linked to morbidity and mortality in several viral infections. We investigated plasma cytokine and chemokine levels of 27 analytes on a luminex platform, in the hospitalized asymptomatic (n=39), mild symptomatic (n=35) SARS-CoV-2 infected patients (early phase of infection), recovered individuals (45-60 days post infection) (n=40) and uninfected controls (n=36), from Pune city. Levels of pro-inflammatory cytokines IL-1β, IL-6, TNF-α and chemokine CXCL-10 were significantly high, while anti-viral cytokines IFN-γ and IL-12 p70 were significantly low in both asymptomatic and mild symptomatic patients compared to controls. Comparison among the patient categories revealed no difference in the levels of cytokines/chemokines except for CXCL-10 being significantly high and IL-17, IL-4 and VEGF significantly low in mild symptomatic patients. Interestingly, levels of all key analytes were significantly low in recovered individuals compared to both patient categories. Nevertheless, the level of CXCL10 was significantly high in the recovered compared to controls indicating that immune system of SARS-CoV-2 patients may take longer time to normalize. Our data suggest that IL-6, IL-1β, TNF-α, CXCL-10 and reduced anti-viral cytokines could be put forward as biomarkers of SARS-CoV-2 infection (Table 1) shown below.

Analytes	Cut-off (pg/mL)	Sensitivity (%)	Specificity (%)	AUC Value	*p*-value
TNF-α	29.19	98.65	83.33	0.9508	<0.0001
CXCL-10	124.9	97.3	91.67	0.9917	<0.0001
IL-1β	0.545	91.89	86.11	0.9202	<0.0001
IL-4	1.395	97.30	66.67	0.9336	<0.0001

II (ii). ***Peripheral lymphocyte subset alterations in COVID-19 patients with different clinical manifestations***

Investigators: Tripathy AS, Gurav YK, Potdar VA, Chaudhury ML, Mokashi ND, Patsute SD, Abraham P

Funding: Intramural

The aim of this study was to assess the peripheral lymphocyte phenotype and subset distribution in COVID-19 patients with differential clinical manifestations. Percentages of peripheral lymphocyte subsets were measured by flowcytometry in hospitalized asymptomatic (n=53), mild symptomatic (n=36), moderate & severe (n=30) SARS-CoV-2 infected patients, recovered individuals (n=40), and uninfected controls (n=56) from Pune. Percentages of CD4+Th cells were significantly high in asymptomatic, mild symptomatic,

moderate & severe patients and in recovered individuals compared to controls. Percentages of Th memory (CD3+CD4+CD45RO+), Tc memory (CD3+CD8+CD45RO+) and B memory (CD19+CD27+) cells were significantly high in recovered group compared to asymptomatic, mild symptomatic patient and control groups. NK cell (CD56+CD3-) percentages were comparable among moderate +severe patient and uninfected control groups. Observed lower CD4+Th cells in moderate+severe group requiring oxygen support compared to asymptomatic+ mild symptomatic group not requiring oxygen support could be indicative of poor prognosis (Fig 3). Our data suggest that immunological reaction triggered by SARS-CoV-2 is mainly T lymphocyte mediated. Higher Th memory, Tc memory and B memory cells in recovered individuals compared to mild symptomatic patient groups could be put forward as markers of recovery from mild infection; though remains to be established if the persistence of any of these cells could be considered as a correlate of protection.

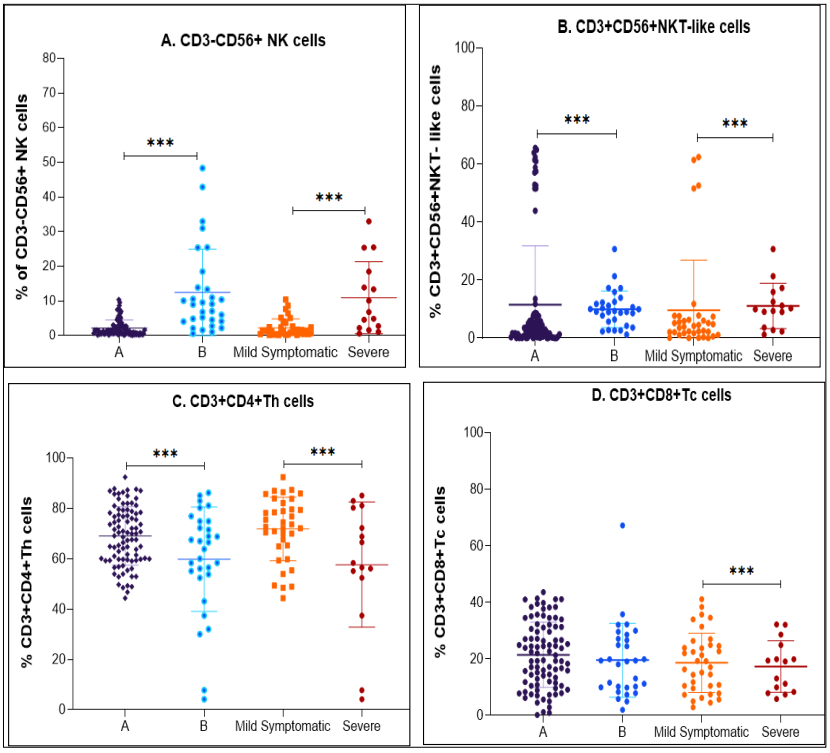

Figure 3: Flow cytometric analysis of NK/NKT-like cells, T cell subsets among patients not requiring O_2 support, patients requiring O_2 support, mild symptomatic and severe COVID-19 patients.Flow cytometric analysis of NK/NKT-like, T cell subsets. Peripheral blood mononuclear cells (PBMCs) from 89 patients not requiring O2 support (Asymptomatic+Mild symptomatic), 30 patients requiring O2 support (Moderate+Severe), 36 mild symptomatic, 15 severe patients and 56 uninfected controls were stained and acquired on flowcytometer. Vertical scatter plots denote the comparisons of frequencies of immune cells and their subpopulation among different study groups: (A) CD3-CD56+ NK cells (B) CD3+CD56+ NKT-like cells (C) CD3+CD4+Th (D) CD3+CD8+Tc cells Data are presented as percentage of immune cells out of lymphocytes. The dots represent individual values and bars represent Mean+SD values. (*p-value <0.05,

**p-value <0.005, ***p-value <0.0001) [Study group A: patients not requiring O_2 support (Asymptomatic+Mild symptomatic), Study group B: patients requiring O_2 support (Moderate+Severe)].

II (iii) Understanding the spectrum of viral infections associated with specific humoral or cell-mediated immunodeficiency and its correlation with clinical implications

Investigators: Mohanty M, Desai M, Madkaikar M, Taur P, Barose S, Sawant

Funding: Intramural

Reports of prolonged viral carrier state in immunocompromised patients have underlined the risk for within-host variant generation as seen in the SARS-CoV-2 B.1.1.7 variant. Children with Inborn Errors of Immunity (IEI) commonly referred to as Primary Immunodeficiency Disorders (PIDs**)** pose a higher risk of COVID-19 infection and may act as a reservoir excreting SARS-CoV-2 virus**.** The objective of the study was to investigate SARS-CoV-2 excretion in stool samples of pediatric patients with PID, asymptomatic for COVID-19 infection.

Our investigations in 34 asymptomatic children suffering from PIDs detected SARS-CoV-2 in gastrointestinal tract for a prolonged period (Table 1). Stool samples of four patients tested positive for SARS-CoV-2. Patient 1 diagnosed with Hyper IgM syndrome was observed to shed the virus for about 99 days from the day first tested positive. This was the first report of prolonged detection of SARS-CoV-2 in the GI tract of patients with PID. Patient 2 diagnosed with Wiskott Aldrich Syndrome shed the virus for 54 days. Co-infection with enteroviruses, noroviruses and human adenoviruses was observed in all four patients. Prolonged fecal shedding of SARS-CoV-2 observed in our study highlights the potential risk of within host variant generation and feco-oral transmission in asymptomatic pediatric patients with PID. Since diarrhea is a frequent symptom in patients with IEI, there is a need for rapid and effective modification of the screening and diagnostic algorithms of COVID-19 for these patients.

Table 1: Fecal shedding of SARS-CoV-2 in pediatric patients with primary immunodeficiency

Day wise collection	**Sample No.**	**C_t Values**		**Interpretation**
		E gene	**Orf1b gene**	
P1: Hyper IgM Syndrome				
01	01	25.15	26.97	Positive
32	02	25.51	27.7	Positive
59	03	30.27	32.24	Positive
99	04	33.02	34.19	Positive
176	05	36.38	36.13	Negative
205	06	UD	UD	Negative
P2: Wiskott Aldrich Syndrome				
01	01	20.53	22.98	Positive
22	02	34.59	35.8	Positive

54	03	29.84	31.63	Positive
141	04	UD	UD	Negative
P3: Chediak Higashi Syndrome				
01	01	30.89	33.85	Positive
40	02	37.99	UD	Negative
P4: Combined Immunodeficiency with XMEN				
01	01	29.97	32.83	Positive
71	02	UD	UD	Negative

II ***(iv) Innate immune evasion by SARS-CoV-2: evaluation of the role of immune sensors in disease progression in children and adults***

Investigators: Mohanty M, Varose S, Sawant U, Fernandes M

Funding: Intramural

Upper respiratory mucosa is the entry point of SARS-CoV-2 and cells at this site constitute the first line of defense against pathogens. Innate immune response at this site is crucial for controlling the replication and symptoms in early stage of virus infection. The objective of the present study was to study the expression of innate immune receptors in upper airway cells as biomarkers for COVID-19. Increased expression of Toll like Receptor-2 (TLR2), MDA5 (Melanoma Differentiation-Associated protein 5) and ACE2 was detected in cells present in nasal swabs of SARS-CoV-2 infected patients in comparison to controls ($p< 0.02$). MDA5 expression was significantly higher in asymptomatic and mildly symptomatic SARS-CoV-2 patients than patients with severe symptoms ($p=0.04$; $p= 0.004$ respectively) (Figure 3A and 2B). Further, the asymptomatic group showed significant induction of type 1 Interferons than the symptomatic group. The findings suggest that increased MDA5 in Nasopharygeal cells of asymptomatic SARS-CoV-2 positive patients subsequently induce type 1 Interferons to protect the individuals from further clinical severity of SARS-CoV-2 infection.

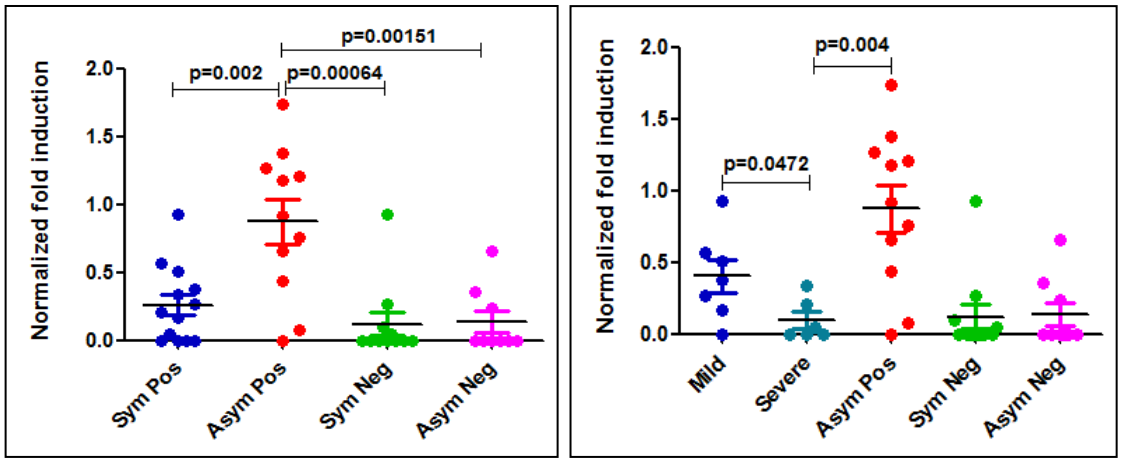

Figure 4: Differential expression of MDA-5 in symptomatic and asymptomatic SARS-CoV-2 positive individuals

3. Epidemiological studies

Molecular epidemiological analysis of SARS-CoV-2 circulating in different regions of India

Investigators: Yadav PD, Gupta N, Shete AM, Nyaynit D, Centers of VRDL

Whole-genome sequencing of SARS-CoV-2 has helped to identify the geographic distribution of different virus clades/ variants across the globe. Availability of genomic sequences helps in understanding different nucleotide as well as amino acid variations in their genetic make-up. To identify the variations in SARS-CoV-2 genomic sequences received from different parts of India during January to August 2020.

Clinical data of 1603 samples received from twenty-five states/Union Territories was analysed. Complete SARS-CoV-2 sequence was retrieved from 689 samples and found the following key facts *viz*., i) Predominance of 'G' clade and its variants (GH and GR) in different states of India, and ii) three mixed SARS-CoV-2 clade variants (G-S, GR-GV, and GH-GR) were specifically identified and found to be circulating in India. However, the observed nucleotide variation in the SARS-CoV-2 genome is <1% as compared to the original L clade of Wuhan, China. The sequence divergence detected within a short frame of time suggests that SARS-CoV-2 is continuously evolving.

Molecular characterization of SARS-CoV-2 virus circulating in India

Investigators: Potdar VA, Chaudhury ML, Bharadwaj SD

Funding: Intramural

SARS-CoV-2 viral genome sequencing and genetic characterization have become an integral part for epidemiological investigation, which revealed the emergence of new variants. The main objective of the present work was molecular characterization of SARS-CoV-2 virus and its variants circulating in India through VRDL network.

ICMR-NIV has sequenced 3673 SARS CoV-2 positive samples out of 6238 received from different states through VRDL network and IDSP, using next generation sequencing platform and submitted 1561 sequences to GISAID. Introduction of S, L and O clades to India were from China and Iran while G clade was from Italy. However, since September 2020, G clade and its sublimates (GH, GR, GV, GVR) became predominant as seen globally. The predominant clades (Pangolin/GISAID) circulating in India are the B.1.1.32/GR, B.6/O, B.1/G, B.1.1/GR, B.1.113/GH and B.1.1.8/GR. From Jan 2021, Variants of Concern (VOC) detected were B.1.1.7 (Alpha), B.1.351 (Beta), B.1.617.2 (Delta), AY.4 and Variants of Interest (VOIs) detected were B.1.617.3, B.1.617.1, P.2, B.1.526, B.1.525, B.1.429. Figure 5 shows temporal distribution of major SARS CoV-2 lineages circulating in India with timelines.

In January 2021 Indian SARS-CoV-2 Genomics Consortium (INSACOG) was established with joint collaboration of MoHWF, DBT, CSIR and ICMR using 10 regional genome sequencing labs and 18 satellite laboratories to identify the variants through continuous surveillance alongwith epidemiological and clinical correlation. NIV Pune being one of the RGSL doing genome surveillance for Maharashtra, Gujarat and Goa had sequenced 5072 samples

till date. Since the emergence of Alpha variant, NIV has sequenced 385 samples from different states and detected 212 (55.1%) positives with 140 alpha variants and a few Beta and Gama variants (Fig 5). In February 2021, eastern parts of Maharashtra reported increased SARS CoV-2 positivity and genome sequencing revealed emergence of Delta and Kappa variants with signature mutations L4542R, E484Q and P681R (Fig 6).

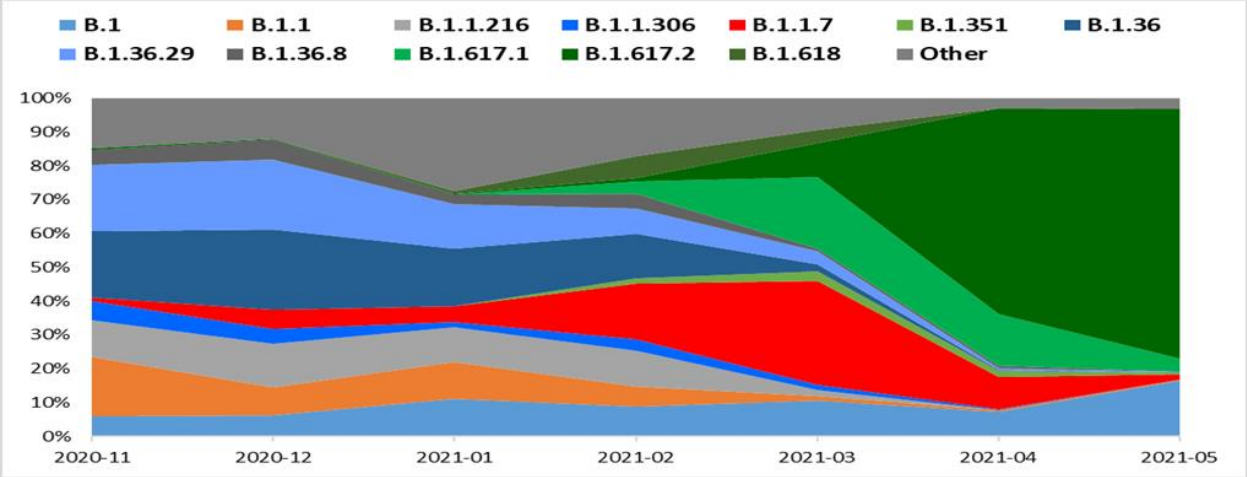

Figure 5: Temporal distribution of major lineages of SARS-CoV-2 in India

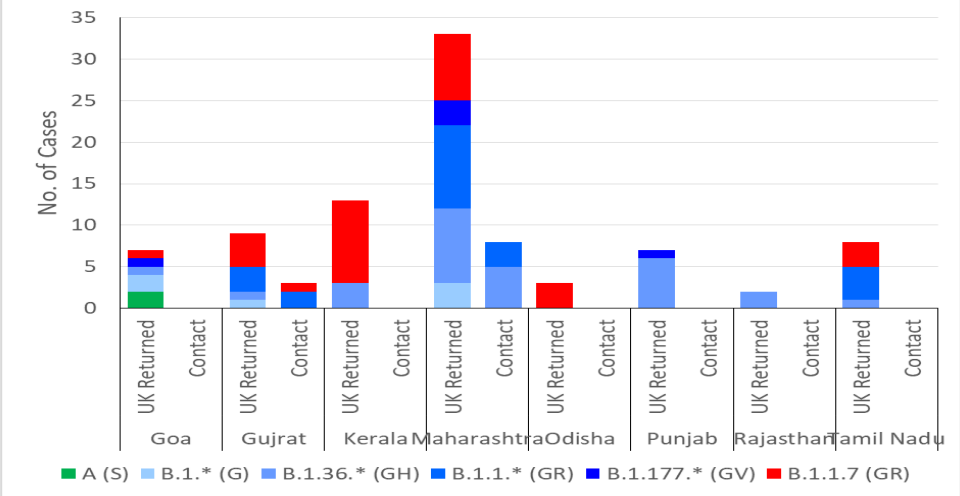

Figure 6: Detection of SARS-CoV-2 variants in India from UK returnees

Seroprevalence of SARS-CoV-2 among healthcare workers, laboratory personnel and general population in Maharashtra India

Investigators: Gurav YK, Sapkal GN, Deoshetwar AR, Pawar SD, Vishwanathan R, Walimbe A

Funding: Intramural

ICMR-NIV has conducted sero-surveys among health care workers, laboratory workers and general population in Pune. The study objective was to determine the seroprevalence of SARS-CoV-2 among health care workers, laboratory personnel and general population. Blood samples were collected from 724 study participants comprising general population (154), health care workers (435) and laboratory workers (135) involved in COVID-19 diagnosis at reference centers and screened for anti-SARS-CoV-2 IgG antibodies by COVID-19 Kavach ELISA. The age of health care workers ranged from 18 to 60 with a mean age of 37.7. Anti-SARS-CoV-2 IgG antibody prevalence was found similar in general population (24.7%, 38/154; 95% CI 18.1 – 32.3) and health care workers (23.9%, 104/435; 95%CI 19.2-27.1) while it was low among laboratory workers with 17.8% (24/135; 95% CI 11.7 – 25.3). The F:M ratio was 1:0.85. The age groups showed increasing sero-positivity with age, lowest in the youngest age group of 18-30 (16.4%) and the highest among older age group of 51-60 (34.1%). The seroprevalence of SARS-CoV-2 among health care workers and general population was found higher in comparison to laboratory personnel.

Proportion of exposure to SARS-CoV-2 among non-healthcare workers -essential service providers in Pune city

Investigators: Deoshetwar AR, Gokhale MD, Sapkal GN, Potdar VA

Essential service providers or the frontline workers rendered their services through the national and local lockdowns and are at higher risk of contracting and transmitting SARS-CoV-2 infection. The objective was to understand the level of exposure of the 'Frontline workers' [FLWs] and compare the estimates among different categories of FLWs. A questionnaire on possible risk factors was administered to the participants. These workers were chosen from areas close to containment zones for which data was available with ICMR.

A total of 221 FLWs were found positive among 636 enrolled. The sero-prevalence among frontline workers was higher than the general population of Pune city but was lower than that in containment zones. The frontline workers who sanitized their hands after every interaction with their customer / client were found to have significantly lower seroprevalence [OR=1.88; 95% CI: 1.31 – 2.70]. People residing in hutments or kachcha house had higher seroprevalence than those living in flats [OR=2.87; 95% CI: 1.58 – 5.21]. The FLWs associated with lower socio-economic status [SES] (vegetable/fruit vendors and Swachh karmacharis) had a high seroprevalence than the FLWs [OR=3.21; 95% CI: 2.22 – 4.64]. FLWs who followed social distancing and hand sanitization had lower seroprevalence. The exact reasons for higher seroprevalence among FLWs from low SES need to be studied further.

COVID-19 serosurvey in Karnataka

Investigator: Ashok M

Funding: Intramural

As a part of COVID-19 sero survey for the state, we tested serum samples from Bangalore urban, Tumkur and Chikkabalapur districts. During the first phase, 1861 serum samples were tested for COVID-19 IgG antibodies and confirmed 361 positives. During the second phase, 3744 serum samples were tested for COVID-19 IgG antibodies and confirmed 759 positives.

Mathematical epidemiology studies on COVID-19: district-wise estimation of basic reproduction number Ro for COVID-19 in India during the initial phase (first wave)

Investigators: Shil P, Tandale BV, Atre NM, Abraham P

A retrospective study on the epidemiological features and spatial spread of COVID-19 in India from February 2020 to March 2021 was conducted. For each district, the cumulative number of confirmed COVID-19 cases was fitted to exponential growth model for the initial phase of the outbreak (the first 7 to 15 days). From this we estimated the exponential growth rate of the epidemic. Other epidemiological parameters like basic reproduction number (R_0) and epidemic doubling time (τ) were determined based on the said growth rate. Using Q-GIS software, we have generated all India distribution maps for R_0 and τ. COVID-19 spread rapidly covering majority of the districts of India between March and June 2020 (**Fig. 7**). As on 1st March 2021, a total of 715 out of 717 districts have been affected. The R_0 range was found to be at par with the global averages. Intense transmission was recorded (R_0>7) in a few districts, where outbreaks were caused by migrant workers returning home. Ranking of districts based on R_0 is indicated in Figure 7. We also found that the spread of COVID-19 was not uniform across the different districts of India.

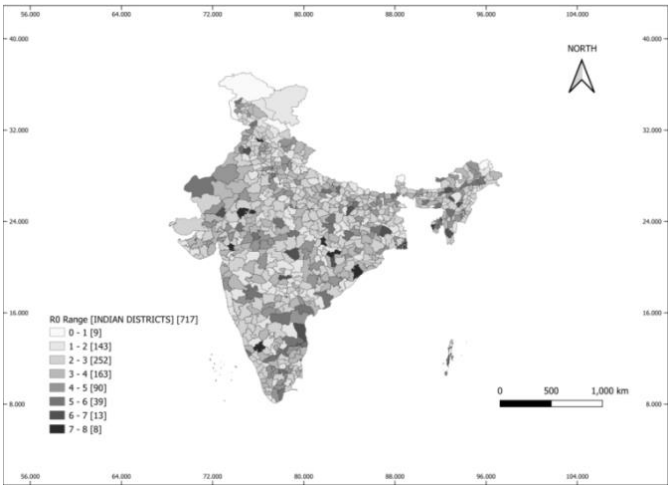

Fig 7: Distrubution in India based on R_0

4. Bioinformatic studies

Structural bioinformatics approaches for functional analysis of SARS-CoV-2 spike mutations in the second wave of COVID-19 in Maharashtra India

Investigators: Cherian S, Potdar V, Jagtap P, Kasabe B

A heatmap of mutations noted in the spike protein revealed that B.1.617.1 lineage possessing distinct signature mutations L452R, E484Q and P681R could be linked to the surge of cases in February 2021 in eastern Maharashtra. The structural analysis of the effect of the spike receptor binding domain (RBD) mutations, L452R and E484Q, towards ACE2 binding revealed a decrease in intramolecular and intermolecular contacts with respect to the wild type strain. However, the hydrophobic L452 residue mutation to the hydrophilic 452R might help in interactions with water molecules and overall stabilization of the complex, as was reflected in the lower minimum energy of the mutant complex (Fig. 8a). The effect of the mutation L452R on ACE2 binding was thus observed as enhanced stabilization of the RBD–ACE2 complex. Structural analysis further showed that the two RBD mutations L452R and E484Q may decrease the binding ability of REGN10933 and P2B-2F6 antibodies to the variant strains, compared to that in the wildtype strain (Fig. 8b,c). The third significant mutation, P681R, in the furin cleavage site resulted in enhancement of the basicity of the poly-basic stretch, and the likely facilitation of additional contacts with furin for S1–S2 cleavage (Fig. 8d). This could help in an increased rate of membrane fusion, internalization and thus better transmissibility. Further *in vitro/in vivo* studies would help confirm the phenotypic changes of the mutant strains.

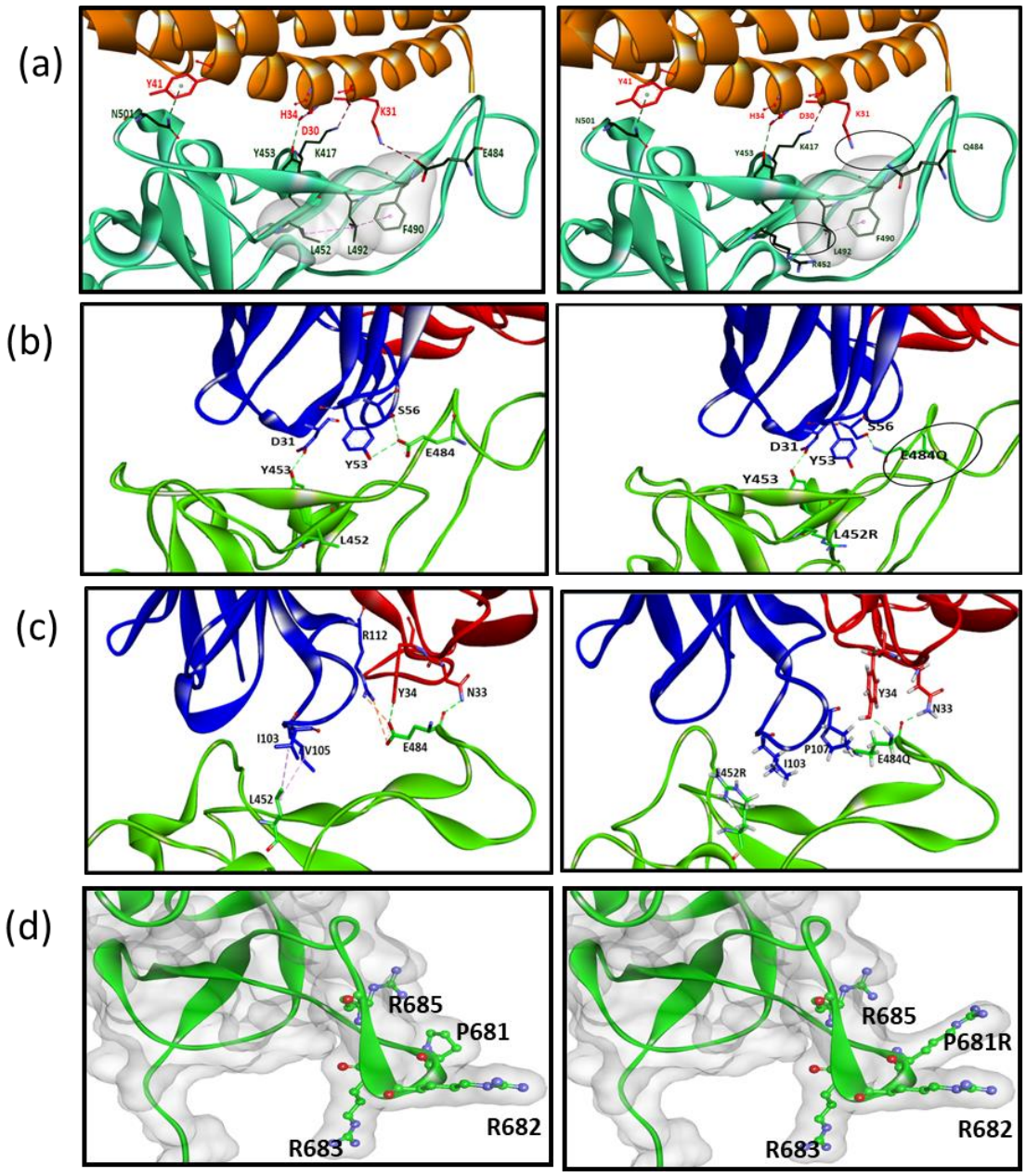

Figure 8: (a) Key interactions between ACE2–RBD involving mutations L452R and E484Q in theRBD (b) Interactions between RBD–mAb REGN10933 (c) Interactions between RBD–mAb P2B-2F6 (d) Furin cleavage site of the spike protein showing the effect of P681R mutation. Wt=wildtype strain; mt= mutant strain. In (a) the intra-molecular contacts in a hydrophobic patch of the RBD region are shown in the surface displayed in grey color. In (b) and (c), blue represents the antibody heavy chain and red represents the light chain.

5. Antiviral studies

Screening of potential agents against SARS-CoV-2

Investigators: Mullick J, Mathapathi BS, Yadav PD, Shete A, Pawar SD
Funding: Intramural

The rapid spread of COVID-19 in India warranted an urgent need of anti-SARs-CoV-2 virus drugs especially in the absence of an effective vaccine. As identification of new drugs being time consuming, repurposing of approved drugs developed for other uses has become a priority. Obtained training on optimization of MTT assay and antiviral testing using Lopinavir & Hydroxychloroquine. Successfully evaluated 12 compounds ranging from repurposed drugs (Chlorophyllin, Indomethacin), peptide (Covapep), antihistamine (Rupatidine fumarate), purified plant extracts (JNTGBRIO4, JNTGBRIO5 & Emodin), novel molecule (Savmax-fatty acid ester), non-toxic glycoprotein (Lactoferrin), immunostimulant (Methisoprinol), skin cream (Efflornithine) and shortlisted 4 drugs based on their effectiveness as antivirales in *in vitro* testing. The reports have been issued to the clients through ICMR.

Prediction of potential siRNA molecules for silencing of the spike gene of SARS-CoV-2

Investigators: Panda K, Alagarasu K, Cherian SS, Parashar DP
Funding: Intramural

RNA interference (RNAi)-based strategies, can be a promising treatment option to combat SARS-CoV-2 infection. This study was aimed to predict potential siRNA molecules for silencing the spike gene of SARS-CoV-2 virus. Four potential siRNAs were predicted using three different siRNA prediction servers and additional validation based on *in-silico* tools (Fig 9). This would ensure that the predicted siRNAs would have the ability to interact efficiently with the target sequence with minimal non-specific binding. The predicted siRNAs may be useful in developing RNAi-based therapeutics against SARS-CoV-2 if found effective by *in-vitro* and *in-vivo* studies.

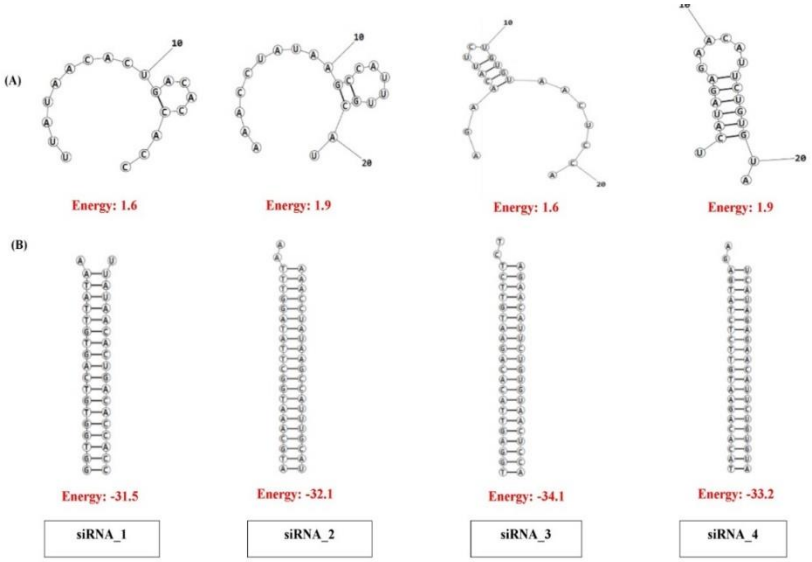

Figure 9: **(A)** Secondary structure prediction and free energy of folding of the predicted siRNAs**; (B)** Lowest free energy structure upon binding of the predicted siRNAs with the target sequences

Drug repurposing of SARS-CoV-2 using structural bioinformatics and systems biology approaches

Investigators: Cherian SS, Jagtap P

Funding: Intramural

The main protease of severe acute respiratory syndrome coronavirus 2 SARS-CoV-2 is a promising viral target for the design of drugs. Biological evaluation of marine natural products such as phlorotannins isolated from edible brown algae *Ecklonia cava* had shown SARS-CoV-1 main protease (3C-like protease) inhibitory activities. It would be important to evaluate these compounds against SARS-CoV-1 3CLpro as well. The purpose of the present study was to apply computational structural bioinformatics approaches for drug repurposing.

We analyzed the binding potential of a few marine natural products against the Mpro/3CLpro of SARS-CoV-2, using computational docking studies. The X-ray structure of SARS-CoV-2 3CLPro/Mpro in complex with the covalent inhibitor, Boceprevir at 1.35Å resolution was used. Docking of the compounds was undertaken using Glide module in the Schrödinger suite 2020. Further, to understand the stability of the interactions within the protein-ligand complex, molecular dynamics simulation was performed using Desmond molecular dynamics program (Schrödinger). A representative figure of the docked pose is shown in Figure 10a. The MD trajectory data for the ligand-protein complex showed that the ligand interacted with 3CLpro active site forming hydrogen bonds/

hydrophobic contacts with the catalytic dyad residues HIS41 and CYS145 (Fig. 10b). The compounds may be further tested *in vitro* and to enhance the binding efficacy of the compounds, derivatives of these compounds may be designed and evaluated.

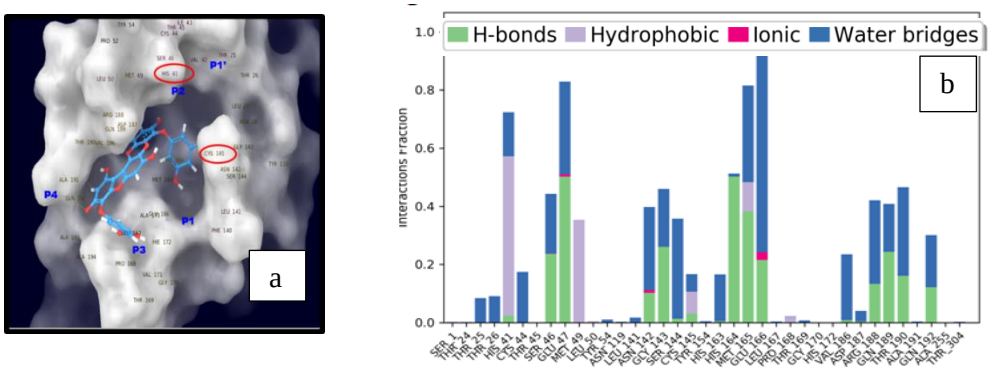

Figure 10: Interaction analysis of docked phlorotannin compound in the substrate binding cavity of SARS-CoV-2 main protease (3CLpro) (a) Pose of the inhibitor in the catalytic site. The subsites of the catalytic site cavity are labelled P1', P1, P2, P3 & P4. (b) Histogram showing the protein-ligand contacts

6. Environmental Studies

Study to assess the transmission of SARS-CoV-2 through fecal material of COVID-19 postive patients and their potential role in virus transmission

Investigators: Mallika L, Potdar V, Yadav PD, Sawant P, Mohandas S, Ranshing S (ICMR-NIV); Padbidri V, S Patwardhan, S Lalwani, Rege S, Palkar S.

The primary routes of transmission of SARS-CoV-2 are through respiratory droplets and close person-to-person contact. Gastrointestinal symptoms have also been reported among COVID-19 patients. This is a hospital-based study to investigate SARS-CoV-2 shedding in excreta of COVID-19 patients during treatment and post recovery. The main objective of this study was to investigate SARS-CoV-2 virus shedding in excreta of patients during and after infection.

Screening of 251 stool samples from COVID-19 positive patients at different time points revealed SARS-CoV-2 RNA positivity in 61.75% cases. Gastrointestinal symptoms were seen in 30.5% of the patients. We found 58.8% positivity in 34 samples collected after 30 days after throat swab positivity. This positivity was not associated with the presence of gastrointestinal symptoms and the severity of illness. The viral shedding profile in Fig 11 showed that viral shedding increased slightly on day 6th, peaked on 14th after the onset of illness and then dropped gradually to lower levels on day 55. Observations from this study highlight the potential presence of SARS-CoV-2 in faeces and its possible role in fecal-oral transmission.

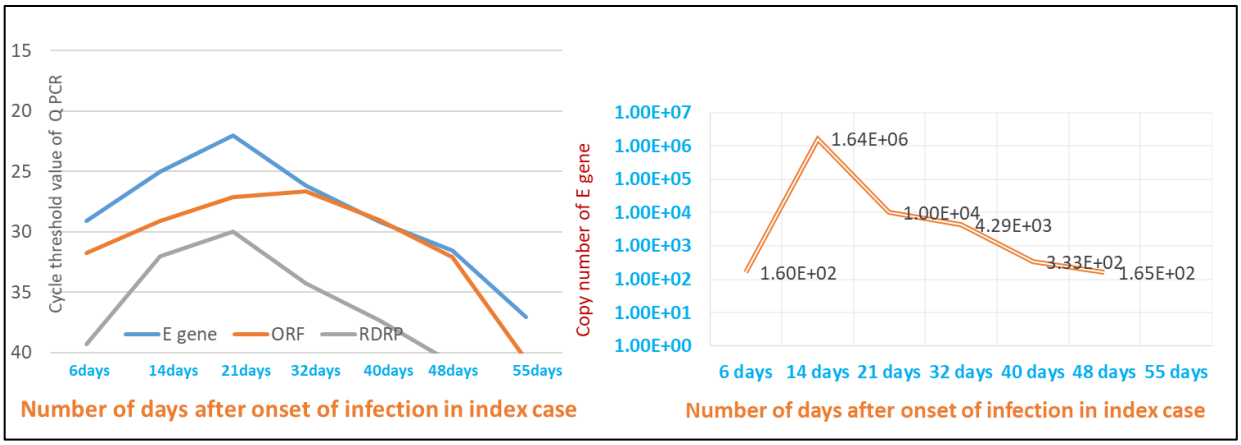

Figure 11: Shedding of virus in stool specimen and Ct and log values for patients positive for COVID-19

Standardization of methodology for SARS-CoV-2 detection from sewage samples

Investigators: Sharma D, Nalavade UP, Kalgutkar K, Gupta N, Deshpande JM.

Though SARS-CoV-2 spreads mainly via droplets of respiratory secretions, it was also detected in stool specimens of patients indicating active infection of the gastrointestinal tract. Presence of SARS-CoV-2 RNA in sewage samples was published in February 2020 indicating the possibility of using environmental water surveillance for monitoring SARS-CoV-2 activity in infected areas.

A total of 20 sewage samples collected from six wards in Mumbai before the spread of SARS-CoV-2 infections and during 11th to 22nd May 2020 when COVID-19 epidemic was already established, were processed using the phase separation method. SARS-CoV-2 was found to concentrate only in the middle phase. All samples collected before 16th March 2020 was negative for SARS-CoV-2 RNA. However viral RNA was detected in sewage samples collected during the ongoing COVID-19 epidemic in all the six wards. Hence, PEG-Dextran phase separation method was effectively used to concentrate SARS-CoV-2 in domestic waste-waters to detection levels. The findings indicate that it would be feasible to initiate sewage surveillance for SARS-CoV-2 by testing sewage samples to generate data about the virus transmission in various epidemiologic settings.

7. Product development and diagnostic services

One-tube-diagnostic assay for SARS-CoV-2

Investigators: Potdar V, Chowdhari M, Bharadwaj S, Abraham P.

RT-PCR assay for SARS CoV-2 was further modified & standardized to a single tube assay which contained four sets of primers and probes, thereby enhancing the throughput testing at a reduced turnaround time for timely reporting. The assay has been validated by three

other labs and is currently used by 10 VRDL labs for environmental monitoring of SARS CoV-2 in sewage water. The technology has been transferred to industry for scale up and commercial production of SARS CoV-2 diagnostic kits.

Development of a combo real-time RT-PCR kit for the detection of SARS-CoV-2, influenza A and B

Investigators: Potdar V, Chowdhari M, Bharadwaj S, Abraham P

Surveillance for influenza during the SARS-CoV-2 pandemic is also important and therefore a multiplex single tube assay was developed to diagnose Influenza and SARS-CoV-2 infections simultaneously. The assay is established In-house and validated by three non-ICMR institutes. The external validation was performed on a panel consisting of 85 negatives and 75 positives representing Influenza types and SARS-CoV-2 viruses of high, medium and low viral load. The kit was further validated for presence of more than two viruses using different permutations of all three virus types. The technology has been transferred to industry for scale up and commercial production.

Development of a RT-LAMP assay for the detection of SARS-CoV-2

Investigators: Nandi SS, Lambe U, Sawant S, Gohil T, Deshpande J

Real time RT-PCR (rRT-PCR), the gold standard is being used to detect SARS-CoV-2 virus due to its sensitivity and specificity. However, sophisticated instrumentation, requirement of skilled manpower and high costs limits its usage and warranted new economical technologies. An RT-LAMP assay was developed, using two primer sets targeting, E gene and N gene in two separate tubes. A total of 253 throat swabs were tested using the RT-LAMP assay and the assay detected 40 copies of SARS-CoV-2 RNA per reaction. The diagnostic sensitivity and specificity of the LAMP assay was 98.46% and 100%, respectively, as compared to the rRT-PCR. The assay can be performed using a heating block ($65\pm1^{O}C$) in a span of 30 minutes. The results can be interpreted visually and no sophisticated instruments are required.

Kit validation

Investigators: Lole KS, Alakarasu K, Potdar V.
Funding: Intramural

During the COVID-19 pandemic, validation of RT-qPCR kits, viral RNA extraction kits, virus transport medium (VTM), swabs and Enzyme Master mix for

one-step RT-qPCR has been performed by ICMR-NIV. Total kits validated in different categories are as below.

Sr No.	Category		No. validated	Kits yielding satisfactory results
1	RT-qPCR and LAMP kits (n=194)	Indigenous (made in India)	139	40
		Imported	55	25
2	RNA extraction kits (n=176)	Indigenous	126	66
		Imported	50	33
3	VTM and Swabs (n=107)	Indigenous	107	94
		Imported	0	0
4	Enzyme master mix for one-step RT-qPCR (n=12)	Indigenous	0	0
		Imported	12	6

Procurement and distribution of reagents/kits:

Coordinators: Basu A, Potdar V, Meena VK, Ranawade S

Procurement for the centralized distribution of reagents of NIV's real time diagnostic kit has commenced since March 2020. ICMR-NIV Pune also functions as ICMR's central depot as well as a regional depot for supply of kits to government laboratories in Maharashtra. RT-PCR kits were also provided to state government laboratories for validation of antigen kits as well as phase III trials of COVAXIN™ and COVISHIELD™. Prior to despatch, quality control (QC) checks were done meticulously at all stages. ICMR-NIV Pune has supplied 42, 84,000 PCR reactions, 22, 17, 500 RNA reactions and 15,88,500 VTMs to government laboratories.

Diagnostic services

Investigators: Potdar V, Chaudhury ML, Bharadwaj SD

Clinical specimens of suspected SARS CoV-2 and influenza cases were referred for diagnosis by different clinics/hospitals from all over Maharashtra. Screening of 299816 referred samples by real time RT-PCR revealed SARS-CoV-2 positivity in 61195 (20.4%) samples. Male to female ratio among the positive cases was 1.32:1. Maximum positivity was noted among elderly population (>60Years, 27.9%) while 14.8%

positivity was observed in children less than 5 years. Percent positivity among symptomatic and asymptomatic patients was 27.6% vs 19.3 % (P=0.0001). Cough was the most common symptom with 58.6% followed by fever (34.3%). Of the 61195 positives, 12533 (20.5%) were hospitalized and the major symptoms experienced was cough (11.2%) and fever (8.3%). First wave of SARS CoV-2 peak was observed during August to September 2020 and second wave commenced in February 2021 (Fig 12). A total 3784 samples from 98 COVID-19 testing laboratories were received for quality control (ILQC) through ICMR portal. Majority of the laboratories had ≥90% concordance with our results. For the exemplary service, ICMR-NIV received India Today's **'Best Testing Facility'** award**.**

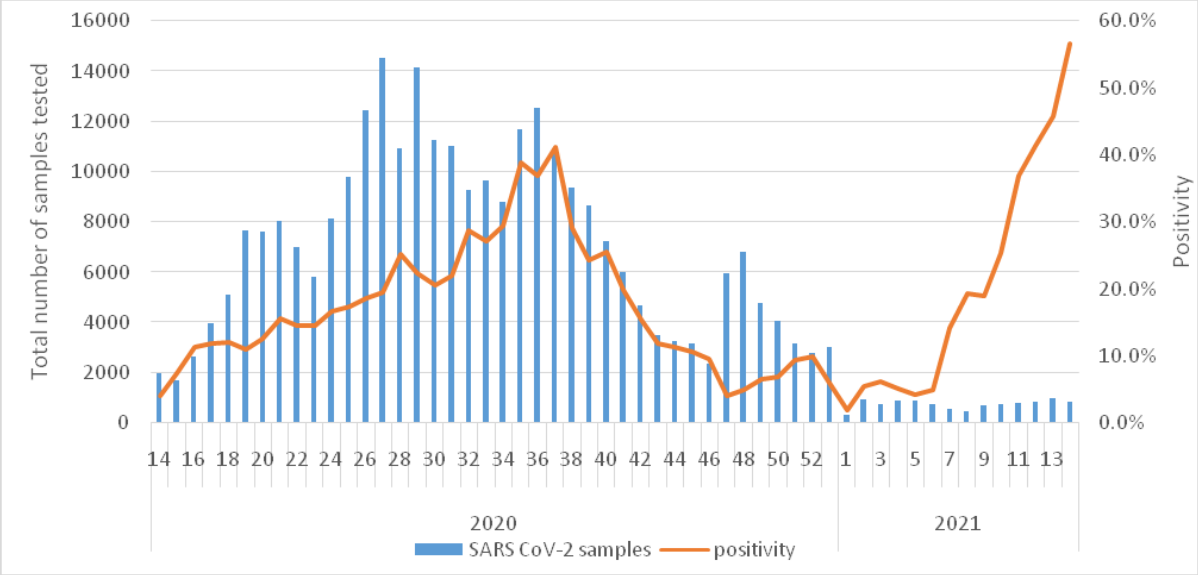

Figure 12: Weekly distribution of COVID-19 positive cases.

8. Miscellaneous studies

Mapping the Raman spectroscopy signature of SARS-CoV-2 full length glycoprotein using solid phase substratum

Investigators: Meena VK, Prasad SS, Basu A (ICMR-NIV); Galawat M, Poddar P (CSIR-NCL)

Funding: Intramural

SARS-CoV-2 is an enveloped virus with a size range of 60-140 nm and has a complicated molecular replication pathway in the host cell. It has a robust envelope glycoprotein projection as visualized by cryo electron microscopy that is made of assemblies of the

structural subunits of the spike glycoprotein that binds to the ACE2 receptor. This glycoprotein is a key molecular determinant of infectivity of the virus making it a target for vaccine and antiviral development. The urgency to develop faster diagnostic assays for SARS-CoV-2 including non-invasive point-of-care high throughput testing systems is a need-of-hour requirement. Molecular spectroscopy techniques specially Raman spectroscopy, have been shown to have potential in developing non-invasive point-of-care diagnosis for viruses. In the present study we used surface enhanced Raman spectroscopy (SERS) to probe purified full length spike glycoprotein of SARS-CoV2 in an attempt to identify "fingerprint" Raman spectral signatures that might be useful for developing a biosensor platform-technology for non-invasive detection of the virus in clinical and environmental samples.

In summary, the full length SARS-COV-2 GP spike protein was observed and analyzed using SERS at 200-3000 cm^{-1} and the characteristic spectrum mapped. It can be concluded that the aromatic amino acids, including phenylalanine, tryptophan, and tyrosine residues, are major generators of the SERS Raman spectra. This finding can be very informative for an advance understanding of the molecular basis of spike protein interaction with ligands and has the potential for developing novel therapeutics z

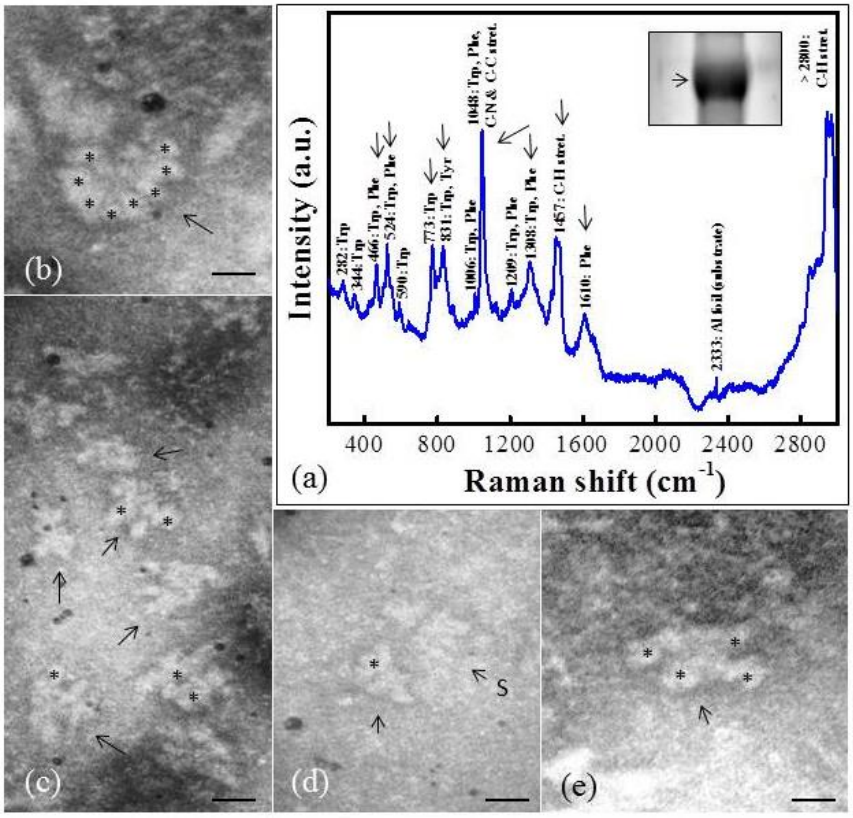

Figure 13: Surface Enhanced Raman Spectrum and negative stained transmission electron microscopy of purified SARS-CoV-2. (a) The representative SERS spectrum of the full length SARS-CoV-2 GP showing characteristic peaks (arrows). (b-e) Representative TEM images of

the protein as described in the text. The inset in (a) is SDS-PAGE profile of the purified glycoprotein. 50 nm magnification scale bars inbuilt into micrographs.

SARS CoV-2 Panel preparations for the evaluation of commercial kits, development of serology assays and follow up

Investigators: Sapkal GN, Deshpande G, Gurav YK, Yadav PD, Shete A, Kaushal H, Tripathy A, Dange V, Patchpute SD

The major objective of this program were (a) to generate SARS-CoV-2 sera panels of negative and positives to validate commercial rapid diagnostic kits using serology assays (b) to generate a gold standard panel of positive and negative sera to study antibody kinetics in patients.
In summary:

- ***Biobanking of serum samples*****:** More than 3000 serum samples were collected from COVID-19 patients/recovered patients and tested for SARS CoV-2 specific IgM and or IgG antibody for serum panel preparation.
- ***Standardization and comparison of virus neutralization assays*****:** SARS-CoV-2 plaque reduction neutralization assay (PRNT) and Micro neutralization test (MNT) for detection of neutralizing antibodies was standardized. This will help in understanding antibody response and natural infection and also in vaccine trials
- ***Preparation of validation panel*** **(IgG/IgM):** Anti-SARS CoV-2 IgM & IgG positive and negative serum panels were developed for commercial kit/equipment validation**.** This has helped in validation of 225 commercial serodiagnostic kits (Rapid/ELISA & CLIA) of which 46 were satisfactory.

Section 3: Scientific work reports (NON- COVID)

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Mr.S.Poharkar

Maintenance of Experimental Animal Facility

Dilip R. Patil & Sidharam Fulari

Background

Animal House Group is a core service department catering to the needs of animal experimentation in the institute. Large and small animal facilities at the Institute are registered with CPCSEA under "Research for education and breeding for in house use" vide Registration No. 43/GO/ReBi/SL/99/CPCSEA having validity until March 2022. Ten strains of mice (Inbred: BALB/c (Jax and CRL), C57BL/6, DBA/2, C3H, Outbred: CD-1, Immuno-deficient: CD-1 *nu/nu,* BALB/c *nu/nu,* RAG-1 KO and AG129) and Golden Syrian hamsters are being maintained, bred in the individually ventilated caging (IVC) system and supplied in filter top cages for in house research.

Work Done

During the report period, a total of 640 mice and 184 Hamsters (for COVID-19 research) were supplied to institutional scientists against 15 IAEC approved research projects. Other species of laboratory animals *viz:* guinea pig, rabbit, fowl, turkey, goose are procured from CPCSEA authorized sources as per requirement. A total of 511 ml blood from different species of laboratory animals, as diagnostic reagent in various assays was supplied to institutional scientists. The animals are maintained under controlled environmental conditions (temperature (22±2ºC), relative humidity (50 ±10 %), 12:12 h light and dark cycle with 100 % of fresh air exchange in animal rooms) with uninterrupted power supply.

Significant contribution was made by the Animal House Group in conducting pre clinical studies on SARS CoV-2 vaccine candidates (BBV152 and ZyCoV-D) in rhesus macaques. The team also contributed to the important task of breeding and supply of laboratory mice and hamsters followed by evaluation for their suitability as animal model in pre clinical studies pertaining to SARS CoV-2.

Rhesus monkeys are currently under rehabilitation post experimentation at the eco friendly group housing enclosures. Annual health monitoring for monkeys (N=36) was conducted. During which, physical examination, body weight, hemogram, hepatic profile, renal profile, lipid profile, tuberculin testing, chest X ray examination was carried out. Routine husbandry and veterinary care was provided and health records were maintained individually. Daily observation and prompt separation of injured / sick animals and their treatment was meticulously followed. Beside institutional care programme for primates, services of consultant veterinarian were also sought.

Institutional animal ethics committee reviews the research protocols and also ensures compliance with the CPCSEA norms. The work by the group involved, scheduling the meetings, compilation of projects and verification of checklists, Form D (animal usage form) and progress reports, guidance to Investigators for filling applications, preparation and finalization of minutes, submission of large animal protocols for CPCSEA approval and communication with CPCSEA, issue of IAEC certificates to Investigators. Accordingly, seven meetings of IAEC were conducted for evaluation of animal experimentation projects. A total of 10 research protocols pertaining to SARS CoV-2, involving the use of non human primates and golden Syrian hamsters were evaluated during the meetings.

Fig 1: Golden hamster

BACTERIOLOGY

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Mr Rajendra Khedkar	Multi Tasking Staff

Project Staff

Ms Shradha Maheshwari	Technician C (from January 2020)
Mr Gajanan Zhambre	Field Worker (from January 2020)

- Core facility for bacteriology.
- Pertusis vaccination early in pregnancy boosts maternal and neonatal immunity.

Project 1(a): Seroepidemiology, maternal immune status and missed diagnosis of pertussis among young infants in India: a multicentric study

Investigators: Viswanathan R in collaboration with clinical partners

Funding: The DBT Wellcome India Alliance

Duration: 2019-2024

Background: Protection due to whole cellular pertussis vaccine wanes within 12-15 years. Women of childbearing age group therefore do not possess sufficient immunity to pertussis, making their newborns vulnerable to the disease.

***Objective*:** To evaluate pertussis antibody status in pregnant women and their newborns, and to understand the impact of antenatal immunization and factors influencing placental transfer of antibodies.

Methods: A hospital based observational study was carried out at an urban tertiary care centre. Pertussis antibody titres in mothers and their newborns were determined. Vaccinated and unvaccinated mothers and their newborns were compared for baseline characteristics, geometric mean titres (GMT) and placental transfer ratio of antibodies. Multivariate logistic regression was performed to understand the influence of different factors on protective antibody titres.

Results: Of the 284 mother infant pairs, 75 mothers and 73 newborns were found seropositive for anti PT IgG antibodies. 94 women were vaccinated in pregnancy; 51 (54.3%) of these mothers and newborns were PT IgG positive, compared to 24 (12.3%) women (and 22 newborns) not vaccinated during pregnancy. Women vaccinated in pregnancy and their newborns had higher GMT (30.88 and 32.54 IU/ml), compared to women who were not vaccinated (12.63%, 2.24 IU/ml) and their newborns (11.58%, 2.53 IU/ml). Placental transfer ratios in newborns of mothers vaccinated in pregnancy and those who had childhood immunisation or natural immunity were similar (1.05 and 1.12 respectively). Protective titres of antibodies at birth (>20 IU/ml) were observed in 72.3% vs 21% of newborns of vaccinated and unvaccinated pregnant women respectively; influenced by mother's vaccination status and seropositivity (Table 1).

Conclusion: Protection against pertussis is low in newborns of mothers who are immunized during childhood. Vaccination early in pregnancy boosts maternal and neonatal immunity.

Table 1: Seropositivity and GMT of anti PT IgG antibodies in vaccinated and unvaccinated subsets

	Vaccinated (n=94)	**Unvaccinated (n=190)**	***p* value**
Seropositive mothers anti PT IgG) *n*%)	51 54.26)	24 12.63)	<0.001*
Seropositive infants*n*)	51 54.26)	22 11.58)	<0.001*
Maternal anti PT IgG GMT IU/ml) 95% CI)	30.88 21.23-44.91)	2.24 1.47-3.91)	<0.001*
Cord anti PT IgG GMT IU/ml 95% CI)	32.54 22.85-46.33)	2.53 1.68-3.81)	<0.001*
Placental Transfer ratio	1.05	1.12	0.555
Maternal anti PT IgG titre>100IU/ml, *n* %)	22 23.4)	10 5.26)	0.0417*
Cord anti PT IgG titre >20IU/ml, *n* %)	68 72.3)	40 21.0)	<0.001*

Project 1(b): To explore the aetiology of prolonged cough in young infants from an urban pediatric centre in western India, focusing on *Bordetella pertussis,* other species of *Bordetella and* respiratory viruses (Preliminary data from Pune site)

Investigators: Viswanathan R, Choudhary ML in collaboration with clinical partners,
Funding: 1(a)

***Background*:** Pertussis is a major public health problem in Southeast Asia estimated to contribute the most to childhood disease burden. Whole cellular pertussis vaccine is given in India, at 6, 10 and 14 weeks of birth with booster doses at 16-24 months and 4-5 years of age. There is paucity of information on pertussis and infection by other species of *Bordetella* from India.

***Objective*:** To explore the aetiology of prolonged cough in young infants from an urban pediatric centre in western India, focusing on *Bordetella pertussis,* other species of *Bordetella and* respiratory viruses.

Methods: Young infants presenting with afebrile or minimally febrile cough lasting more than two weeks with at least one of the following signs or symptoms: Paroxysms of coughing/ inspiratory whoop/ post-tussive vomiting or Apnea (with or without cyanosis for infants below one year of age) were recruited at a tertiary care centre. Nasopharyngeal swab samples were collected. Multiplex real time PCR assay was performed for identification of *Bordetella pertussis, B.parapertussis and B. holmesii. B. pertussis* was further confirmed by detection of *ptxS1* by real time PCR assay. Real-time qRT-PCR assay was also performed for influenza A [A (H1N1)pdm09 and A(H3N2)], influenza B parainfluenza (PIV) virus 1, 2, 3, 4, human metapneumovirus (hMPV), respiratory syncytial virus (RSV A&B), adenovirus and rhinovirus.

***Results*:** 45 participants were included for final analysis. Six of the 45 cases were confirmed with *Bordetella* infection; 03 *B.pertussis* and 03 *B. holmesii.* Co-infection of *B.holmesii* with hMPV was observed in two children while one patient also had Rhinovirus. Of the three cases of pertussis, two occurred in a pair of 15 month old twins. Seventeen cases were positive for one or more viruses (Table 2). The most commonly detected etiological agent was RSV-A followed by hMPV.None of the children with pertussis had completed age appropriate vaccination, with case 1 being a child of migrant worker without immunization records. All the cases were managed symptomatically and recovered uneventfully.

Conclusion: Our study confirms occurrence of pertussis in Western India, which can present with mild symptoms. *B.holmesii* and several viruses can present with similar clinical features and need to be differentiated. Laboratory capacity for pertussis diagnosis needs to be expanded and strengthened.

Table 2: *Bordetella* species and viruses causing prolonged cough illness

Case	Age(m)/Sex	Duration of cough (weeks)	Type of cough	Age ppropriate vaccination for pertussis	*Bordetella* spp. detected	Virus detected
1.	8/M	3	Productive/paroxysmal/post tussive/Whoop	No	*B.pertussis*	No
2.	15/M	3	Dry/nocturnal/ post tussive	No	*B.pertussis*	No
3.	15/M	3	Dry/nocturnal/ post tussive	No	*B.pertussis*	No
4.	24/F	3	Dry/ post tussive	No	*B.holmesii*	No
5.	24/F	3	Dry/nocturnal	Yes	*B.holmesii*	hMPV
6.	9/F	4	Productive/paroxysmal	Yes	*B.holmesii*	hMPV, Rhinovirus
7.	18/M	3	Productive/nocturnal	Yes	Not Detected	Adenovirus
8.	8/M	3	Dry/ paroxysmal	No	Not Detected	Adenovirus
9.	24/F	3	Productive/ paroxysmal	Yes	Not Detected	Rhinovirus
10.	15/M	3	Productive/ paroxysmal	Yes	Not Detected	hMPV
11.	15/M	3	Productive/ paroxysmal	Yes	Not Detected	hMPV
12.	24/M	3	Dry/whoop	Yes	Not Detected	hMPV
13.	9/M	3	Dry/paroxysmal	No	Not Detected	RSV-A
14.	15/F	3	Dry/paroxysmal	Yes	Not Detected	RSV-A

15.	18/M	3	Productive	Yes	Not Detected	RSV A, PIV 3
16.	24/M	3	Productive	Yes	Not Detected	Rhinovirus
17.	4/M	3	Dry/paroxysmal	Yes	Not Detected	hMPV, Adenovirus
18.	6/M	3	Productive/paroxysmal/whoop	Yes	Not Detected	RSV-A
19.	3/M	3	Productive/paroxysmal/whoop	No	Not Detected	Rhinovirus
20.	24/F	3	Productive/paroxysmal	No	Not Detected	RSV-A
21.	8/M	3	Dry/paroxysmal	No	Not Detected	RSV-A
22.	7/M	3	Productive/nocturnal	Yes	Not Detected	Influenza A
23.	14/F	4	Dry/paroxysmal	Yes	Not Detected	PIV-3

1. **Diagnostic Services for Bacteriology**

   Investigators: Viswanathan R

   Funding: Intramural Service Project

   Diagnostic services for diarrheal disease were provided for sporadic cases of pediatric diarrhea in collboration with Enteric Viruses Group. A case of hypervirulent *Klebsiella pneumoniae* was detected in a one and half month old infant with intractable diarrhea who did not respond to symptomatic treatment. As the mother was COVID positive, the child was being fed on formula prepared in unhygenic manner. No other virus or bacteria was detected. Following confirmation of the hypervirulent strain, the child was treated with colistin and recovered. Further characterization of the isolated strain is ongoing.

BIOINFORMATICS & DATA MANAGEMENT GROUP

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Dr Nitin M. Atre	Research Associate (ICMR extramural project)

Core facility services

Investigators: Cherian SS, Shil P, Walimbe AM, Jadhav SM

Regular services to the various experimental groups at NIV and MCC, Pashan, in the areas of statistical data analyses of epidemiological and serological data, bioinformatics sequence and structure analyses, mathematical modeling etc. were provided. Management and maintenance of computers, servers, laptops, printers, computer peripherals, network and internet services was done on regular basis. Regular maintenance of connectivity between NIV, MCC and three field units is being done for data transfer, AIMS, LIMS Software and intercom services. NIV website is being monitored on a regular basis. Audio and video conferencing units are maintained on a regular basis. Technical support has been provided for conferences and workshops held by NIV. The indigenously developed softwares like payroll, pension, supplementary bills, arrears calculator, computer complaint register etc. were also been maintained.

Project 1: Structure-based design and evaluation of the antiviral activity of potential lead compounds against the Chikungunya virus

Investigators: Cherian SS, Agarwal M, Parashar D

Collaborators: Mainkar P & Kumar N (CSIR-IICT, Hyderabad)

Duration: 2017-2021

Funding: Extramural (ICMR)

Background: Natural polyphenolic compounds, *i.e*., flavonoids and xanthonoids possess wide ranging bioactivities such as anti-inflammatory, anti-oxidative, anti-bacterial, anti-fungal and antiviral activities. Such compounds are known for their wide spectrum antiviral activity against multiple viral protein targets of different viruses such as dengue virus, HCV virus, herpes virus, etc. and can also be investigated for possible anti-chikungunya (CHIKV) antiviral activity.

***Objectives*:** (i) To identify and shortlist drug compounds with antiviral properties by screening of available compound libraries and evaluation of antiviral activity of selected compounds against CHIKV (ii) Identification of viral drug targets of the effective compounds.

Findings: Docking studies to understand the molecular mechanism of action of a few natural and synthetic compounds bearing benz-oxazole scaffold that were found to be effective against CHIKV by *in vitro* studies. CHIKV peptidase C9 domain of nsP2 protein which is known for its cysteine protease activity is required for the proteolytic cleavage of non-structural polyprotein precursor into the four mature nsPs. The catalytic dyad residues are Cys1013 and His1083. Docking of benzoxazole compounds, EM-GU-BOT-5 and EM-GU-BOT-11 (**Fig. 1**) to nsP2 peptidase C9 domain (3TRK.pdb) showed pi-sulfur interaction between Cys1013 and the aromatic ring of benzo-oxazole group as well as hydrogen bonding interaction with Asn1082, a residue which is in the immediate vicinity of His1083, resulting in good binding affinities (-8.1 and -8.3 kcal/mol respectively). *In vivo* studies are in progress.

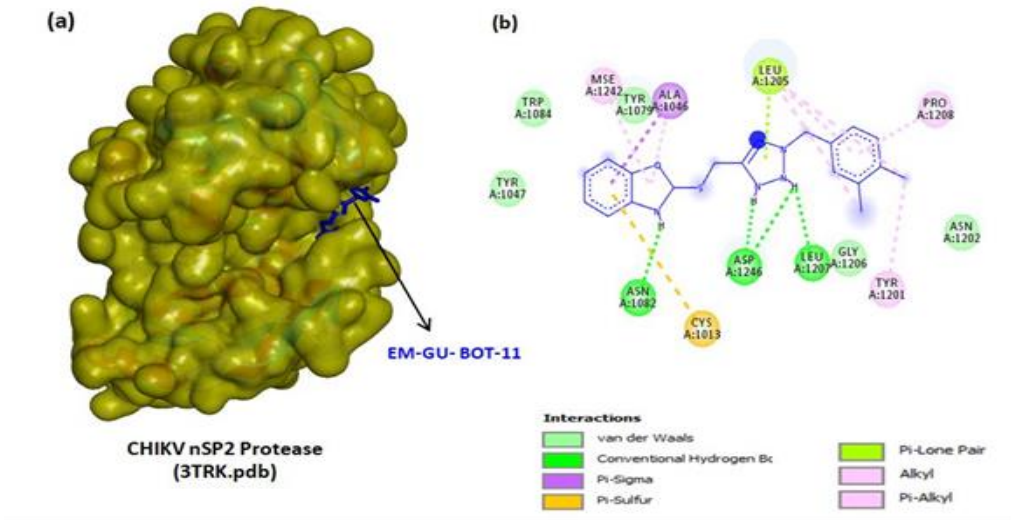

Figure 1: Molecular docking of compound EM-GU-BOT-11 that showed *in vitro* antiviral activity with nsP2 peptidase C-9 domain (3TRK.pdb), the putative target of CHIKV (a) Surface overview of the docked compound (b) 2D interaction diagram

Project 2: Repurposing of drugs towards anti-dengue and chikungunya viruses using systems biology approach

Investigators: Cherian SS, Kasabe B, Parashar D, Alagarasu K.

Funding: Extramural (ICMR)

Duration: 2019-2022

***Background*:** Studies based on computational approaches using systems biology data for shortlisting potential FDA-approved drugs have only recently been initiated, and experimental testing has not yet been undertaken for the predicted drugs. Further multitarget drug repurposing to develop drugs that are able to interfere with multiple pathways involved in pathogenesis of co-infections has not been undertaken. There is need to use a systems biology approach for analysing "multi-omics" data to repurpose FDA approved and investigational drugs against these viruses to identify effective novel drug candidates.

***Objectives*:** (i) Identification of the differentially expressed signature gene/ protein profiles for dengue and chikungunya viruses (DENV/ CHIKV) and also common signatures based on available literature and appropriate databases (ii) Identification of specific metabolic pathways involved and shortlisting of FDA-approved drugs to be repurposed for DENV/ CHIKV using computational systems biology approach.

Findings: Based on the analysis of available DENV proteomic datasets we obtained >500 differentially expressed proteins. Further literature mining and use of the 'DenvInt' database revealed experimental evidence of interaction between human proteins and DENV proteins. From the interactomics data, 489 human-DENV interacting proteins were identified. Further, using STITCH as a target based method to identify drugs that can interact with the signature viral proteins/ human proteins were identified. A representative interaction network of the protein BRD4 which shows interaction with several compounds including flavopiridol, a flavonoid derived from an indigenous plant in India is shown in **Fig. 2a**. The proteins reported in the

DENV–human PPIs study included all the 10 DENV proteins. The top five DENV proteins in terms of the number of interactions with human proteins were NS5, NS3, C, NS2A, and NS2B, which displayed 71, 67, 30, 25 and 18 interactions respectively as shown in **figure 2b**.

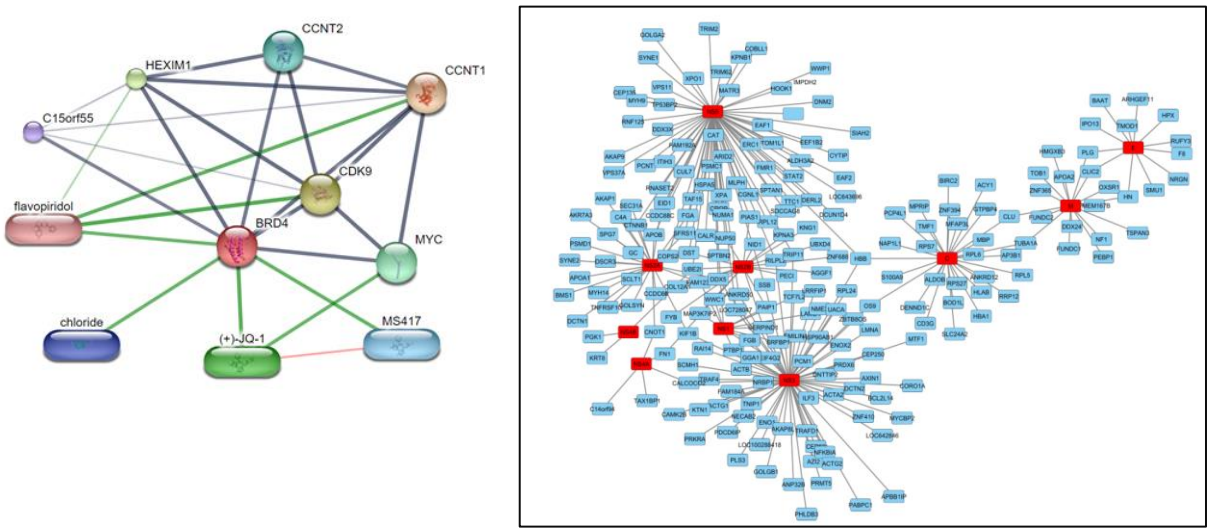

Figure 2: (a) STITCH network generated for BRD4 which shows interaction with flavopiridol. The polygons depict small molecules and spheres are the interacting proteins **(b)** Interaction network of human and dengue virus proteins. Red nodes represent dengue viral proteins and are labelled with corresponding gene names. Blue nodes represent the human proteins and are labelled with the corresponding UniProt ID. The black edges show the interactions between human proteins and dengue viral proteins as determined by the interactomics analysis

Project 3: Bioinformatics characterization of Chandipura virus proteins

Investigators: Shil P, Tripathy AS; Pavitrakar D (Ph.D. student)

***Background*:** Chandipura virus (CHPV) is responsible for acute encephalitic syndrome in pediatric population and is endemic to India since 2003. Since no vaccine or therapeutics is available against the disease, alternative approaches of therapeutics could be the inhibition of host proteins involved in the regulation of virus replication.

Objectives: *(i)* Design a multi-epitope peptide construct based on B-cell and T-cell epitopes of different antigenic proteins of CHPV like nucleoprotein (N), phosphoprotein (P), matrix protein (M) along with the immuno-dominant glycoprotein (G).

(ii) Investigate the role of Cyclophilin A as a possible host modulator in CHPV infection

***Work done & findings*:** Epitopes possessing high immunogenicity which were also non-allergenic and non-toxic were considered. The final multi-epitope construct named as: MEC-CHPV, comprised of β-defensin adjuvant at N-terminal for enhancement of immunogenicity followed by fourteen B-cell epitopes, four Helper T-cell epitopes and six Cytotoxic T-cell epitopes. Characterization of MEC-CHPV was carried out in terms of antigenicity, allergenicity and physicochemical parameters. Molecular docking and molecular-dynamics simulation of the MEC-CHPV with human Toll like receptors (TLR-8 and TLR-3) showed stable interactions (**Fig. 3**). *In silico* cloning of MEC-CHPV in pET30a (+) expression vector was also conducted using codon optimization. *In silico* immune-simulation using C-IMMSIM package indicated a typical immune response against MEC-CHPV when used as a potential vaccine. This study provides a cost-effective way to design a peptide vaccine candidate against CHPV using immuno-informatics approach.

We also evaluated the effect of Cyclophilin A (CypA, an immunophilin bearing peptidyl-prolyl cis/trans-isomerase activity) in inhibition of CHPV. *In vitro* inhibition of CypA using Cyclosporin A demonstrated a 3-log reduction in CHPV titer and non-detectable level of CypA, as against controls. *In silico* studies with molecular docking and dynamics simulations indicate stable binding of CHPV N protein with host CypA, thus corroborating *in vitro* observations. For the first time we have generated evidence on the role of CypA in CHPV replication, thus making it one of the potential host factors to be explored in future anti-viral studies.

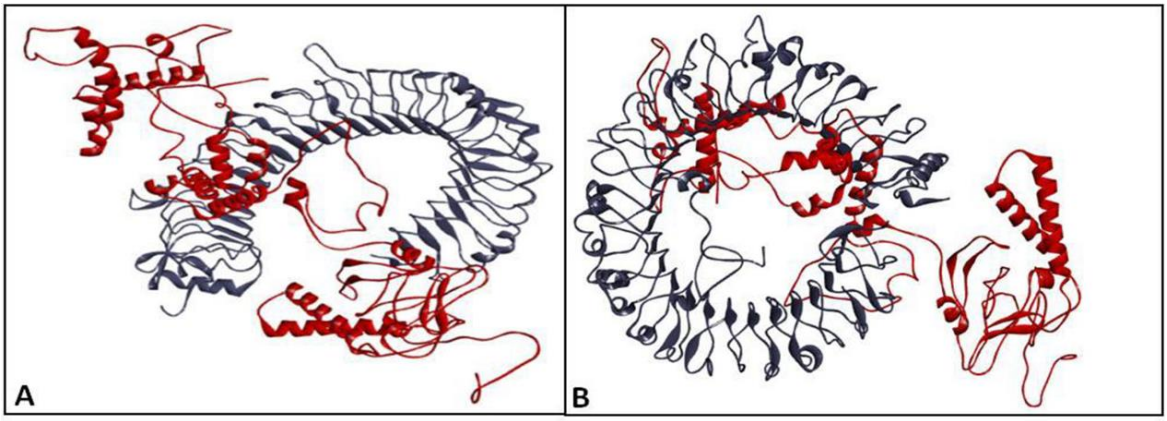

Figure 3: Molecular docking of MEC-CHPV with A) human TLR3 and B) human TLR8.

Project 4: Development of forecasting models for dengue and chikungunya in the Pune region
Investigators: Shil P, Awate P, Jagtap M
Funding: MATRICS, SERB-DST.
Duration: 2020 – 2023
***Background*:** With the changing climate scenario, India shows huge burden of dengue and chikungunya. The vastness and diversity (both geographic and climatic) of India necessitates regional level studies on dengue transmission.
Objectives: To study the influence of climate factors on dengue occurrences in the Pune Urban Zone ***Work done & findings*:** Analyzing the epidemiological and meteorological data (2009-2018) obtained from Government sources, climate driven biological factors affecting dengue transmission, *viz.*, human to vector transmission probability, mosquito to human transmission probability and dengue extrinsic incubation period (EIP) was estimated. The lagged effects of meteorological parameters like maximum and minimum temperatures, rainfall etc., on dengue occurrence time-series were estimated and then incorporated as external regressors into SARIMA based models. We found SARIMA $(1,1,1)\times(1,1,1)_{12}$ to be the best model to explain dengue time-series in PUZ and used to forecast dengue occurrences (ahead-of-season projections) (Fig 4). The efficacy of the model-based forecast was evaluated in terms of the mean absolute percentage error (MAPE) defined as: $MAPE = \frac{1}{n}\sum_{t=1}^{n} | (x_t - x_f) \div x_t |$where x_f is the forecasted number of monthly cases, x_t is the reported number of monthly cases and n denoted the number of months. Fairly accurate projections were obtained for the year 2018 as indicated by the very low value of MAPE = 0.67. The methodology developed can be utilized as an early warning system for projection of dengue, which can benefit the policy-makers in implementing mitigation measures.

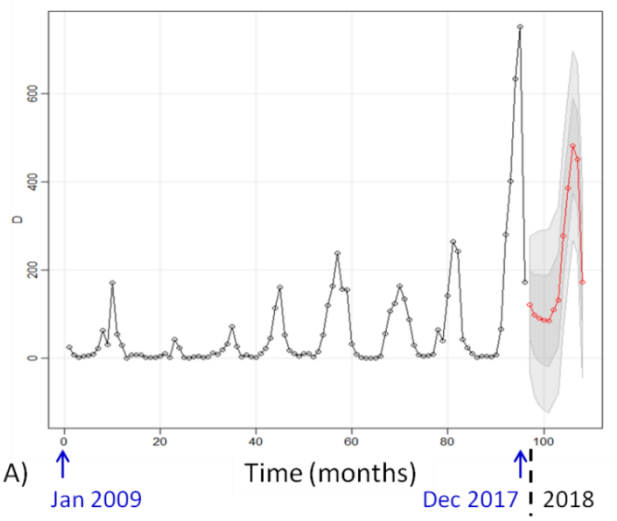

Figure 4: Dengue time-series based on multivariate SARIMA $(1,1,1)\times(1,1,1)_{12}$ incorporating climate factors. Projections for 2018 are shown in red color.

In addition, attempts have been made to understand the role of regional climate phenomenon like the Indian Ocean Dipole (IOD) and the El-Nino Southern Oscillation (ENSO) on dengue occurrences. The DMI (index for IOD) showed a weak association with dengue time-series. The Nino 3.4 SST (index for ENSO) showed a correlation with dengue time-series during 2013-2017. The wavelet transform analyses supported these observations (**Fig. 5**).

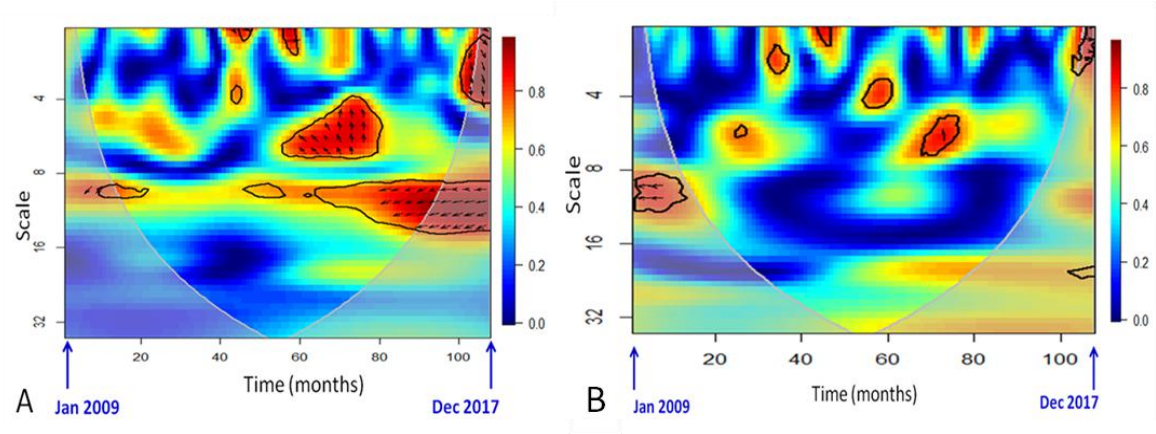

Figure 5: A) Bivariate wavelet coherence analyses of dengue incidences and the Nino Index (SST NINO 3.4). B) Bivariate wavelet coherence analyses of Dengue incidences and the Indian Ocean Dipole Mode index. The solid black lines indicate the cone of influence and significantly coherent time-frequency regions ($p < 0.05$).

DENGUE - CHIKUNGUNYA GROUP

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Mr. YogeshBalaKarthik	Project Technical Assistant

- Apex referral laboratory for National Vector Borne Disease Control Programme
- Studies using molecular genotyping suggest prevalence of Dengue 2 virus in eastern and southern states, Dengue 3 in western and some northern states, Dengue 4 in Andamans and North Maharashtra and Dengue-1 in Maharashtra.
- Phylogeographic studies suggest that India is an endemic reservoir for Chikungunya virus and global transmission could have originated from here.
- Lipid nanoparticles were seen to have higher efficacy in delivery of anti siRNA against chikungunya virus in mice model.

Apex referral laboratory activity for National Vector Borne Disease Control Programme

Investigators: Alagarasu K, Parashar D, Patil JA, Kakade MB, More A, Shah PS

Funding agency: NVBDCP/Intramural

D**uration:** 2020-2021

A. Molecular characterization of dengue viruses circulating in India

***Background*:** A large number of dengue outbreaks were reported from Maharashtra as well as other states of India during 2020-21. Information on circulating serotypes is an essential component of surveillance of dengue.

Objective: To find out the distribution of serotypes of circulating dengue virus and genotypes within serotypes in Maharashtra and other states.

***Findings*:** During 2020-2021, 400 dengue suspected samples referred by the state of Maharashtra were tested by real-time RT-PCR for dengue and chikungunya. Serotyping of the 83 positive cases revealed 23 as DENV-1, 7 as DENV-2, 01 as DENV-3. Fifty two samples tested positive for chikungunya virus. Screening of 40 samples from Goa showed 17 samples positive for dengue (three DENV-1, 10 DENV-2, and four DENV-3). Out of 56 samples received from Patna, Bihar, 35 were positive for DENV-1 and one for DENV-2. The study suggested that DENV-1 is the dominant circulating serotype in the states of Maharashtra and Bihar while the dominant serotype in Goa was DENV-2 during 2020-21.

B. Molecular characterization of chikungunya viruses

***Background*:** A large number of chikungunya cases were reported from Pune district during 2020-2021

***Objective*:** To perform molecular characterization of chikungunya virus cuirculating in Pune.

***Findings**:* Fifty seven samples out of 400 tested positive for chikungunya virus by real-time RT-PCR. Isolation attempts yielded 09 isolates. Whole genome sequencing (sanger sequencing) and phylogenetic analysis showed all the isolates as Indian ocean lineage belonging to East Central South African genotype (Fig 1).

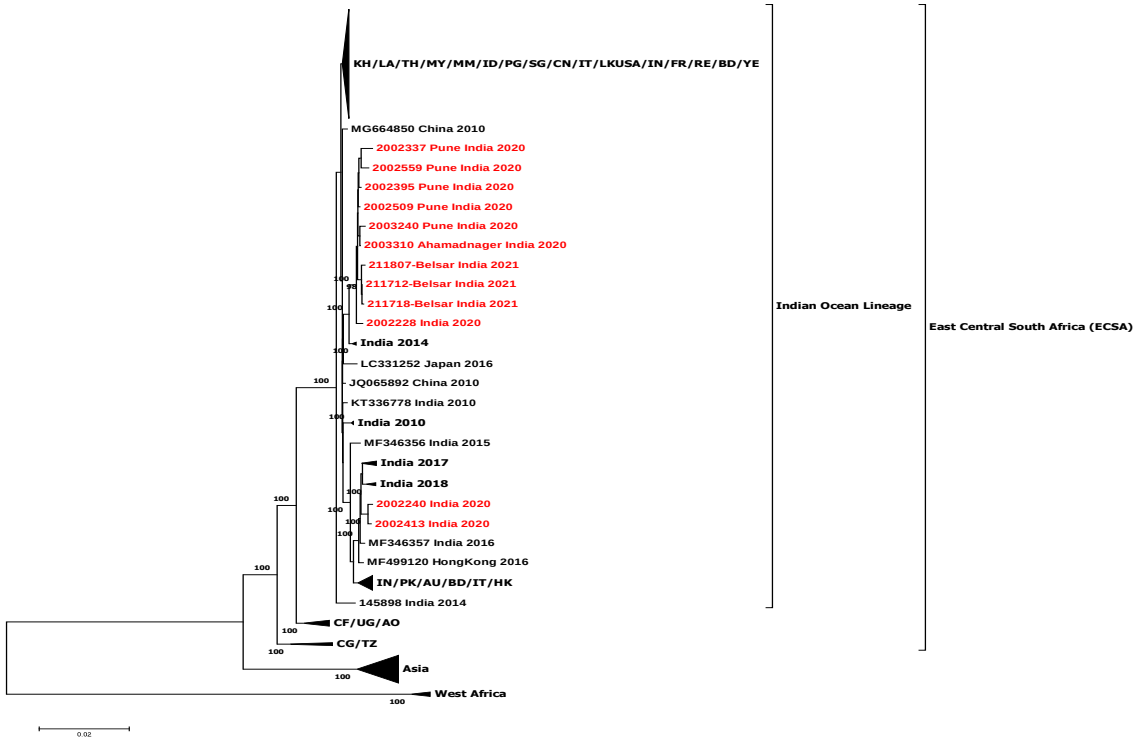


Fig 1: Phylogenetic tree of CHIKV based on complete genome sequence. Sequences generated during 2020 and 2021 are labeled in red colour.

Project 2: Development and evaluation of an in-house multiplex real time RT-PCR assay for simultaneous detection of dengue, chikungunya and Zika viruses in clinical samples

Investigators: Alagarasu K, Parashar D, Yadav PD

Funding agency: Intramural

Duration: 2020 - 2023

A fourplex assay for detection of dengue, chikungunya, ZIKV and internal control (B-actin) was standardized. Primers and probes for detection of dengue serotypes and Zika virus (ZIKV) were designed afresh while the earlier designed and validated chikungunya primers and probes were used. The assay was tested against dengue and chikungunya panel supplied by Quality control for Molecular Diagnostics, UK. Core samples of both dengue and chikungunya panels were detected as positive by the assay. However, one sample positive for chikungunya in the educational panel was detected as negative. ZIKV positive samples in both dengue and chikungunya panels were not detected by the assay. Our dengue virus serotyping assay as well as pan dengue primer assay correctly identified the serotypes in the dengue panel and did not cross react with ZIKV.

Project 3: Antiviral activity of approved drugs and natural peptides against dengue and chikungunya virus

Investigators: Parashar D, Alagarasu K, Cherian SS.

Funding agency: Intramural

Duration: 2020 - 2023

***Background*:** Despite the global health significance, no antivirals are available for dengue and chikungunya. Usage of traditional medications as readily available alternatives due to their compatibility

with the body and fewer side effects compared to synthetic drugs (supportive therapy) has become popular globally.

***Objectives*:**

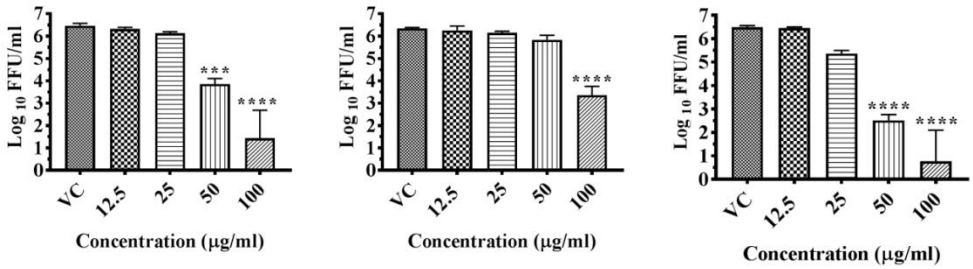
1. Evaluation of *in-vitro* antiviral activity of commercially available *Carica papaya* products against DEN & CHIK virus.
2. Evaluation of antiviral activity of herbal plant extracts including papaya extracts against DEN & CHIK virus.

***Findings*:** Primary screening of papaya products and extracts have been done by post treatment. Effective extracts were further tested as an antiviral against dengue and chikungunya viruses in different conditions *i.e*. Pre-treatment, Co-treatment and Post treatment. Results showed that commercially available products containing papaya as an active ingredient did not show activity. Conversely, papaya nanoparticle and papaya extracts showed significant inhibition of DENV while papaya powder showed significant reduction of CHIKV titeres in cell culture assay (Fig 2). This study confirms the effectiveness of *Carica papaya* against DENV and CHIKV infection in *in vitro* system.

In-vitro antiviral activity of α-Mangostin, a natural compound against dengue virus serotype -2

α-Mangostin, a xanthanoid, was observed to exert antiviral activity against DENV-2 under pre, co and post treatment conditions. Complete inhibition of DENV-2 was observed at 8 µM under co-treatment condition (Fig.3). Molecular docking experiments indicate that α-Mangostin can interact with multiple DENV protein targets *i.e.,* NS5 RdRp domain, NS5 methyltransferase, NS2-NS3 protease, NS3 helicase and E glycoprotein. *In-vitro* and *in-silico* findings suggest that α-Mangostin has the potential to inhibit DENV growth at different points of its replication cycle and might act as a prophylactic/therapeutic agent.

(a)



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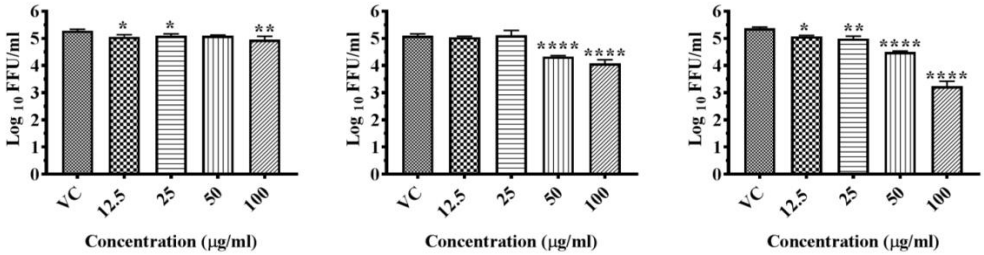

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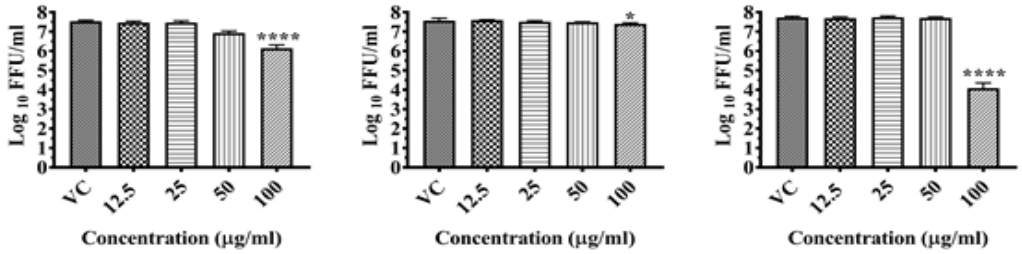


Fig.2. Effect of papaya nanoparticles (a), papaya extract (b) on DENV-2 virusand papaya powder (c) on chikungunya virus by focus forming unit assay.

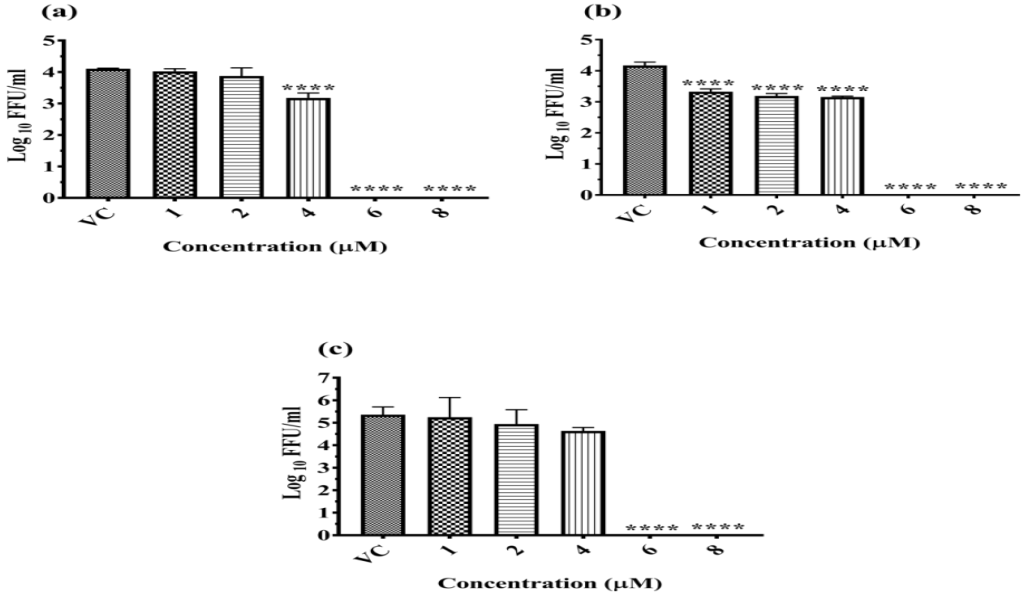
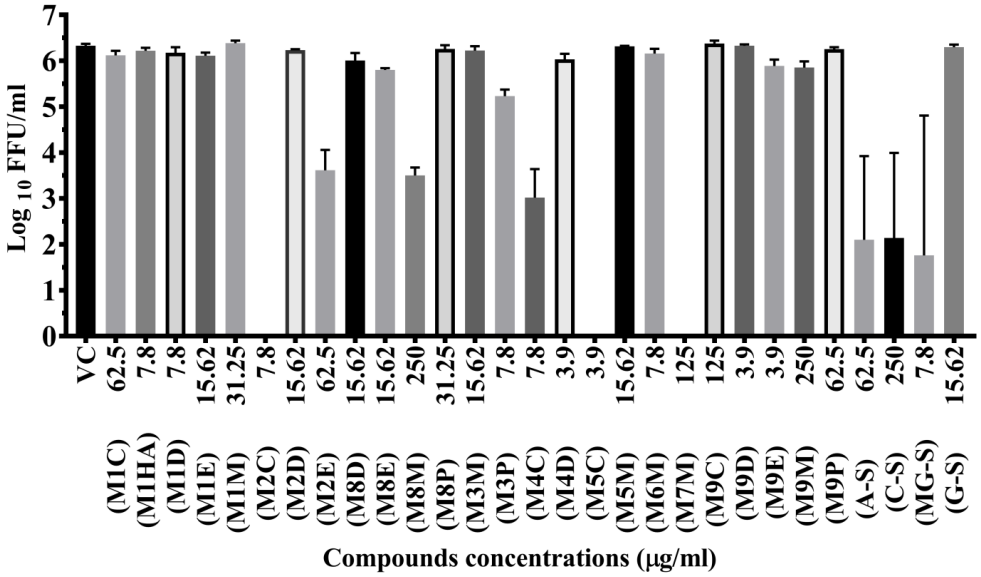


Fig. 3. Effect of α-Mangostin on DENV-2 by focus forming unit assay under different conditions.

Herbal plant extracts from ICMR- National Institute of Tropical Medicine **(NITM), Belagavi:**

Twenty nine plant extracts were received from NITM Belagavi under a collaborative project and screened for anti-DENV and anti-CHIKV activity. Nine extracts showed anti-DENV activity while five demonstrated anti CHIKV activity (Fig 4).

(a)



(b)

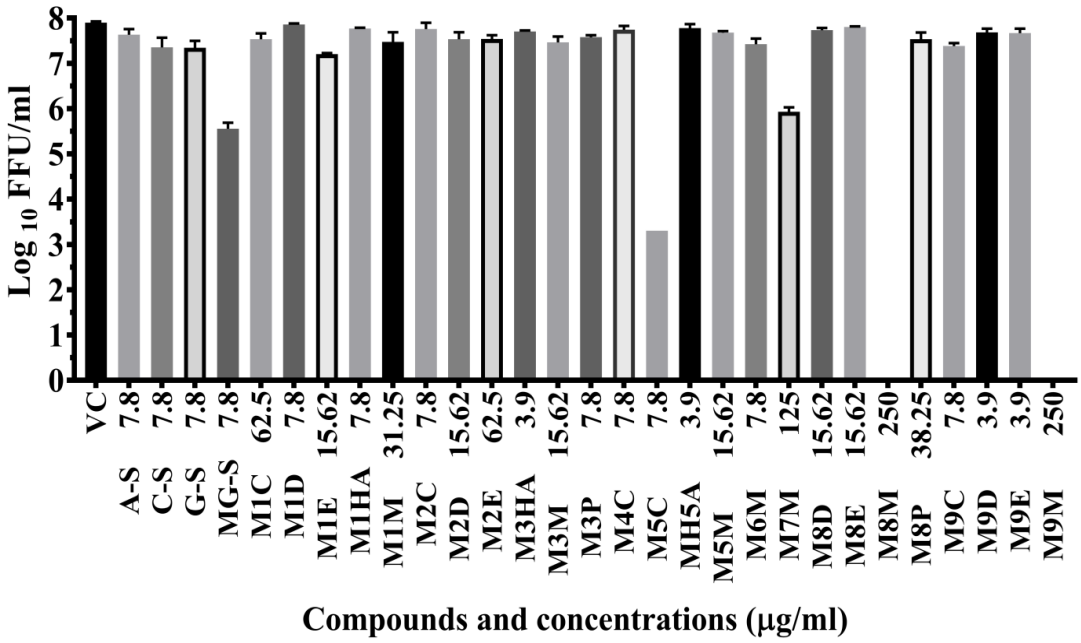


Fig.4. Primary screening of plant extracts using Foci forming unit reduction assay against DENV (a) and CHIKV (b)

Project 4: Use of lipid nanoparticles for effective delivery of siRNA in Chikungunya virus

Investigators: Parashar D, Jeengar MK, Patil P, More A; Ramakrishna S, Kurakula M (CSIR-IICT, Hyderabad)

Funding agency: Extramural (DST Nano Mission) **Duration:** 2017- 2020

Background: Previously NS1 and E2 siRNAs administration showed CHIKV inhibition in *in vitro* as well as *in vivo* systems. The main challenge for siRNA therapy is its inability to reach their intended targets in

the cytoplasm to exert gene silencing activity. Therefore, there is a need for novel siRNA delivery systems to overcome these challenges. Cationic lipids are promising for designing safe non-viral vectors beneficial in treating chikungunya.

***Objectives*:** To explore solid lipid nanoparticles for their suitability as siRNA delivery system.

***Findings*:** Four (F1, F2, F3, and F4) nano-delivery systems (Hybrid polymeric/ Solid lipid nanoparticles) using cationic lipids (Stearylamine, C9 lipid, Dioctadecylamine) and polymers (Branched PEI-g-PEG - PEG) were prepared, characterized, and complexed with siRNA and assessed for stability and potential toxicities against CHIKV. Among the four, F4 containing stearylamine, with induced optimum cationic charge of 45.7 mV in the range of 152.1 nm, allowed maximum siRNA complexation, better stability, and higher transfection, with strong inhibition against E2 and ns1 genes of CHIKV. The study concludes that cationic lipid-like stearylamine with ease of synthesis and characterization, showed maximum complexation by structural condensation of siRNA owing to high transfection alone. Synergistic inhibition of CHIKV along with siRNA was demonstrated in both *in-vitro* and *in-vivo* models (Fig 5). Therefore, stearylamine based cationic lipid nanoparticles can be explored as safe, potent, and efficient nonviral vectors overcoming siRNA *in-vivo* complexities against chikungunya.

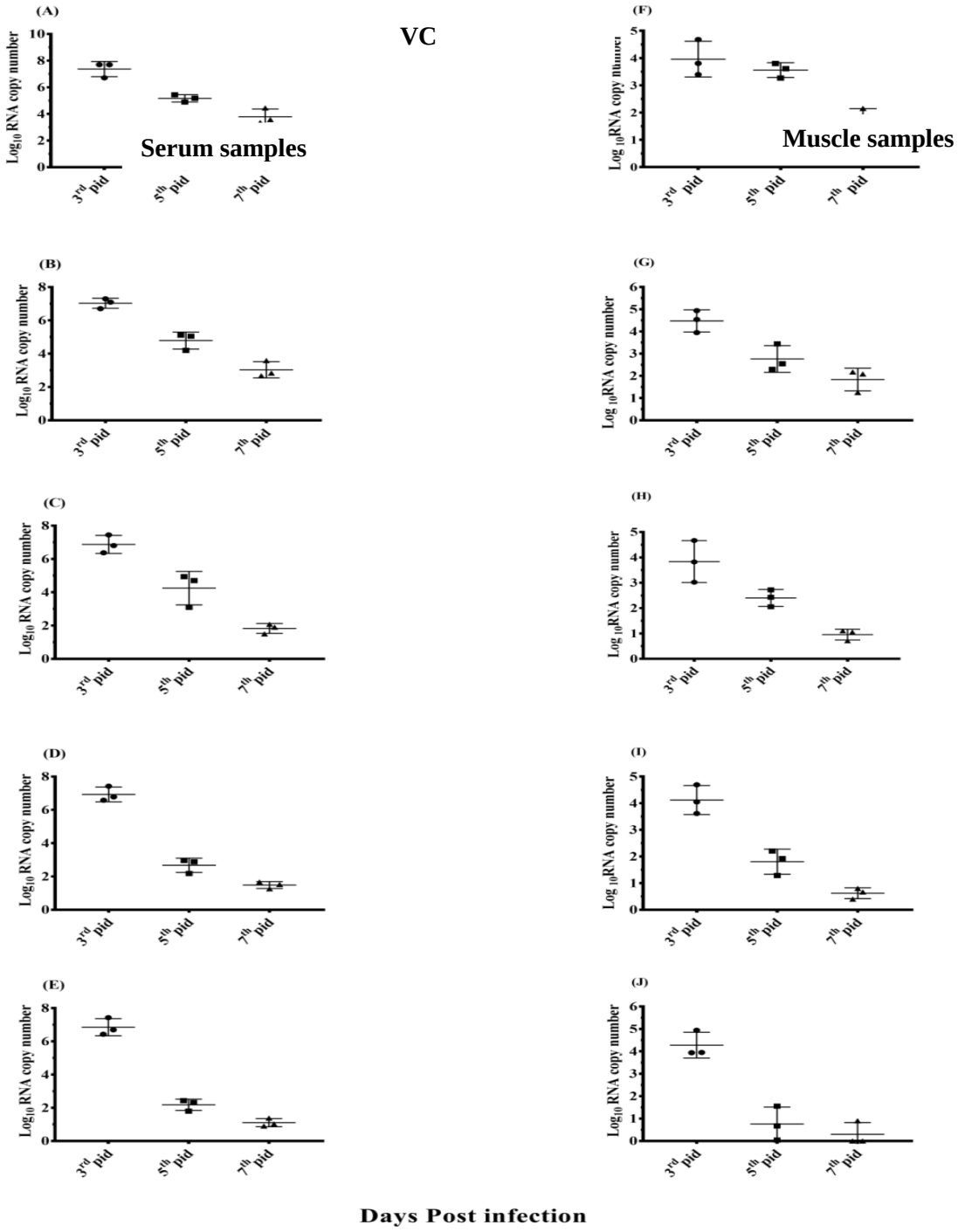


Fig.5. *In vivo* anti-CHIKV activity of siRNA complexed SLN delivery system. The reduction in CHIKV copies/ml in (A) serum and (B) muscle tissue after treatment with siRNA loaded F4 delivery system. nc-siRNA (A, F), F4 Blank (B,G), F4 loaded with CHIK 1 siRNA (C,H), F4 loaded with CHIK 5 siRNA (D,I), F4 loaded with CHIK 1+5 siRNA (E,J)

Project 5: Structure-based design and evaluation of lead compounds targeting Chikungunya virus

Investigators: Cherian SS, Agarwal M, Parashar D, Patil P, More A, Alagarasu K; Mainkar PS & Kumar NV (CSIR-IICT, Hyderabad)
Funding agency: Extramural (ICMR)
Duration: 2017- 20201
***Background*:** Approved antiviral therapies or vaccines for the treatment or prevention of CHIKV infections are not available. Natural polyphenolic compounds like xanthonoids and flavonoids possess wide ranging bioactivities such as anti-inflammatory, anti-oxidative, anti-bacterial, anti-fungal as well as antiviral activities.
***Objective*:** To evaluate certain natural and synthetic compounds in the treatment of chikungunya both *in vitro* and *in vivo*.
***Findings*:** *In vitro* antiviral studies revealed 14 out of compounds with significant inhibitory activity against chikungunya virus. Of these, OCL-101, EM-GU-BTT-1 (Fig 6a & 6b), EM-GU-BTT-7, EM-GU-BTT-16, EM-GU-BOT-5, EM-GU-BOT-8, EM-GU-BOT-11 (Fig 6c & 6d) and EM-GU-BOT-18 were effective under the post-treatment condition while EM-GU-BTT-15 was effective under the pre-treatment condition. Three compounds, OCL-108, OCL-113 and EM-GU-BOT-19 were effective both under pre and posttreatment conditions while OCL-105 and EM-GU-BTT-17 showed effectiveness under all the treatment conditions. Molecular docking analysis of Benzothiazole derivatives, EM-GU-BTT-1 and EM-GU-BTT-17, showed effective binding affinity and significant interactions with CHIKV RNA dependent RNA polymerase enzyme while the benzoxazole derivatives, EM-GU-BOT-5 and EM-GU-BOT-11, showed significant interactions with CHIKV nsP2 protease. Molecular docking analysis of α-Mangostin was in accordance to *in-vitro* studies where it efficiently interacted with the E2-E1 hetero-dimeric glycoprotein and the ADP-ribose binding cavity of the nsP3 macrodomain.

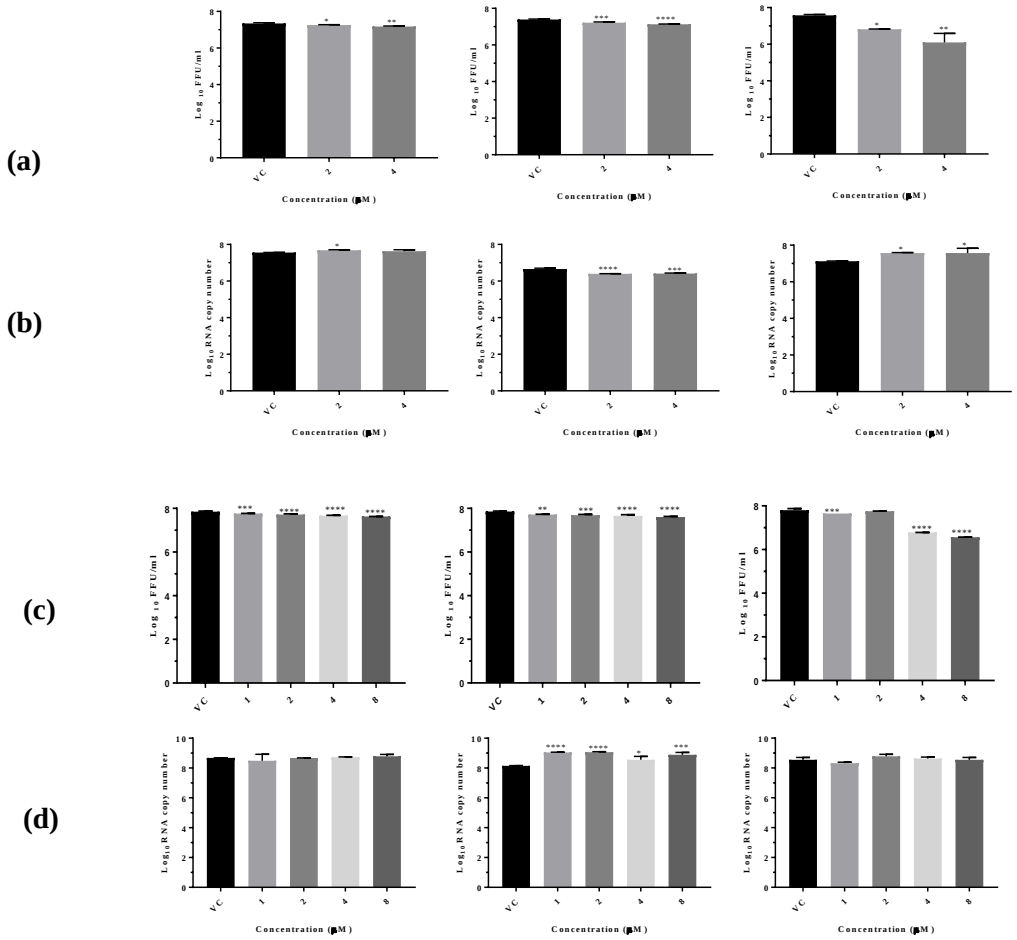


Fig 6: Effect of EM-GU-BTT-1 and EM-GU-BOT-11 compounds on CHIKV as assessed by focus forming unit assay (a & c) and qRT-PCR assay (b & d) respectively for pretreatment, cotreatment and posttreatment at 24h. The titres are present as log_{10} titres ****, p< 0.0001; *, p <0.05. All the values are expressed as mean ± SEM. The experiments were performed in triplicate independently. ****P<0.0001, **P<0.01 and *P<0.05 vs. control

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Project 1: Scale up of facilities for production of Diagnostic kits/ Reagents for detection of JE, DEN & CHIK IgM antibodies

Investigators: PS Shah & Kanchan Kumar Patil

Funding agency: NVBDCP

Project duration: Ongoing service

***Work done*:** MAC ELISA diagnostic kits to detect IgM antibodies of dengue, chikungunya and Japanese encephalitis viruses were produced and supplied to SSHs and 16 Apex laboratories engaged in laboratory diagnosis of vector borne viral diseases in humans. Around 700 Sentinel centers have been identified all over the country. The kits were supplied routinely to the Sentinel centers as well as during emergence of outbreak of these diseases. The number of MAC ELISA supplied to SSH and Apex labs under the national program during the year is given in Table 1.

Table 1: Supply of MAC ELISA kits in 2020-2021

	JE	DEN	CHIK	TOTAL
National Program	469	3508	1469	5446
VRDL (DHR)	159	211	147	517
Others*	12	8	3	23
Total	640	3727	1619	5986

* AES project centers, RMRC-Dibrugarh, Molecular Diagnostic Lab-Sindhudurg Maharashtra

The kits were provided to the centers based on their requirements and additional kits were supplied due to outbreaks in the neighboring area. Monthly distribution of kits is given in Figure 1.

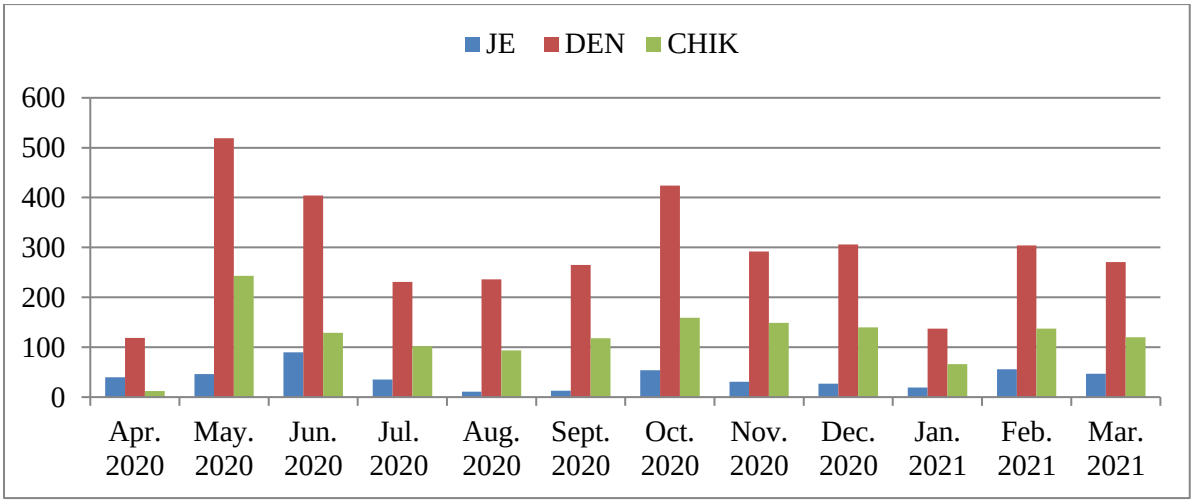

Figure 1: Month-wise supply of MAC ELISA kits under National Program

ELECTRON MICROSCOPY & HISTOPATHOLOGY GROUP

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Shri. Satish Mate	Multitasking staff (EM)
Shri. Ulhas Gadekar	Multitasking staff (Pathology)

- Core Electron microscopy and pathology services
- Inhibition of endothelial cell myristoylation can inhibit dengue 2 virus replication suggesting post translation lipid modifications to play a possible virus-host interplay.
- Dengue virus NS1 protein shows differential affinity to cellular membrane lipids.
- Imaging of SARS-CoV-2 virus particles in clinical sample by transmission electron microscopy

Project 1: Characterizing pathophysiology of Dengue virus induced stress in cultured vascular endothelial cell

Investigators: Basu A, Meena VK, Jain P, Tadkalkar N

Funding: Intramural

NS1 protein can activate endothelial cells (EC). Dengue virus (DV) infection and exogenous exposure of purified NS1 alters glycocalyx morphology and increases cell stiffness. Post-translational myristoylation of NS1 protein can have biological functions in host-DV interaction at EC level. Biophysical characterization of the mechanisms by which NS1 interacts with EC is being studied by AFM and Raman spectroscopy.

ENCEPHALITIS GROUP

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Mr. Kunal Sakhare	Technician A

Research student

Ms. D. N. Mali	Senior Research Fellow

Project 1: Investigations (diagnosis) of referred human clinical specimens during encephalitis outbreaks from different parts of India.

Investigators: Bondre VP, Mahamuni SA, Sakhare KS, Sankararaman V, Butte D.

Funding Agency: Intramural (Institutional service project) Duration: Ongoing

Background:

The Encephalitis Group of ICMR-NIV is involved in investigation of encephalitis outbreaks and provides diagnostic support to samples of suspected Acute Encephalitis Syndrome (AES) patients and contacts referred from across the country. Diagnostic support is also provided to samples of domestic/ wild animals and arthropods of medical importance referred by state health authorities to understand their role in transmission of encephalitic viruses. Suspected AES samples were investigated according to symptoms and undergo screening for Japanese encephalitis, Chandipura, dengue, human herpes viruses (1, 2, 6, etc), cytomegalovirus, Epstein Bar virus, etc. AES cases reported with rash and neurological symptoms were investigated for Measles, Mumps, Rubella, Vericella Zoster virus, *Orientia tsutsugamushi* (Scrub typhus) and spotted fever group rickettsia by serological or molecular diagnosis assays. Virus isolation was also attempted using multiple cell lines and CD1 mice. Genomic characterization of viral isolates from clinical samples was also done, to study their molecular evolution.

***Findings*:**

a) **Investigations and surveillance on AES**

During the reporting period, 851 clinical specimens (CSF= 418; sera= 433) from 534 suspected AES cases were investigated. Anti-JEV IgM and anti-DENV IgM antibodies were detected in 27 cases (5.05%) and 38 (13.66%) cases respectively. Among the 119 AES cases referred from Gujarat and Maharashtra states, CHPV IgM antibodies were detected in 02 samples (one each) while CHPV genome was detected in 04 (CSF=1 and sera = 03) cases referred from Gujarat. One sample tested positive for HSV-1. Samples were also screened for West Nile virus, enteroviruses, cytomegalovirus, Chikungunya virus, Epstein Barr virus, and *Orientia tsutsugamushi*, but found negative.

b) Surveillance of JEV and CHPV transmission in endemic areas:

Under the project entitled, 'Japanese encephalitis epidemiology in Central Part of India', 385 clinical specimens (205 sera and 180 CSF) collected from 240 suspected AES cases from Maharashtra and Telangana were screened for JE, dengue, CHPV and HSV-1/2 that revealed positivity for JE IgM in 27 cases, dengue IgM in 27 and CHPV IgM in one case. Among the 38 human sera from close contacts showed anti-CHPV NT antibodies in 10 samples by plaque reduction neutralization test (PRNT) while none tested positive for JEV and WNV. PRNT performed on 121 animal sera referred from Vidarbaha region of Maharashtra showed presence of anti-JE NT antibodies in 29 samples, but did not show anti-CHPV NT antibodies.

c) Virus isolation attempts from human clinical specimens.

Clinical specimens collected within a week of onset of infection and transported in cold chain were processed for virus isolation in different cell lines and infant mice. However, none of the 110 specimens (71 CSF and 39 sera) yielded virus isolation.

Project 2: Role of Histidine residues of envelope protein in membrane fusion of Japanese encephalitis virus.

Investigators: Bondre VP and Mali DN.

Funding: Institutional

Duration: 2018-21

Background &Findings:

In continuation to that reported in Annual Report 2019-20, envelope gene of JEV genotype I was expressed through cloning of Signal-prM-E cassette under the transcription control of CMV promoter which resulted in stable expression of JEV E protein in BHK-21 cells as virus like particles (VLPs). The biologically active E glycoprotein was confirmed by antigen capture ELISA and Western blot analysis while VLP formation was confirmed by transmission electron microscopy (Fig. 1). Individual mutations to alter the flavivirus specific conserved H residues located at (E-144, E-246, E-319, E-395, E-397) were introduced in the construct by site directed mutagenesis and the interaction of native VLP, genetically altered VLPs with the host cells was studied in comparison to the wild type virus.

Generation of VLPs with mutated His residues: In the VLP coding cassette, the five conserved histidine residues individually replaced by Alanine (A) through incorporation of site directed mutations in H coding codon. The genetically altered plasmids expressing VLP confirmed for the mutation by RT-PCR and DNA sequencing. Transfected cells stably expressing mutated VLPs were purified by the single-cell dilution method. Three of the selected cell clones secreting maximum amounts of mutated VLPs in media (SDM-5 G2A10, SDM-1 IB6 and SDM-2 IA7) were amplified and cryopreserved (Table.1). These findings confirmed by antigen capture ELISA through capture of Fig. 1. Transmission Electron Micrograph of expressed JEV envelope glycoprotein taking the shape of 40-50 nm diameter virus-like particles.the coated VLPs as antigen by anti-flavivirus monoclonal antibodies (HX-2) using positive and negative controls (Fig 1).

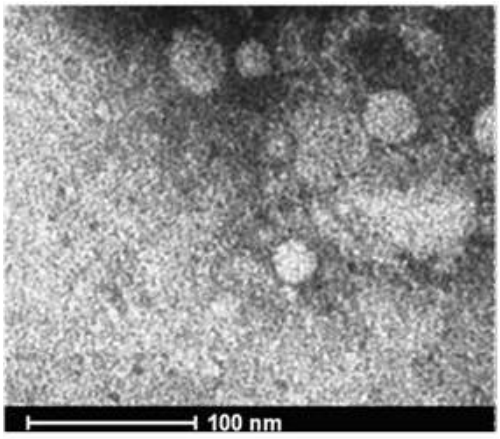


Fig 1. Transmission Electron Micrograph of expressed JEV envelope glycoprotein showing 40-50 nm diameter virus-like particles

Table1. Details of clones further selected for host–VLP interaction studies to define the role of H residues in membrane fusion

Clones ID	OD@450	Clone Name	OD@450
SDM -5 D2A6	0.371	SDM-2 IB10	0.375
SDM-5 D2F6	0.367	SDM-3 ID3	0.226
SDM-5 E2E7	0.279	SDM-3 IE3	0.153
SDM-5 G2A10	0.536	SDM-3 IA4	0.481
SDM-1 IA6	0.296	SDM-3 IB6	0.317
SDM-1 IB6	0.418	SDM-4 ID8	0.484
SDM-1 IC6	0.323	SDM-4-IF9	0.160
SDM-2 IA7	0.514	SDM-4 ID11	0.476
JE PC	**2.625**	**NC**	**0.09**

Project 3: To study the mechanism of endosomal membrane fusion process in Japanese encephalitis GI strain using DiD labelled virus.

Investigators: Mali D, Bondre VP

Funding: Intramural

Duration: 2016-21

Lipophilic fluorescent probe 1,10 -dioctadecyl-3,3,30 ,30-tetramethylindodicarbocyanine, 4-chlorobenzenesulfonate salt (DiD), a far-red fluorescent lipophilic carbocyanine (excitation at 644 nm/emission at 665 nm) with a high extinction coefficient, moderate fluorescence quantum yield, and short excited-state lifetime in lipid environments is used to labels the viral membrane. DiD has advantage over other dyes as at longer excitation wavelengths the cell auto-fluorescence background levels are low resulting in reduction of the signal-to-noise ratio. When incorporated in the viral membrane at a relatively high surface density, the emitted fluorescence level largely quenched, but single DiD-labeled virus particles could be clearly seen. Membrane fusion of virus particles labeled with a relatively high surface density of DiD can be observed as a fluorescence dequenching due to the lateral diffusion of the DiD probe from the viral membrane into the target cell membrane.

JEV GI (strain 0945054) labelling achieved by mixing of 1 ml virus preparation (titer: 3 X 10^6 pfu/ml) with 10 µl of DiD (10µM/ml final concentration) solution with stirring (1000 rpm for 30 min) at room temperature. The labeled virus preparation was purified by size exclusion chromatography (Sephadex G-25 column) and analyzed by ELISA using DiD labelled virus as antigen. The purified and labelled virus was concentrated through 100kDa cut off columns and assessed for virus infectivity by plaque assay. No major effect of labeling on the infectivity of the virus was seen as it yielded 4 X 10^5 pfu / ml in comparison to 3 X 10^6 titer of the parental virus (Fig. 2). Uniform DiD labeling of the virus membrane was confirmed by allowing the labeled virus to attach on the host cell monolayer at 4ºC for 1 hr followed by IFA analysis (Fig. 3a and 3b).

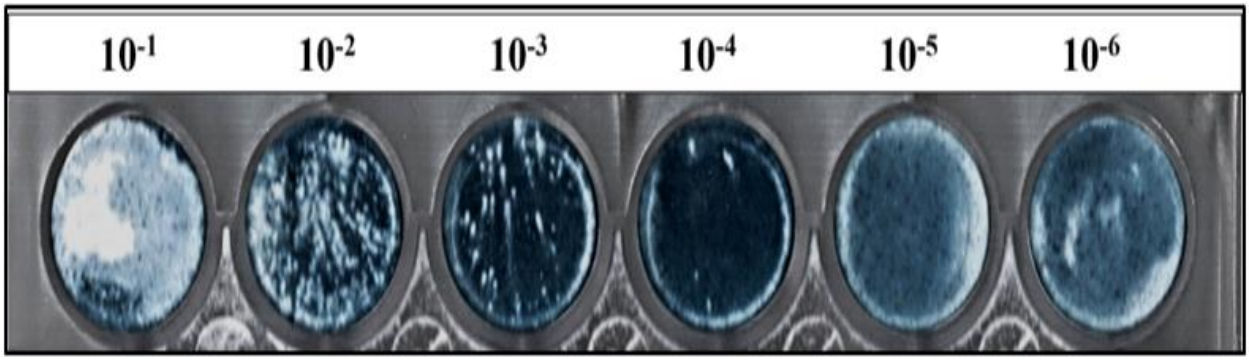


Fig. 2. Plaque titration of labeled virus JE 0945054 to confirm the infectivity

Using the standardized protocol, wild-type VLPs and genetically altered VLPs for H residues were labeled with DiD dye. For tracking experiments, synchronized infection of DiD labeled virus was achieved by maintaining the Neuro2A monolayer at 4ºC for 20-30 min before infection. Post infection (or exposure to VLPs), the cells were incubated on ice for 1 hr, washed with pre-warmed media and incubated at 37 ºC for 90 min to internalize the surface adsorbed virus / VLPs. The infected cells on coverslips were fixed at an interval of 15 min, probed with primary antibodies [against Caveolin (surface interaction), EEA1 (early endosome membrane), LAMP1 (lysosome associated membrane protein), etc.] followed by secondary antibodies and analyzed through Leica SP8 Spectral Confocal laser scanning microscope at 63 X magnification under oil immersion. Co-localization of the labelled virus, wild type VLP and mutated VLPs with different cellular compartments recorded and the results were compared.

At 15 min post internalization, the labeled virus was found co-localized with Caveolin; at 45 min post-internalization, with the early and late endosomes while at 60-90 min post internalization, it was found accumulated on the surface of nuclear membrane indicating that after membrane fusion the viral nuclear material was released, and the waste (DiD labelled lipids) was targeted to ER pathways for degradation. Similar results were obtained with the wild type labeled VLP and four of the genetically altered labeled VLPs (H144A, H246A, H395A and H397A). The results demonstrated that replacement of H residues located at 144, 246, 395 and 397 positions of JEV E glycoprotein does not have any impact on virus-host interactions. However, with H319A, mutation of H residues affected the virus-host interaction as the VLP internalization and fusion with endosomal membranes was found aborted (Fig. 5). The H319 is located in the fusion loop of E protein that is conserved amongst all flaviviruses, essentially playing a vital role in virus entry, virus-cell membrane fusion and elicits a strong flavivirus cross-reactive immunity. Further studies are in progress.

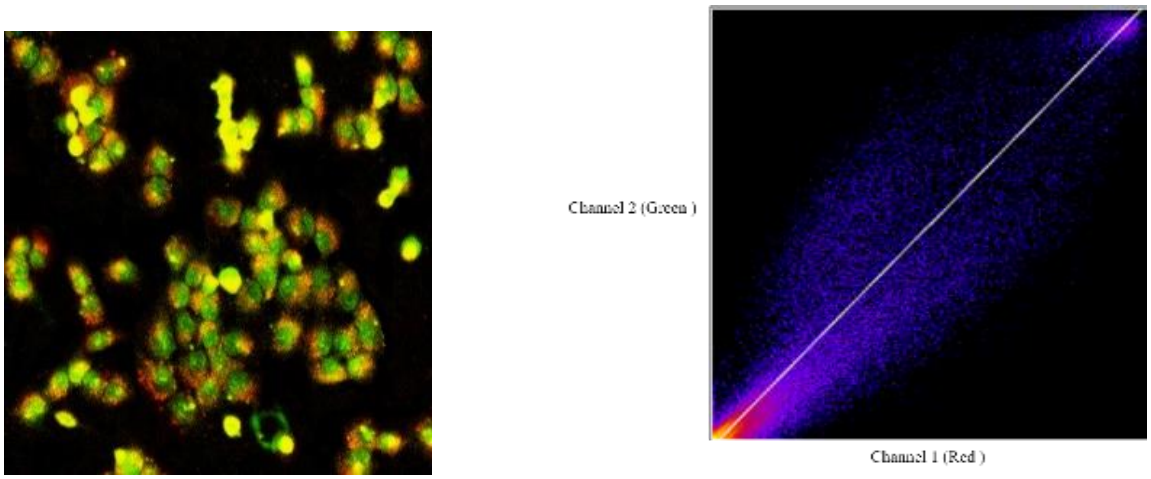
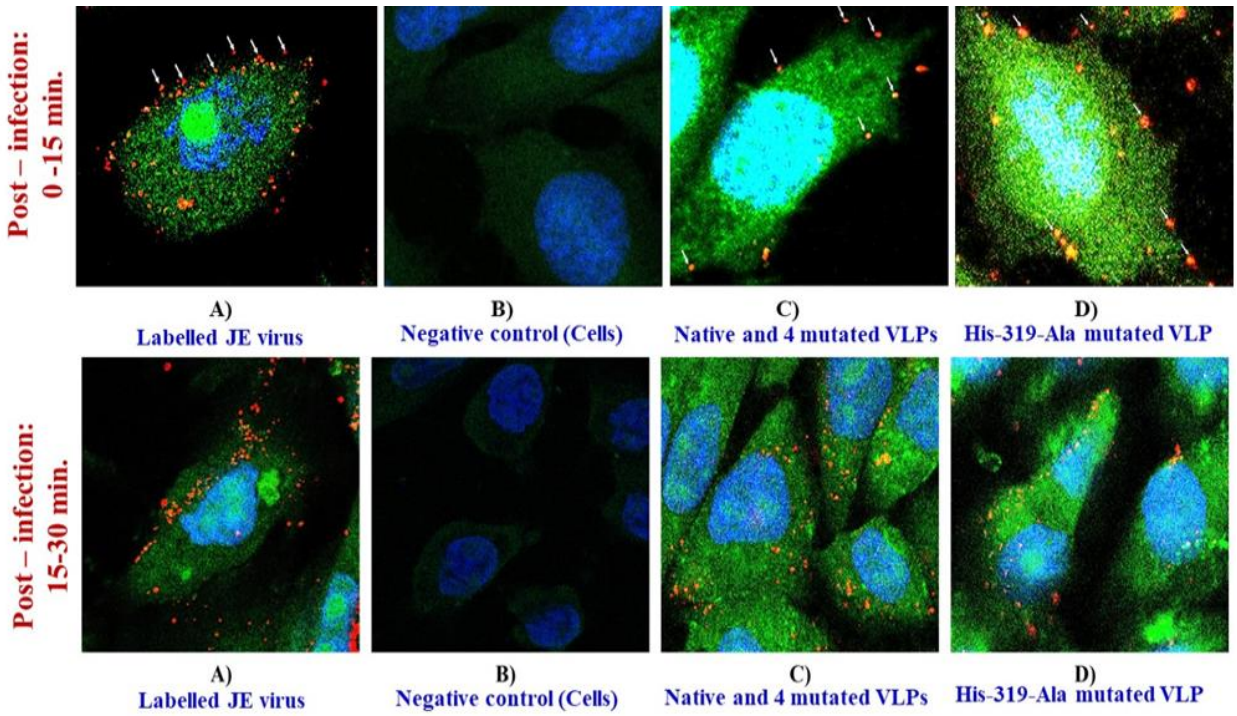


Fig 3: A. Co-localization of red (DiD labelled JEV) and green fluorescence (anti-JE MAbs-FITC) in IFA of cell monolayer infected with the DiD labeled virus by using JE specific monoclonal antibodies. B. Channel 1 (red) represents the labeled virus and channel 2 represents the labeled virus and MAbs labeled with FITC. The Pearson's correlation coefficient of the two-color channels was 0.92, which confirms the good quality uniform labeling of the virus as estimated using ImageJ software (https://imagej.net).



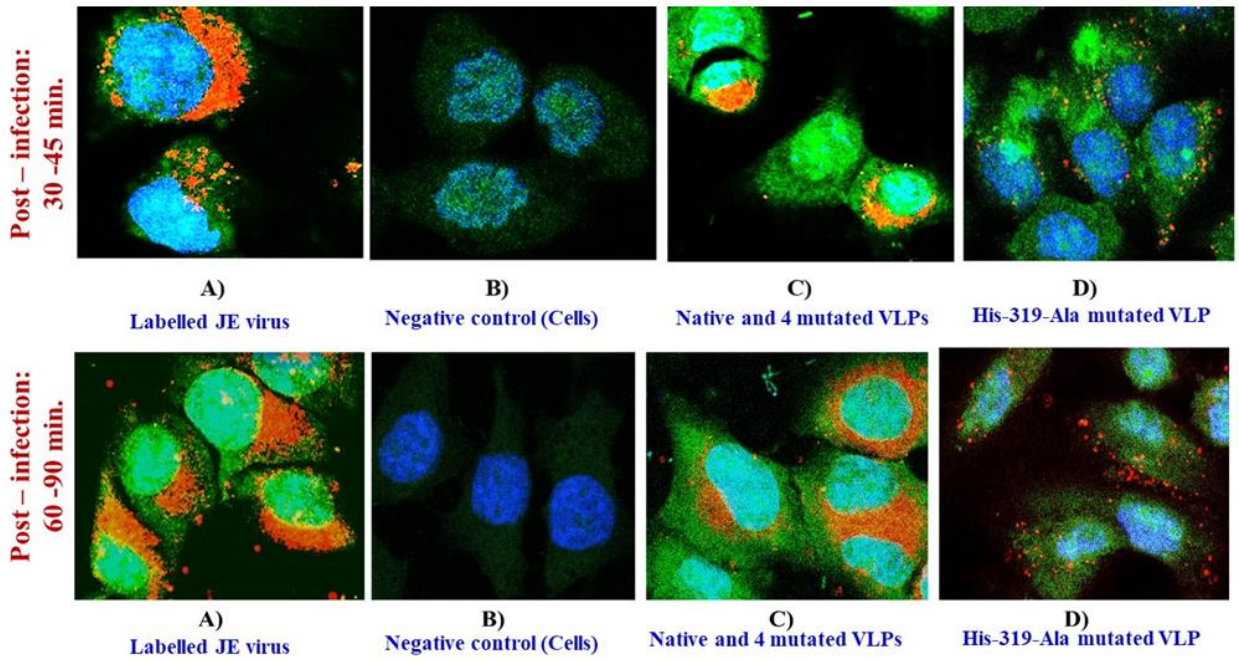


Fig. 5. Confocal microscopic images of endocytosis of DiD labelled JEV, native VLP generated from JEV, and genetically altered VLPs at different time intervals post synchronized internalization. A: The labelled JEV and all 6 VLPs (native and five genetically modified) found on the cell surface co-localized with the caveolin during 0-15 min. B: Beginning of endocytosis of JEV and 5 VLPs (native and 4 mutated) during 15-30 min intervals. Panel C: JEV and 5 VLPs fused with the late endosomal membrane resulting in de-quenching of DiD dye due to lateral diffusion at 30-45 min post internalization. Panel D: At 60–90 min, the DiD labeled lipid of virus envelope / 5 VLPs accumulated on the surface of nuclear membrane suggesting that it did not undergo endocytosis and located on the cell surface even at 90 min of exposure.

Project 4: Expression of Japanese encephalitis virus genotype-1 envelope and non-structural proteins to explore in early diagnosis.

Investigators: Bondre VP, Pavitrakar D, Sankararaman V, Mali DN.

Funding: Intramural

Duration: 2020-22

Background: JE infection primarily diagnosed through detection of anti-JEV IgM antibodies (mostly directed against virus envelope protein) in specimens collected during acute phase of illness. The existing JE MAC ELISA kit of ICMR-NIV uses cell culture derived virus (antigen) which affects assay specificity and hence replacement of the whole virus with purified envelope (E) glycoprotein is necesary to eliminate cross reactivity and bio-safety risk.

Findings:

a) Recombinant JEV E protein as an antigen:

JEV GI envelope glycoprotein extracellularly expressed using mammalian expression was found reactive with the standard panel of sera used in JE IgM ELISA kit. The IgM capture ELISA developed using recombinant JEV E protein as antigen and JE specific monoclonal antibody (HS-3) as the antigen capture antibody, specifically identified 150 positive sera detected by the existing JE MAC-ELISA kit.

Further evaluation of the newly developed JEV E protein based ELISA assay performed using a panel containing 10 sera each from JE IgM positive, JE IgM negative, JE IgM indeterminate by NIV- JE MAC ELISA kit. Using the newly developed assay, all the 20 JE positive and negative sera (tested by NIV MAC ELISA kit) samples was found concordant while 8 of the 10 indeterminate sera were confirmed as negative for JEV.

Another set of sera tested by the existing ELISA as dual positive for JE and dengue IgM were evaluated by the newly developed JE E protein based MAC ELISA assay for specificity. Among the 10 dengue and JE IgM dual positive sera, with the new assay, all dengue IgM positive sera tested positive for dengue while only 2/10 tested positive for JEV (against all 10 JE positive by the old assay). These results indicate that the JEV E protein antigen based assay is more specific than the existing JE MAC ELISA Kit.

To achieve absolute specificity of the JE MAC ELISA assay developed using expressed JEV E glycoprotein, the E protein coding gene cassette in pcDNA 3.1 was genetically altered by site directed mutagenesis. JEV GI envelope glycoprotein clone was modified at AA 106 G-K (GGG-AAG) and another modification at AA107 L-D (CTT-GAT) using site directed mutagenesis. The mutated plasmid were transfected in BHK-21 cells and stable cell clone expressing either E106 or E107 AA mutation located at fusion loop of E protein were selected under G418 antibiotics. Selection of stably expressing cells was carried out by IFA using JEV specific monoclonal antibodies (HS-3). Furthermore, the clones were adapted for stable expression under G418 antibiotic pressure (Fig. 6). The culture supernatant from single cell clones expressing modified E protein was also tested in JEV MAC ELISA using JEV specific detector monoclonal antibody (HS-3). The detection of fusion loop mutated E protein has failed in the presence of existing JEV cross-reactive MAB (HX-2), currently being used in JE MAC ELISA. The ELISA OD of mutated expressed E protein with HS-3 antibody was comparable to that of kit antigen. The suitability of these recombinant E protein modified at fusion loop will be explored further for testing patient sera.

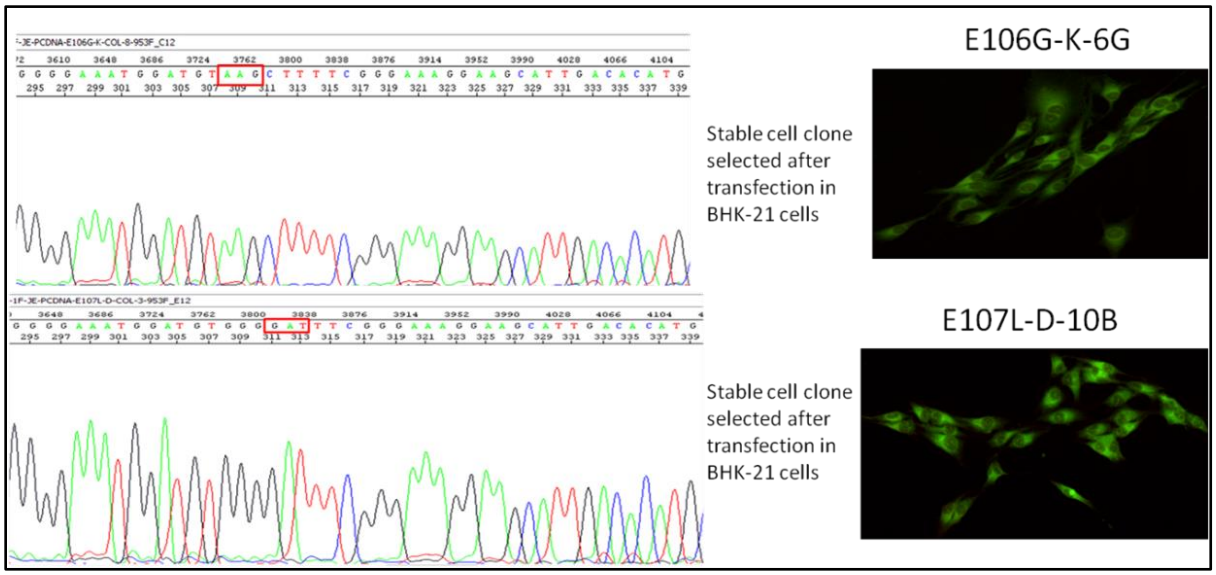


Fig. 6: Site directed mutations introduced in AA 106 G-K (GGG-AAG) and AA107 L-D (CTT-GAT) residues located in the fusion peptide of hairpin loop of JEV E protein and expression of the mutated proteins in cell culture as detected by IFA.

Recombinant JEV NS1 protein as diagnostic antigen:

To explore JEV NS1 as an antigen in detecting NS1 specific antibodies in patient's serum, NS1 protein coding gene cassette was amplified along with necessary signal sequence and directionally cloned under the control of CMV promoter in mammalian expression vector pcDNA3.1 (+). The recombinant plasmid was verified for presence of JEV NS1 insert by PCR and transfected in BHK-21 cells using Lipofectamine 3000 (transfection reagent) and Geneticin (G418). The transfected BHK-21 cells analyzed for expression of NS1 protein by IFA and western blotting using JEV specific polyclonal serum in comparison to JEV infected and mock cells (Fig. 7 A, B, C). Cells expressing JEV NS1 protein were screened by limiting dilution to obtain clones harboring recombinant plasmid on their chromosomes (Fig. 7D). Further studies on to develop early diagnosis system and development of monoclonal antibodies are in progress.

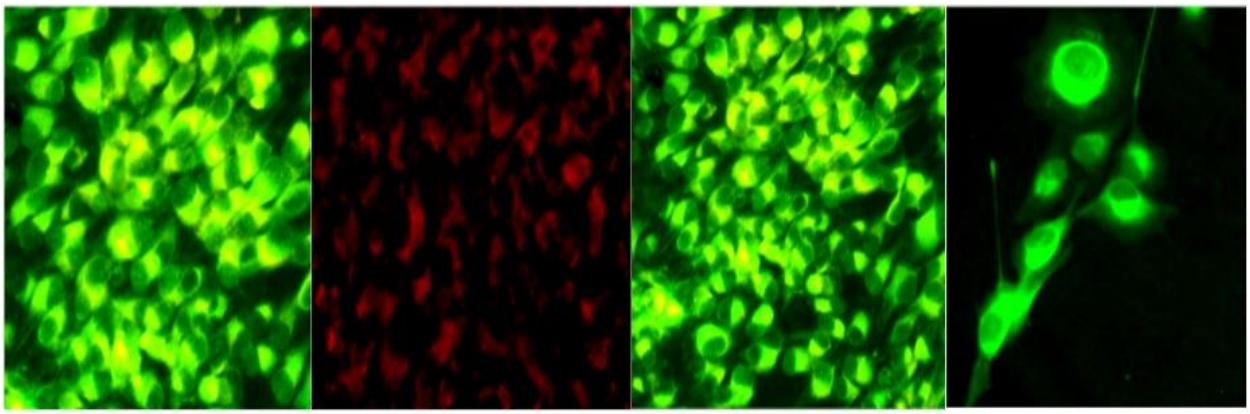


Fig.7: Expression of JEV NS1 protein in BHK-21 cells as detected by IFA
A. JE infected cells B. Un-infected BHK-21 C. Transient; D. Stable expression of NS1.

Project 5: To determine antiviral activity of viral RNA dependent RNA polymerase inhibitors against Chandipura virus infection.

Investigators: Bondre VP, Pavitrakar D, Sakhare K.

Funding: Intramural

Duration: 2020-22

Background:

Chandipura virus (CHPV) is an emerging viral infection associated with acute neurological illness of the paediatric age group with high case-fatality rate. To date, no therapeutics is available against it. CHPV, being a negative-stranded RNA virus, encodes an RNA-dependent RNA polymerase (RdRp) enzyme for viral genome transcription and replication; antiviral compounds targeting viral RdRp activity are of current research attraction. Accordingly, well-characterized RdRp inhibitors, *viz*., Ribavirin, Favipiravir, Remdesivir, and Galidesivir were tested to determine the anti- CHPV activity through *in vitro* and *in vivo* systems.

Findings:

In the present study, Remdesivir did not reduce the viral plaque number in Vero cells within the non-toxic range of the drug, *i.e*., up to 40µM concentration in plaque reduction assay. On the contrary, the EC_{50} of

Galidesivir by plaque reduction assay in Vero cells was 300µM with a substantial reduction in plaque size, emphasizing the possible anti-CHPV effect of Galidesivir (Fig. 8).

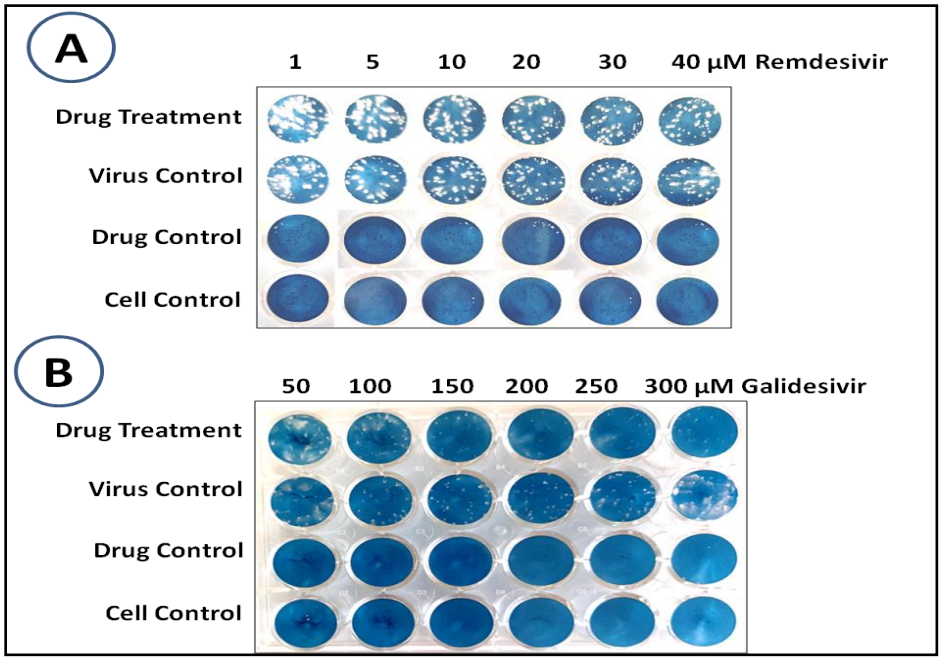


Fig. 8. A. Remdesivir shows no inhibitory effect in CHPV plaque number in Vero cells **B**: Determination of 50% Effective concentration (EC_{50}) of Galidesivir in CHPV infection by plaque reduction assay. Reduction in plaque size observed from 150µM concentration, 300µM concentration showed both 50% reduction in plaque number and size.

Pursuing the *in vitro* studies carried out with Favipiravir as a successful RdRp inhibitor against CHPV replication, studies were carried out in ten-day-old CD1 mice. Mice were inoculated with 10000 PFU of CHPV by intraperitoneal route. Preliminary studies demonstrated administration of 100mg/kg/day up to day 7 post-infection showed 60% protection while 300mg and 600mg dose showed 100% protection and hence 300mg/kg/day dose was found suitable. Mice treated with 100 and 300 mg/kg/day elicited substantial neutralizing antibody response while the survivors of 600mg/kg/day treatment did not show neutralizing antibody response (Fig. 9). With 300mg/kg/day treatment, CHPV could not be detected in the brain as against a titer of $10^{7.3}$ PFU/ml in the brain of untreated mice (Fig. 10). Histopathological and immunohistochemical analysis of the different mouse groups are in progress.

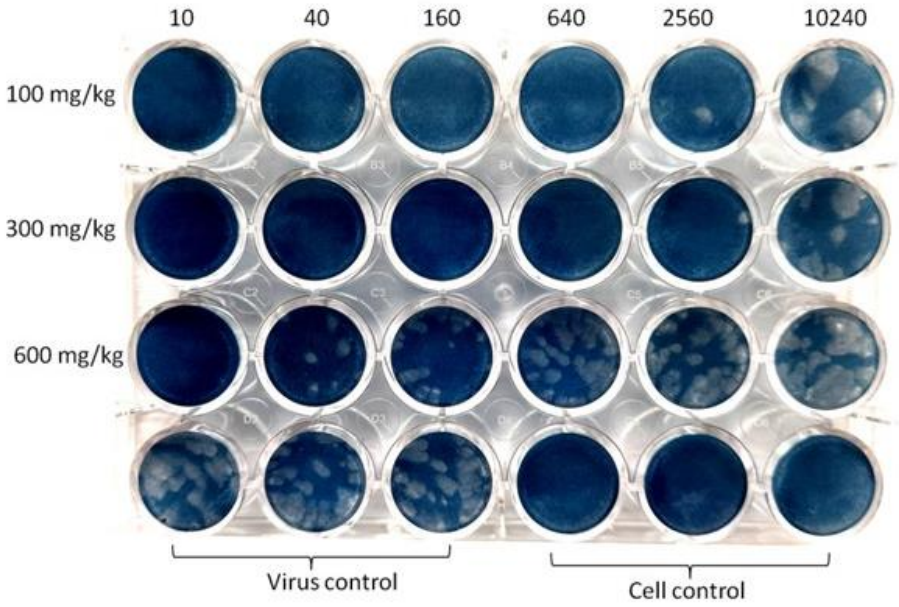


Fig. 9: Development of neutralizing antibody response against CHPV infection in mice treated with different doses of favipiravir. Mice with 300 mg/kg/day elicited protective immunity against CHPV.

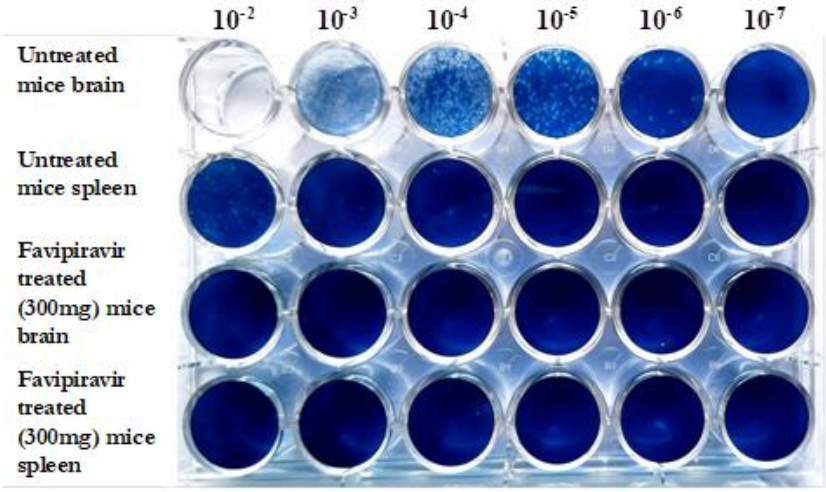


Fig. 10: Plaque assay data of brain and spleen tissues of favipiravir treated (300 mg/kg/day) mice and control mice. The treatment offered protection against lethal dose of CHPV in mice as virus was not detectable in brain and spleen of treated mice in comparison to untreated mice.

Project 6: Development of an indirect ELISA assay for surveillance of Japanese encephalitis.
Investigators: Bondre VP, Mali D, Butte D and Mahamuni S.
Funding: Intramural
Duration: 2020-22

Background: Routine JE surveillance involves sero-surveillance of humans, vector and swine populations for early warning signals. Surveillance also helps in assessment of vaccination impact, efficacy

of vaccination and prevention measures implemented etc. Although, WHO recommends nationwide surveillance throughout the year, only sentinel surveillance is done in India. To meet the requirements of necessary laboratory assays to support routine surveillance, we developed an indirect ELISA using recombinant JEV Egp for detection of anti-JEV IgG antibodies. The assay selectively detected IgG antibodies from convalescent sera of JE patients (tested positive in PRNT, the gold standard assay for comparison).

Findings:

To assess the sensitivity of newly developed assay, paired sera (acute and convalescent, collected 1 week apart) available from 54 JE IgM positive cases (different outbreaks); 61 IgM negative cases and 17 sera giving JE IgM indeterminate results were screened for IgG antibodies. Among the 61 IgM negative patients (acute sera), IgG antibodies were detected in 14 convalescent sera collected within a week while among the 54 IgM positive cases (acute sera), IgG antibodies were detected in convalescent sera from 20 cases. Among the 17 patients with indeterminate results, IgG antibodies were detected in convalescent sera of 7 cases. The OD values of negative controls lies at <0.13 while in IgG positive cases the OD exceeded 0.35. In most of the cases, the IgG ELISA findings correlated with the PRNT results (Table 2). Further work on standardization of assay sensitivity and specificity are in progress

SN	NIV ID Sera	JE IgM ELISA	JE IgG ELISA OD	JE IgG Result	JE PRNT
1	1617597-2	Negative	0.394	Positive	Positive
2	1617610-2	Positive	0.365	Positive	Positive
3	1617614-2	Indeterminate	0.546	Positive	Positive
4	1617618-2	Positive	0.14	Negative	Positive
5	1617576-2	Indeterminate	0.863	Positive	Positive
6	1617599-2	Positive	0.116	Negative	Negative
7	1617603-2	Indeterminate	0.089	Negative	Negative
8	1617606-2	Positive	0.133	Negative	Negative
9	1617608-2	Positive	0.079	Negative	Negative
10	1617612-2	Positive	0.063	Negative	Negative
11	1617620-2	Positive	0.079	Negative	Negative
12	1617622-2	Indeterminate	0.121	Negative	Negative
13	1617575-2	Negative	0.086	Negative	Negative
14	1617580-2	Positive	0.098	Negative	Negative
15	1617582-2	Positive	0.09	Negative	Negative
16	1617584-2	Positive	0.068	Negative	Negative
17	1617595-2	Positive	0.086	Negative	Negative

Table-2: Comparison of ELISA and PRNT results for JEV IgG detection.

Project 7: Establishing Rabies diagnostics and research capabilities.

Investigators: Ullas PT and Bondre VP.

Funding: Intramural **Duration:** Facility Establishment

Background: As per the 2019 and 2020 recommendations of the Scientific Advisory Committee and the ICMR, efforts were initiated to establish a rabies laboratory in the Encephalitis Group. As a preparatory step, all staff of the Group received a complete course of pre-exposure intradermal rabies vaccine, and all

demonstrated protective neutralizing antibody titres, one week post vaccination. Dedicated laboratory areas and equipment were identified for the work on rabies, and procurement of laboratory reagents and test kits were initiated. Simultaneously, we initiated liaisons with the public health department, hospitals, department of veterinary services and animal health, and other rabies laboratories. Following this, we initiated the standardization of various diagnostic assays for rabies.

Findings:

a) **Procurement of a standard reference laboratory strain of rabies virus:** A mouse brain adapted strain of 'fixed' rabies virus was procured from Central Research Institute, Kasauli, for use as a positive control in various diagnostic assays. A seed stock of the virus was prepared by intracerebral inoculation of suckling mice, and stored at -80˚C freezer in safe custody, with restricted access. Further, attempts were made to adapt this virus for growth in several cell lines, to prepare virus stock for *in vitro* neutralization assays. However, despite 3 consecutive passages in different cell lines (BHK-21, Vero and Neuro2a), virus could not be grown, probably due to the long passage history of the stock in mouse brains at the source laboratory.

b) **Fluorescent Antibody Test (FAT) for confirmation of rabies:** This assay, the current global gold standard for rabies diagnosis, was standardized in the laboratory, using a commercial rabies fluorochrome antibody conjugate and a set of known-positive and known-negative brain tissue samples of animals, graciously gifted by Mission Rabies, a non-governmental organization working on rabies control in India. Briefly, acetone-fixed impression smears made from brain tissue samples were stained using the commercial anti-rabies fluorochrome antibody conjugate, as per manufacturer instructions and reactivity examined under a Cell Imaging System (FloiD). The lab infected mouse brain and the naturally infected dog brain showed presence of rabies antigen as detected by bright apple green fluorescence (Fig. 11) indicating successful standardization of the assay.

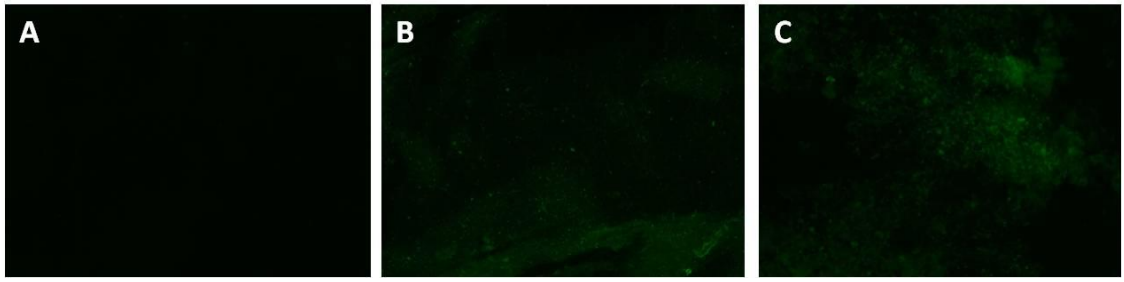


Fig. 11. Staining patterns observed in impression smears of brain tissue stained with LIGHT DIAGNOSTICS™ Rabies DFA Reagent. (A) Normal mouse (B) Suckling mouse inoculated with CVS strain of rabies virus (C) Dog with naturally-acquired rabies infection.

c) Standardization of a Semi-Nested Reverse Transcriptase Polymerase Chain Reaction (snRTPCR) for detection of rabies virus nucleoprotein (N) gene

This assay was successfully standardized in the laboratory, using a protocol published earlier, and RNA extracts prepared from serial 10-fold dilutions of an inactivated cell culture rabies vaccine. Fig. 12. shows the results from the standardization experiment.

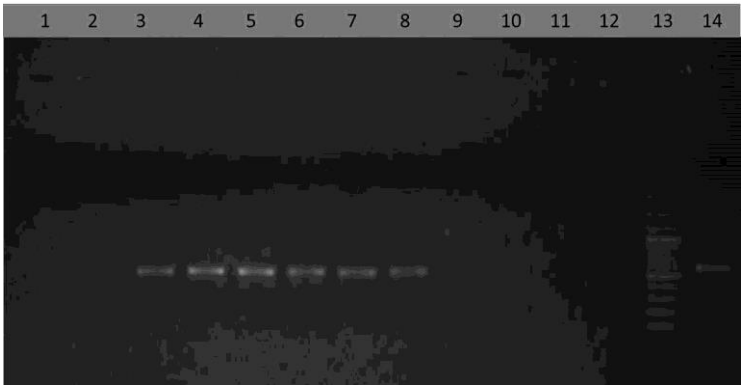


Fig. 12. Amplification of rabies virus nucleoprotein gene using a semi-nested RTPCR. Test results obtained from Extraction Control (Lane 1), No-Template Control (2), RNA extracts of an undiluted cell culture rabies vaccine (Lane 3) and serial 10-fold dilutions (10^{-1} to 10^{-9}) of the same. Lane 13: 100bp DNA ladder. Lane 14: Positive control.

d) Standardization of a Semi-Nested Reverse Transcriptase Polymerase Chain Reaction (snRTPCR) for the detection of rabies virus Large Protein (L) gene

Another semi-nested RTPCR assay for detection of rabies virus L protein also was standardized. Fig. 13 shows the results from the experiments.

Fig. 13. Amplification of rabies virus large protein (L) gene using a semi-nested RTPCR. Test results obtained from Extraction Control (Lane 1), No-Template Control (2), RNA extracts of an undiluted cell culture rabies vaccine (Lane 3) and serial 10-fold dilutions (10^{-1} to 10^{-10}, Lanes 4 to 13) of the same. Lane 14: 100bp DNA ladder. Lane 15: Positive control.

e) Standardization of a real-time RT-PCR assay for detection of rabies virus

A real-time RT-PCR assay for quantitation of rabies virus N gene was also successfully standardized using a protocol reported earlier. Appropriate controls, including no-template control, positive control and specificity controls (viral RNA extracts from Chandipura virus, Japanese Encephalitis Virus and West Nile virus) were included in the assay. Initial experiments were performed with RNA extracts made from serial 10-fold dilutions of an inactivated cell culture rabies vaccine, and showed successful amplification of the N gene. No non-specific amplification was observed with RNA extracts from related viruses. Fig. 14 shows the successful amplification of rabies N gene using the real-time RTPCR assay.

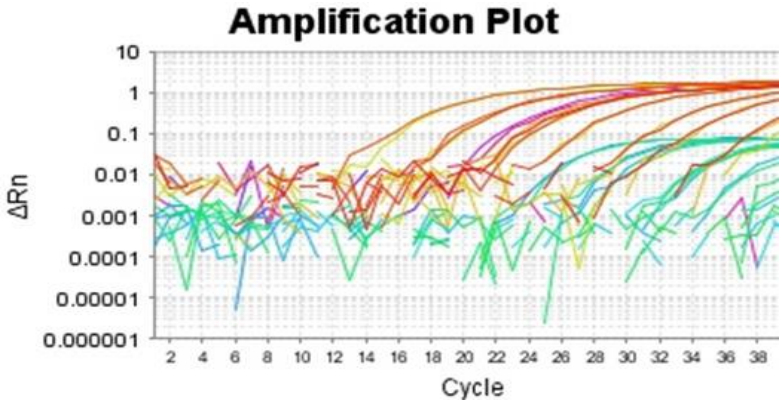


Fig. 14. Real-time RTPCR for detection of rabies virus. Fluorescence growth curves represent successful amplification of the nucleoprotein gene of rabies virus, from RNA extracts prepared from undiluted and serial, 10-fold dilutions of an inactivated cell culture rabies vaccine.

A positive control for the real-time RT-PCR also developed, using an *in vitro* transcript prepared for rabies virus N gene. Experiments showed that the real-time assay had a sensitivity of detection of up to 10^{-10} dilution of the *in vitro* transcribed RNA. The assay was also validated using RNA extracts made from known positive and known-negative animal brain tissue samples. 09/10 negative brain tissue samples and 10/10 positive brain tissue samples gave successful amplification in the real-time RTPCR assay.

f) Development of a Rapid Fluorescent Focus Inhibition assay

Currently we are focusing on the standardization of the Rapid Fluorescent Focus Inhibition Test (RFFIT) for the detection and quantification of rabies virus neutralizing antibody levels. Since this is an *in vitro* neutralization test, a cell culture adapted rabies virus strain is required as the challenge virus. We have approached several institutes for its procurement, and are waiting to receive the strain.

ENTERIC VIRUS GROUP

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Project 1: Diversity of non-rota enteric viruses in patients with acute gastroenteritis.
Investigators: Gopalkrishna V, Joshi MS, Lavania M
Staff: Chavan NA
Funding Agency: Intramural
Duration: 2017-2020
Background: In India, disease burden and molecular epidemiology of Rotavirus A (RVA) is well studied and documented. However, the role of non-RVA viral agents among acute gastroenteritis patients are reported in limited studies.

Objective: Identification and molecular characterization of non-RVA viral agents *viz.*, Norovirus (NoV), Adenovirus (AdV), Astrovirus (AstV) in patients with acute gastroenteritis (AGE).
Findings: Retrospective analysis of fecal specimens collected from children hospitalized for AGE in Maharashtra (Pune, n=300; Mumbai, n=300) and Gujarat (Surat, n=300; Ahmedabad, n=300) during 2013-2016 was carried out using AstV specific RT-PCR. The AstV detection rate was 6.1% (n=73) with 8, 24, 17, 24 cases in Pune, Mumbai, Surat and Ahmedabad respectively. Circulation of seven different AstV genotypes with predominance of AstV-5 followed by AstV-4 and AstV-1was observed.

i) Adenovirus positive fecal specimens (n=61) collected from Western India indicated high adenoviral load in acute gastroenteritis patients infected with subgroup F (n=32) as compared to patients infected with other adenovirus subgroups. This is the first report of HAdV-18 of adenovirus subgroup A from AGE patients in India.

Project 2: Hospital based surveillance of rotavirus strains in children with acute gastroenteritis
Investigators: Gopalkrishna V, Joshi MS, Lavania M,
Staff: Shinde MS, Chavan NA
Funding Agency: Intramural **Project Duration:** 2017-2020
Background: Rotavirus infections are the major cause of diarrhea in children (<5 years) despite the availability of two oral vaccines, *viz.,* RotaTeq and Rotarix. In India, RotaVac vaccine has been introduced and implemented in the National Immunization program since 2016. Epidemiological and molecular studies would be helpful to define the effect of rotavirus vaccines in diarrhea control.

Objective: To determine circulation of the prevalent and unusual rotavirus G-P genotypes, seasonal distribution and disease burden

Findings:A retrospective study conducted on 1297 stool specimens of AGE patients from Western India [Mumbai (n=298), Pune (n=402), Surat (n=300) and Ahmedabad (n=297)] using the NSP2 gene-based RT-PCR and nucleotide sequencing and phylogenetic analysis revealed 1% positivity for Rotavirus B (RVB). The RVB strains belonged to G2 genotype and clustered with strains from the Indian-Bangladeshi lineage. This is the first report of detection of RVB from Ahmedabad City.

Another retrospective study with 257 stool specimens collected from AGE patients from Western India [Mumbai (n=153), Pune (n=104)] using partial VP6 based RT-PCR detected two Rotavirus C (RVC) positive strains from Pune City. Phylogenetic analysis indicated clustering of one strain with I2 genotype of human RVC while the other with I7 genotype of porcine RVC strain [Fig 1]. This is the first report of detection of porcine RVC in human stool specimens. Further studies are in progress.

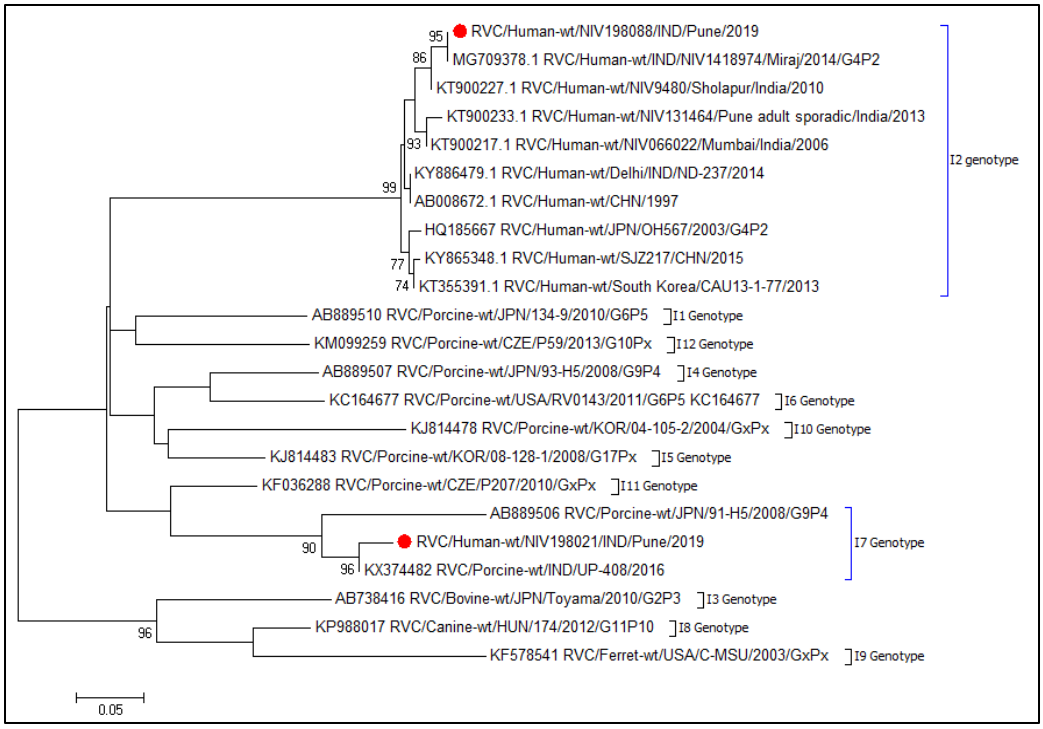

Figure 1: Phylogenetic dendogram constructed based on partial nucleotide sequences of the VP6 gene of RVC by General time reversible model +G+I. The strains from present study are indicated by red circular bullets. Scale indicates genetic distance

Project 3: Identification and molecular characterization of group C rotaviruses in infected humans and animals from Western India: A retrospective study

Investigators: Joshi MS, Gopalkrishna V

Funding Agency: Intramural

D**uration:** 2015-2021

Background

Group C rotavirus (GCR), is a known causative agent of sporadic and outbreak gastroenteritis cases globally. Zoonotic potential of GCR has been well documented (porcine GCR in humans). GCR was detected porcine fecal samples in earlier studies (AR 2019-20) and all the eleven genes of GCR strains were characterized to identify cross species transmission and reassortment events.

Objectives

- To detect and characterize GCRs from humans, bovines and porcines with acute gastroenteritis and elucidate evolutionary relationship and time scale stasis or dynamics

Findings

Molecular characterization of all the 11 genes of single GCR positive strain was carried out using different primer pairs. The nucleotide sequence data for 80% (14198 bp) of genome was obtained from different genes of GCR and with more than 70% sequence data for each gene with the exception of VP3 (53%) and NSP5 ((19%) genes. The percent nucleotide identity values and phylogenetic analysis classified RVC strain as G1, P1 , I7, R1, C1, M3, A1, N5, T5, E5, H1 genotype of the VP7, VP4, VP6, VP1, VP2, VP3, NSP1, NSP2, NSP3, NSP4 and NSP5 gene respectively.

Project 4: Identification and Molecular Characterization of non-rota enteric viruses in Neonates admitted at Neonatal Intensive Care Unit (NICU).

Investigators: Gopalkrishna V, Ranshing S, Lavania M, Shinde MS, Chavan NA
Funding Agency: Intramural **Project Duration:** 2016-2021

Background
A few reports have been published on outbreaks of diarrhea in neonates due to AstV and AdV. No reports are available from India.
Objectives
To determine the prevalence of AstV and AdV infections among neonates and to characterize RV strains to understand their genotypes.
Findings
RT-PCR screening of 700 stool specimens collected from 621 neonates from two local hospitals in Pune during April 2016 to March 2018 revealed presence of noroviruses (GI and GII), enteric adenovirus and human astroviruses. Genotyping (by sequencing) of positive specimens showed Genotype B3 (Human mastadenovirus B) and genotype AstV1 (astrovirus) was detected in 1% and 1.7% neonates respectively, suggestive of the prevalence of single genotype of both AstV and AdV.

Project 5: Study of cross neutralization of neonatal G12P[11] strain against convalescent phase sera from children vaccinated against current Rotavirus vaccines.
Investigators: Gopalkrishna V, Ranshing S, Lavania M,
Funding Agency: Intramural **Project Duration:** 2016-2021

Background: In India, in addition to commercially available vaccines, *i.e.* Rotarix™, the monovalent vaccine (GSK) and RotaTeq® pentavalent bovine-human reassortant vaccine (Merck), two indigenously developed vaccines *viz*., ROTAVAC® (a monovalent vaccine by Bharat Biotech, India) and ROTASIIL® (a thermostable bovine-human reassortant multivalent vaccine, Serum Institute of India, India) have been used to immunize children. No data is available on the neutralizing antibody responses to common RV types.
Objectives:To determine the neutralizing antibody (NAb) responses against the most common RV types (G1–G4 and G9) and G12P[11] among ROTAVAC® and ROTASIIL® vaccinated infants and **a** comparative analysis of homotypic (genotype-specific) /heteretypic (cross-reactive) responses between the two vaccines.
Findings: Clinical specimens were obtained from three hospitals; Bharati Hospital and KEM Hospital in Pune and Christian Medical College, Vellore. Group 1, sera (n=36) were obtained from RotaSIIL® vaccinated infants one month after the last dose and Group 2 composed of sera (n=31) from ROTAVAC® vaccinated infants. In the Group 1, 97.2%, 94.4%, 91.7% and 80.5% vaccinees were found seropositive for NAbs against strains G9P[8], G1P[8], G4P[6] and G3P[8] respectively, while only 47.2% recipients seroconverted to the strain G2P[4] demonstrating homotypic response. In contrast, only 30.5% (n=11) infants revealed serum NAbs to fully heterotypic strain, G12P[11]. Concerning the Group 2, 80.6% recipients seroconverted to the partial homotypic strain G9P[8]. Almost 77.4% vaccine recipients were seropositive against heterotypic strain G1P[8]. Significantly lower heterotypic response to strains; G2P[4], G3P[8] and G4P[6] was observed. Only 35.48% sera cross-neutralized partial heterotypic strain, G12P[11]. A comparative analysis of the log GMTs between the two groups indicated significantly higher serum NAbs

against the evaluated strains; G1P[8], G3P[8], G4P[6] and G9P[8] in subjects receiving RotaSIIL® vaccine compared to ROTAVAC® vaccines (Fig.2).

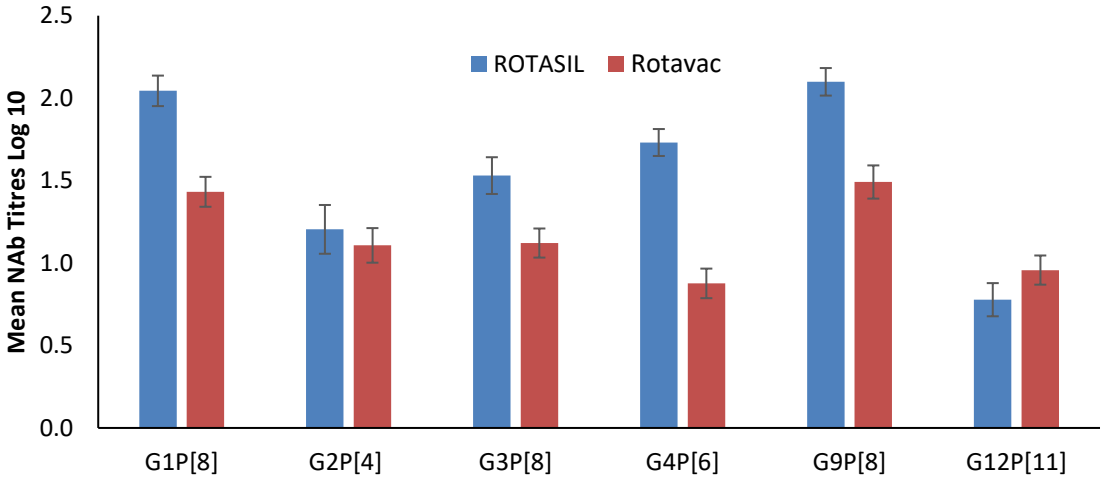

Fig 2: Comparison of mean NAb titres between RotaSIIL® and ROTAVAC® vaccine recipients against RV strains

Project 6: Detection and molecular characterization of potentially zoonotic enteric viruses in animals
Investigators: Sawant P, Gopalkrishna V & Lavania M
Funding Agency: Intramural **Duration:** 2017-2021
Background: Enteric viruses are mainly associated with gastroenteritis in humans and animals and transmitted by the fecal-oral route, person-to-person contact, contaminated food, water and surfaces. Some enteric viruses are considered to have zoonotic potential, thus constituting a risk for humans by direct transmission from animals, milk and dairy products. Zoonotic potential has been reported for RV, AstV, AdV, NoV and SaV, because of the close genetic relationship of strains detected in humans and animals. However, no data is available from India.

Objectives: To detect the prevalence of enteric viruses in bovine, porcine, canine and feline species and their molecular characterization to understand genetic diversity.

Findings: Screning of diarrheic samples from bovine (153), and porcine (60) and canine (80) collected during 2017-2021, revealed presence of RVAs in 27 bovine samples (17.64%) with a predominance of G10P[11] (51.85%), followed by previously unreported genomic constellations, G6P[14] (14.81%), and, G6P[4] (7.40%) and G10P[33] (3.70%). The G10P[33] was bovine-simian (SA11-like) reassortant strain, G6P[4] was bovine-human reassortant strain while G6P[14] strain is increasingly reported in humans. Real-time RT-PCR and ELISA studies showed 06 porcine (6/60) samples positive while all the canine samples tested negative for RVA. Besides RVA, other porcine viruses detected were enterovirus G15 and porcine teschovirus-17.

Project 7: Hospital based surveillance of enteric viruses/strains in children with acute gastroenteritis
Investigators: Lavania M, Joshi MS, Shinde MS, Chavan NA
Funding Agency: Intramural **Project Duration:** 2020- 2023

Background: Acute gastrointestinal (AGE) infections contribute to approx. 446,000 deaths every year in preschool children especially in low and middle-income countries. Rotavirus (RVA) infections are the major cause of severe dehydrating diarrhea in children <5 years old despite the availability of vaccines. Other enteric viruses associated with AGE include rotavirus B, rotavirus C, Caliciviruses (Noro and Sapovirus), enteric adenovirus (AdV), human astroviruses (AstV), aichiviruses, toroviruses, coronaviruses, picobirnaviruses, enteroviruses, and Sali/Klassi viruses. In India, RotaVac and ROTASIIL vaccines have been implemented in a phased manner in the National Immunization program since 2016. Epidemiological and molecular studies of RVA and non-RVA viral agents would be helpful to define the effect of rotavirus vaccines in diarrhea control.

Objectives:

- To identify cases of rotavirus among hospitalized AGE patients and to determine the circulating genotypes using sentinel hospital-based surveillance.
- To characterize all enteric viruses through molecular methods to understand their diversity.
- To identify risk factors among AGE cases compared to controls and to evaluate the association of enteric pathogens with AGE

Findings: Investigation of fecal specimens from 15 children hospitalized with AGE at Pune city (Maharashtra), showed 53.3% (n=8) positivity to RVA by ELISA. Eight different G-P types of RVA were identified with dominance of G3P[8] and G2P[4] (n=3 each) followed by G1P[6] and G2P[NT]. The samples were also tested for NoV- genogroup I and II by RT-PCR. Nucleotide sequencing and phylogenetic analysis using partial ORF1 and ORF2 regions, confirmed presence of GII.P16-GII-4 genotype of Genogroup II in two specimens. AstV was detected in five specimens. Nucleotide sequence analysis using partial ORF1 and ORF2 regions confirmed presence of HAstV-1 (n=4) and HAstV-8 (n=1). Dual infection was observed in two specimens; one with RVA and AstV and the other with NoV and AstV. All the specimens were negative for AdV when tested with hexon gene specific PCR. Etiology remained unknown in two specimens. The study highlights circulation of NoV and AstVs in AGE cases in addition to RVA.

EPIDEMIOLOGY GROUP

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Project 1: Japanese encephalitis epidemiology in Central part of India: enhanced sentinel surveillance for etiological contribution and burden following vaccination in Maharashtra and Telangana

Investigators: Tandale BV, Bondre VP, Sapkal GN, Damle RG, Tomar S (ICMR-NIV Pune) & Narang R (MGIMS Sewagram), Qazi M (GMC Nagpur), Padmaja G (KMC, Warangal)

Funding agency: ICMR (Extramural)

Duration: 2018 to 2021

Background:

Central India comprising parts of Maharashtra and Telangana is endemic to viruses like Japanese encephalitis, Chandipura and other viral and bacterial agents. Acute encephalitis syndrome (AES) hospitalizations among children and adults were tracked for incidence trends along with Japanese encephalitis (JE), other viral and bacterial etiologies.

Objectives:

- To estimate incidence of acute encephalitis syndrome hospitalizations
- To determine contribution of Japanese encephalitis and other etiologies of AES
- To estimate JE vaccination coverage among vaccine-eligible age groups
- To assess effectiveness of JE vaccination in preventing JE disease

Findings:

Among 83 AES cases enrolled during April 2020 to October 2020, JE was confirmed in 3 (6.0%) of 50 pediatric and 5 of 33 adults cases. The year-round occurrence of AES hospitalizations was observed along with JE confirmation among children and adults (Fig. 1). JE vaccination coverage in Maharashtra and Telangana states from October to December 2020 was 94.8% and 92.8% respectively. We had 35 JE cases available with 2 controls matched on residence, gender and age groups per case, pair-matched OR was 0.261 (95% CI 0.106 – 0.641). Thus, unadjusted overall JE vaccine effectiveness estimate was 73.9% (95% CI 35.9 – 89.4%). Other viruses/non-viral agents detected were Herpes simplex virus (11), dengue (2); leptospirosis (3), typhoid (2) and Scrub typhus (5).

Conclusion:

Japanese encephalitis contributed as the major agent of acute encephalitis syndrome among children and also adults in medium-endemic states in India despite very high coverage and moderate expected level of effectiveness of JE vaccination.

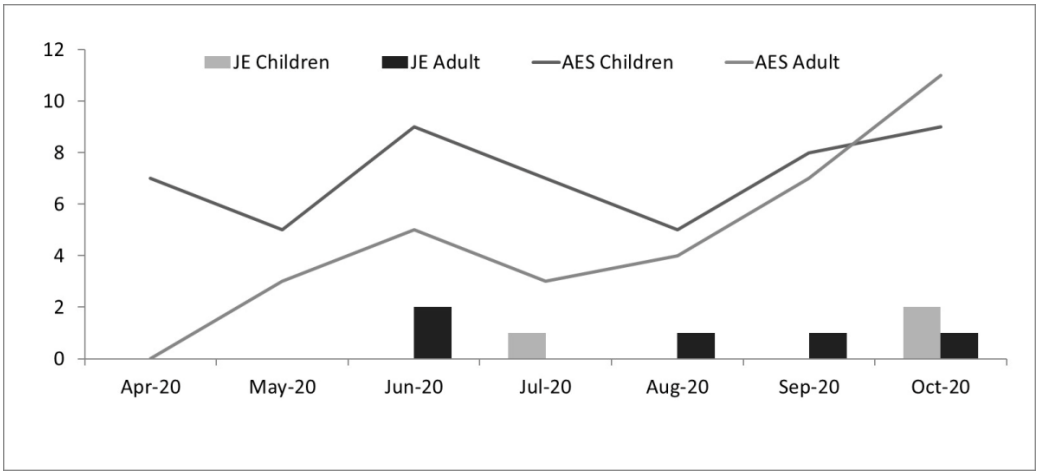

Fig. 1: Acute encephalitis syndrome hospitalizations with JE confirmation, April 2020 – October 2020

Project 2: Mobile Application for Immunization Data in India (MAIDI)

Investigators: Gupta N, Tandale BV
Funding Agency: DBT-BIRAC under GCI-IDIA (BMGF) through ICMR [BT/IDIA0183/04/17]
Duration of study: 2019 to September 2021

Background:

We planned to develop, pilot and evaluate feasibility of integrated mobile application for beneficiaries, healthcare workers and health officials so as to improve uptake of vaccination.

Objectives:

1. To develop an integrated mobile application for beneficiaries, health care providers and health system conceptualized based on thorough situation analysis of the available tools.
2. To validate and pilot the mobile application in selected facilities to assess its ability to
   a. Educate and train the community as well as health care providers/health systems using m-learning platforms.
   b. Capture high quality data from public and private sectors, and
   c. Generate appropriate performance indicators.
3. To study the operational feasibility, acceptability and barriers in use of the application.

Findings:

MAIDI application was developed on reviewing existing apps - ANMOL, eVIN and RISE. Each version was reviewed and comments were provided for consideration of improvements. There were 12 users of this app from public facility; 8 ASHA workers and 4 ANMs.

In public facilities, 42 immunization sessions were observed, monitored and tracked using MAIDI from November 2020 to March 2021. Out of 437 visits by children in due list for sessions, almost 70.7% (285/403) were missed. Among 1136 children from ASHA's areas, 774 children were analysed for timeliness (Table 1).

Schedule time period	Children available	On-time vaccinated	On-time vaccination (%)
At birth (BCG)	4	3	75.0%
At 6 weeks	105	63	60.0%
At 10 weeks	148	64	43.2%
At 14 weeks	156	52	33.3%
At 9 months to 12 months	136	123	90.4%
At 16 months to 24 months	138	126	91.3%
At 5 to 6 years (DPT B2)	64	56	87.5%

Average on-time vaccination was 63.8% with drop over 6-14 weeks period. Vaccination was done on-time in 90.4% children at 9 to 12 months and beyond until 6 years.

Conclusion:

MAIDI application will help in strengthening the immunization programme. We propose to use the lessons learnt so as to develop a scalable country wide acceptable application.

Project 3: Resource Center under Health Technology Assessment in Research Project under the scheme (3103) human resource and capacity development
Principal Investigator: Gurav YK

Funding: Department of Health Research
Duration: 2019-2022
Background: To facilitate the process of transparent and evidence based decision making in the field of health, Govt of India has created an institutional arrangement called the Health Technology Assessment in India (HTAIn) under the Department of Health Research (DHR). HTA resource center is being established at ICMR-NIV Pune with the responsibility to collate to generate evidence related to the clinical effectiveness, cost-effectiveness, safety of medicines, devices and health programs using the Health technology Assessment approach.
Findings: Under the HTAIn at ICMR-NIV Pune, two HTA proposals are prepared in consultation with State health services, Govt. of Maharashtra and experts in the field.

1. Cost effectiveness of Syphilis & HIV combined point of care (POC) testing among pregnant women in Maharashtra
2. Cost-effectiveness of rubella vaccination among women in Maharashtra

The proposals got necessary Institutional approvals (PRC, SAC and Ethics committee) in January - February 2021) and submitted to Technical Appraisal Committee at DHR for approval in August 2021.

Project 4: Cost-analysis of diagnostic tests for COVID-19 at National Reference Laboratory in India.

Investigator: Gurav YK, Sambhare S, Sapkal G, Potdar VA, Shete A, Choudhary M, Bharadwaj S, Ulhas PT, Deshpande G
Funding: Intramural
Duration: 2020-2021

Background:
Diagnostic tests have cardinal and primary role in managing COVID-19 pandemic and developing diagnostics requires well set infrastructure and expertise. Evaluation of cost for development of diagnostic tests is a basic need to carry out economic evaluation which is important in decision making *i.e.* resource allocation and intervention effectiveness.

Objectives:
1. To measure the cost for development of RT-PCR and IgG ELISA diagnostic tests for SARSCoV-2
2. To evaluate the operative cost of RT-PCR and IgG ELISA diagnostic test for SARS-CoV-2

Findings:
Primary data has been collected from three laboratories involved in COVID-19 research at ICMR-NIV, Pune (Influenza group, DVG and BSL-4) under different cost heads *i.e.*, human resources, capital costs, recurrent costs, utility/overhead expenses and Building Infrastructure with a health system perspective. Primary analysis has estimated per unit cost of COVID-19 RT-PCR as INR 574.53. The running (per unit) cost of COVID kavacch IgG ELISA was estimated as INR 340.68. Estimation of developmental cost of COVID kavacch IgG ELISA and RT PCR is underway.

ENTOMOLOGY GROUP

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- Metagenomic analysis of viromes of *Aedes* mosquitoes from India are in progress
- Sand fly fauna of Pune city has been completed
- Dual infection studies of dengue and chikungunya viruses in *Aedes aegypti* mosquitoes are in progress

Project 1: **Metagenomics analysis of viromes of *Aedes* mosquitoes in India**

Investigators: Sudeep AB, Cherian SS, Lole KS

Funding: Extramural (ICMR)

Duration: Oct. 2019- Sept 2022

Background: Mosquitoes harbor a large number of insect specific viruses (ISV) that are highly species specific and non-pathogenic to humans or domestic animals. However, there is a potential threat of these ISVs evolving into human pathogens by genome alterations. Recent studies have shown abundance and diversity of arthropod-associated RNA viruses, demonstrating the role of arthropods in viral evolution, potentially serving as hotspots where insect-specific viruses have evolved into dual-host viruses.

Objectives: To study *Aedes* mosquito viromes from different parts of the country to determine the region-wise prevalence of arboviruses and differential vectorial capacities of the *Aedes* mosquitoes using metagenomics approach.

Findings: Standardization of the MinION Mk1B was conducted with lambda phage using standard library preparation protocol provided by Oxford nano technologies, UK. The recovered and basecalled sequences were uploaded to the online EPI2ME server. The sequenced reads were quality scored and assembled using the workflow for control lambda phage experiment on the EPI2ME platform. A total of 20,641 reads comprising 271.5 Mbases were generated and the average length was found to be 13,154 bases and the mode was 3570 bases. The workflow successfully base-called 19,432 reads with average quality score of 10.4 of which, 19,369 reads aligned to the reference genome with 90.9% average accuracy and 96.6% average identity (Fig 1). From analysis of the RNA samples, it was observed that majority of the reads belonged to Phasi Chaeron-like virus (PCLV) belonging to Phenuviridae family of the order Bunyavirales. The PCLV Genome was further confirmed with Sanger sequencing and the genomes of the three (S, M, L) segments were deposited in GenBank. In addition, the study yielded reads related to members of several other families especially *Flaviviridae* and *Peribunyaviridae.* Further characterization of the reads is in progress.

Project 2: Dual infection studies of dengue and chikungunya viruses in *Aedes aegypti* mosquitoes using molecular approach.

Investigators: Sudeep AB, Ghodke YS

Funding: Intramural

Duration: 2019-2021

Background: Dengue and chikungunya viruses are important human pathogens transmitted by *Aedes aegypti* mosquitoes principally, across the globe. Recent studies have shown dual infection are more pathogenic to humans leading to high morbidity and mortality. A study was initiated to understand the potential of the mosquito to replicate both the viruses using molecular approach.

Objectives: To understand the mechanism of replication of dengue and chikungunya viruses in vector mosquitoes.

Findings: To understand the mechanism of action, five different sets of experiments were carried out. *Aedes aegypti* mosquitoes were infected intrathoracially with one virus followed by oral infection with other virus after an incubation period of five days. The experiment was replicated by reversing the sequence of the two viruses. In another set of infection both the viruses were mixed together adjusting the virus titers equally. Harvesting of samples were done as per desired schedule and stored at -80^{o}C until processing. Control sets infected with individual viruses were also harvested accordingly for comparison.

Project 3: Vector competence of Anopheles mosquitoes to Chittoor virus, an Indian variant of Batai virus (In continuation with last year's report):

Investigators: Sudeep AB, Ghodke YS

Funding: Intramural

Duration: 2018-2021

Background: Chittoor virus, an Orthobunyavirus of the Peribunyaviridae family has been isolated repeatedly from mosquitoes in India since 1957. Though no major outbreaks involving humans has been reported yet, the close antigentic relationship to Batai which is known to cause infections in humans in Europe and Africa. Chittoor virus was once isolated from Anopheles mosquitoes and hence the study.

Objective: To study the vector competence of *Anopheles stephensi* mosquitoes to Chittoor virus alongwith it's potential to transmit the virus horizontally and transovarially.

Work done & findings: Mosquitoes were fed on viremic mouse, sampled and tested for virus growth on pre-scheduled time intervals. Horizontal and vertical (trans-ovarial) transmission of virus was also attempted using standard protocol. The mosquitoes replicated and maintained the virus till 15th day post infection yielding a maximum titre of 4.6 on the 8th day post infection (PI). Virus dissemination studies have shown presence of the virus in saliva from 8th day PI while in legs, the virus could be detected from 2nd day PI only. Virus could not be detected in the wings upto 15th day PI. Midgut was found infected on the 6th day PI onwards while infection of gonads was not observed till 8th day PI. The mosquitoes were able to transmit the virus to infant mouse demonstrating horizontal transmission while TOT could not be demonstrated.

Project 4: Studies on sand fly fauna of Pune city: revisited after 20 years

Investigators: Sudeep AB, Sonawane VA

Funding: Intramural

Duration: 2018-2021

Background: Sand flies are associated with the maintenance and transmission of several viruses of public health importance apart from their primary role as vectors of leishmaniasis. Chandipura virus (CHPV), one of the most pathogenic sand fly-borne viral diseases as far as case fatality rate is concerned, is endemic to parts of Maharashtra, Gujarat and Telengana. A longitudinal study was carried out in Pue city to study the sand fly fauna as well as to determine prevalence of CHPV.

Objective: To study the sand fly fauna of Pune city and to determine the prevalence of Chandipura virus.

Work done: Twenty-six localities covering new townships and recently annexed fringe villages in Pune city were surveyed and collected 5,387 sand flies using hand held aspirators. The sand flies were identified morphologically, pooled according to species, gender and locality and processed for virus isolation. Four species of sand flies were identified, *i.e*., *S. punjabensis* (39.3%), *S. babu* (30.4%), *S. bailyi* (27.2%), and *P. argentipes* (3.2%) were collected. *Phlebotomus papatasi, however,* could not be collected. Most of the collections (89.8%) were made from areas in close proximity to humans, *viz.*, living rooms, toilets, bathrooms etc (Fig 1). Though per man hour density in the fringe villages was found high, the newly urbanized areas also had a substantial abundance of sand flies (Fig 2). CHPV or any other virus could not be isolated despite processing all the sand flies.

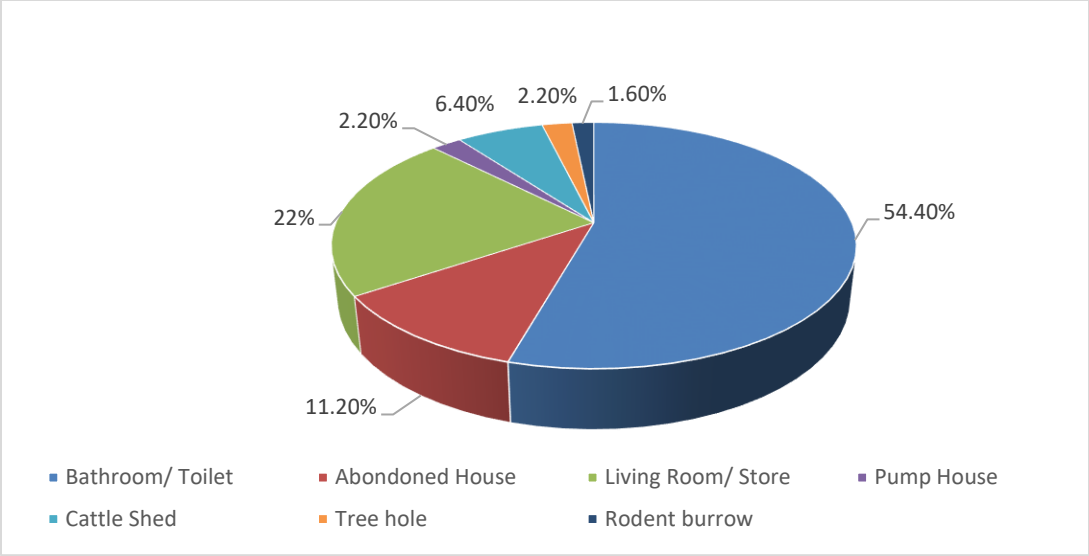

Fig 1: Breeding/resting sites of sand flies in the study area

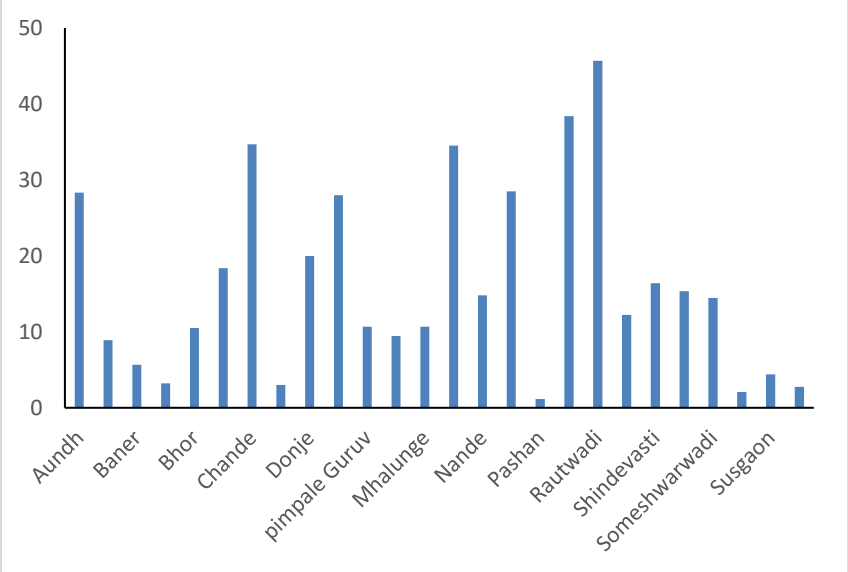

Fig 2: Average PMHD of sand flies in different collection sites in Pune region

Project 4: Surveillance of dengue and chikungunya viruses in *Aedes aegypti* populations in Pune district.

Investigators: Sudeep AB, Lole KS, Nath A (Ph. D. student)

Funding: Intramural

Duration: 2020-22

Background: Pune has become endemic to dengue and chikungunya viruses as evidenced by recurring outbreaks. A study to determine the *Aedes aegypti* population in different parts of Pune district has been initiated with respect to dengue/chikungunya outbreaks.

Work done: Weekly surveys were carried out in different parts of Pune city, *viz*., Bhavani Peth, Phule Nagar, Dange Chowk, Wakad, Aundh, Warje, Bhosari and Dehu Road cantonment areas from where

dengue cases were reported. *Aedes aegypti* larvae were detected in all the searched localities and a few outdoor resting adults were also collected. Indoor search for adults could not be made due to COVID-19 restrictions. Mosquito indices were determined and reported to state health authorities (Table 1& 2). All the mosquitoes/larvae were brought to the laboratory live, adults were screened for presence of the virus and the larvae were reared to adults and also screened. No virus detected in the field collected adults or immature mosquitoes. Further studies with dengue and chikungunya RT-PCRs are in progress.

Table 1: *Aedes aegypti* survey in Pune city and the mosquito indices in different areas

Name of place	No. containers positive/searched	No. houses positive/searched	Container Index	House Index	Breteau index
Dehu Road	7/34	6/24	20.59	25	29.17
Dehu Road Parsi Colony	16/53	6/24	30.19	37.5	66.7
Aundh	4/44	4/28	9.09	14.19	14.19
Dange chowk	6/47	5/28	12.77	18	21.48
Wakad	3/37	3/19	8.1	15.79	15.79
Bhavani Peth	7/40	5/21	17.5	23.8	33.3
Pule Nagar	3/48	3/26	6.25	11.54	11.54
Warje	3/49	3/25	6.12	12	12
Bhosari	6/29	6/23	20.68	26.07	26.07

Table 2: Breeding habitats for *Aedes aegupti* mosquitoes in the study area

Sr No.	Type of container	Indoor/outdoor	Total containers positive/searched	Percent positivity
1.	Metal drums	Outdoor	3/8	37.5
2	Plastic drums/container	Outdoor	41/303	13.5
3.	Tyre	Outdoor	3/8	37.5
4	Flower pots	Outdoor	02/07	29.6

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Project 1: Establishment of a facility for production of standard virus positive controls for diagnostic PCRs and RT-PCRs tests for the important public health viral diseases

Yadav PD, Sapkal GN, Sudeep AB, Mourya DT, Jain R, Majumdar T, Shrivastava R

Funding agency: Extramural [DHR]

Project Duration: 2017-2022 [ongoing]

Background & work done: The project was initiated to support VRDLs in molecular diagnostics especially for high pathogenic viruses as handling of these viruses needed high security laboratories (BSL-3/BSL-4). During the year positive controls (inactivated virus) for seven viruses along with reagents were supplied to national network laboratories/private institutes/companies etc. SARS-CoV-2 live virus was supplied to two commercial partners for vaccine development. List of institutions to which the viruses and reagents were supplied is given in Table 1.

Table 1: List of antigen/reagents/kits and supplied to laboratories during year 2020

Sr. No.	Antigen/live virus	Volume of virus supplied & purpose	The target institutions
1.	KFDV	Anti KFD Human IgM ELISA kit- 5 kit	NIV Kerala Unit
2.	KFDV	Real time RT-PCR primers/probes-500 Rxn RT PCR primers-500 Rxn Positive controls – 500 Rxn	NIV Bangalore Unit
3.	Dengue	Anti dengue human IgM ELISA kit- 1 kit	District Hospital, Sindhudurg
4.	Chikungunya	anti chikungunya human IgM ELISA kit- 1 kit	District Hospital, Sindhudurg
5.	KFDV	anti KFD human IgM ELISA kit- 1 kit	District Hospital, Sindhudurg
6.	KFDV	• Real time RT-PCR primers/probes-500 Rnx • RT PCR primers-500 Rxn Positive controls – 500 Rxn	Virology Laboratory Wayanad, Kerala
7.	SARS-CoV-2	Gamma Inactivated antigen-12074 kits (for SARS CoV-2 IgG ELISA)	Karwa Enterprises Pvt Ltd, New Delhi
8.	SARS-CoV-2	Gamma Inactivated antigen-2868 kits (for SARS CoV-2 IgG ELISA)	Meril diagnostics, Vapi
9.	SARS-CoV-2	Gamma Inactivated antigen-2870 kits(for SARS CoV-2 IgG ELISA)	J.Mitra & Co. Pvt. Ltd, New Delhi
10.	SARS-CoV-2	Gamma Inactivated antigen-5612 kits (for SARS CoV-2 IgG ELISA)	Trivitron Healthcare Pvt. Ltd., Chennai
11.	SARS-CoV-2	Gamma Inactivated antigen-3980 kits (for SARS CoV-2 IgG ELISA)	Cadilla, Ahmedabad
12.	SARS-CoV-2	Gamma Inactivated antigen-50 kits (for SARS CoV-2 IgG ELISA)	Voxtur Bio Ltd., Palghar
13.	SARS-CoV-2	Gamma Inactivated antigen- 50 kits (for SARS CoV-2 IgG ELISA)	Avecon, Haryana
14.	SARS-CoV-2	Gamma Inactivated antigen- 10638 ml (for raising antisera in horse)	Biological E Limited, Hyderabad
15.	SARS-CoV-2	Gamma Inactivated antigen-10012 ml (for raising antisera in horse)	Vins Bioproducts Ltd, Hyderabad
16.	SARS-CoV-2	Formalin Inactivated- 500ml (for raising antisera in horse)	Central Research Institute, Kasauli
17.	SARS-CoV-2	Live virus- 10 ml (for vaccine development)	Bharat Biotech Pvt Ltd, Hyderabad
		Gamma inactivated-10 ml	
18.	SARS-CoV-2	Live virus- 10 ml (for vaccine development)	Zydus Cadilla

19.	SARS-CoV-2	Gamma Inactivated antigen (for R& D)	CCMB, Hyderabad
20.	SARS-CoV-2	Gamma & formalin inactivated antigen (for R& D)	ICMR National Institute of Immunohematology
21.	SARS-CoV-2	Gamma Inactivated antigen (for R& D)	CSIR Institute of Genomics and Integrated Biology, New Delhi

Project 2: Prospective investigation of transmission of Crimean Congo Haemorrhagic Fever (CCHF) amongst close contacts of confirmed CCHF cases

Investigators: Sahay RR, Yadav PD, Shete AM, Upadhayay K, Jain R, Patil S, Majumdar T

Funding agency: ICMR (Extramural Project)

Project Duration: 2019-2021 [ongoing]

Background: CCHF is a tick-borne viral disease with an average case mortality rate of 30-50%. Several outbreaks/sporadic cases of CCHF associated with tick bite or nosocomial infection were reported from Gujarat and Rajasthan since 2012. ICMR-NIV, Pune provides diagnostic support to both the states. ***Objectives***: To understand the likelihood of transmission of CCHF amongst close contacts of CCHF positive patients and their clinico-epidemiological profile.

***Findings*:** Weekly follow up of 17 CCHF survivors were were made till 76th POD to determine presence of viremia. It was observed that anti-CCHFV IgM detection in serum samples commenced from 2nd POD; however, anti-CCHFV IgG antibody was detected only after the 28th POD. Cytokine analysis has revealed significant increase in level of serum IL-6, IL-10 and IFN-γ during the acute phase of infection, but IL-10 lowered to normalcy upon clearance of the virus in the clinically recovered cases. Phylogenetic analysis revealed circulation of a reassortant strain of Asian-West African genotype in humans which has not been reported from India yet.

Project 3: Development of serodiagnostic assays for Nipah virus

Investigators: Yadav PD, Shete AM, Mohandas S, Jain R, Melag S, Chopade Y, Sarkale P, Lakra R, Majumdar

Funding agency: Extramural (ICMR)

Project Duration: 2018-2021 [ongoing]

***Background*:** Nipah virus (NiV) is a highly infectious zoonotic pathogen which has been detected in India since 2001. An outbreak of NiV was reported from Kerala during 2018 with a case fatality rate of approx 90%. This necessitated improved surveillance for human infections using reliable, specific and sensitive laboratory diagnostic assays to identify prevalence of NiV in naïve areas.

***Objectives*:** Development of anti-Nipah Human IgG, anti-Nipah bat IgG and swine IgG ELISA

Findings: ICMR-NIV, Pune has developed, standardized and validated anti-NiV human IgM and anti-NiV human IgG ELISA for detection of NiV antibodies in human samples. The developed NiV diagnostic assays don't require specialized infrastructure and provides complete end-to-end solution for NiV diagnosis and surveillance activities in rural/field settings. With proven ability to work even at Primary Health Centers with wireless data transfer capability, this easy-to-use technology finds application in the management of Nipah virus infection.

Project 4: Identification and characterization of novel viral isolates using Next-generation sequencing platform

Investigators: Yadav PD, Shete AM, Sahay RR, Mohandas S, Nyayanit D

Funding agency: ICMR-NIV, Pune

Project duration: 2020-2021 [ongoing]

Background: Next generation sequencing (NGS) is presently the most advanced approach for identification of unknown/untyped viruses independent of prior sequence information. Detection of novel pathogenic viruses in the clinical specimens has special significance in public health as many recent disease outbreaks have been caused by novel (zoonotic) pathogens.

***Objective*:** Indentification of unknown etiological agent from the clinical samples of a sick tiger

Finding: Nasal swab sample from a sick tiger predominately had Felid herpesvirus 1 reads. Reference mapping of the nasal swab sample reads to the Felid herpesvirus 1 reference (Accession Number: NC_013590) led to the retrieval of 97.8% of the Felid herpesvirus 1 genome (Length of reference: 135,797). Further mapping of the 28s rRNA of Babesia gene led to retrieval of its partial stretch in rectal, nasal swab along with the EDTA blood specimens.

Project 5: Preparation of reagents for highly infectious diseases

Investigators: Yadav PD, Shete A, Mohandas S

Contributing staff: Jain R, Patil S, Majumdar T, Lakra R, Kadam M, Kumar A, Suryawanshi A

Funding agency: Intramural ICMR-NIV, Pune **Project Duration:** 2018-2020 [ongoing]

Objective: Preparation of reagents against high risk pathogens for diagnosis and supply

***Work done*:** Reagents for Anti-KFD Human IgM and IgG ELISA reagents were prepared and provided to Zydus Cadila under the translation program of DHR /ICMR.

NABL accreditation ISO/IEC 17025:2017

Maximum containment facility has developed diagnostic assays for KFD, CCHF under the scope of NABL. Recently the NABL has updated the standards from ISO/IEC 17025:2005 to ISO/ IEC17025:2017. For compliance to the standards audits were conducted by NABL routinely.

Testing and rectification of different components of the BSL-4 facility & preparation of basic documents

MCL is a state of the art laboratory that provides diagnostic support for highly infectious viral agents. Preventive and breakdown maintenance of the equipments and onsite installations are necessary to provide uninterrupted service to the country.As per the Indian Boiler Act, the IBR boiler installed at the BSL-4 facility needs to be inspected every year by boiler inspector from "Directorate of Steam Boiler Department". The inspector visited the site in October 2020 and submitted the fitness certificate on which the Chief Inspector issued a certificate for the use of IBR boiler with validity till October 2021.

OLTC & Transformer Oil filtration & Maintenance**:** The examination includes evaluation of the general condition of oil; consideration of all results together often enables the cause of degradation or the source of a contaminant to be recognized, so that appropriate action can be taken to ensure reliable operation of the equipment. The controller of the OLTC also replaced with a new one.

Installation of new equipments: Various equipments (X-ray scanner machine with detector, Bronchoscope, Oxygen concentrator, CO2 incubators, CCTV camera's, Centrifuges, Microscope, IVC system, Photocopier machine, RNA extractor, RT PCR, Printers (printer & X-Ray, bone marrow drill machine, walky talky sets, de-humidifier, Logitech conference camera etc were installed in the facility.

Routine maintenance and Annual Maintenance Contract visits: Servicing of major utility equipments (Diesel Generator, Boiler, UPS, HT & LT panels) was completed as per schedule. Servicing of laboratory equipments of BSL-4 was also completed.

Annual Shutdown for re-validation: Annual shutdown of BSL-4 facility was carried out for maintenance and the laboratory was re-validated. All the supply and exhaust filters were tested for leakage/damage. Biosafety doors, autoclaves, biological effluent eecontamination tanks and tissue digester were also validated during the period. All pressure / temperature sensors, transducers were calibrated. Servicing of 14 AHU's were also performed.

Project 6: Providing diagnostic support for referred samples of viral hemorrhagic fever and other unknown etiology and outbreak investigation

Investigators: Yadav PD, Shete AM, Sahay RR, Mohandas S, Jain R, Patil S, Maujumdar T, Sarkale P
Funding agency: ICMR-NIV, Pune
Project Duration: Service Project
Background: Mandate of BSL-4 facility is preparedness and diagnosis of emerging and reemerging viral infections and the lab provided diagnostic support to samples referred from across the country. ***Objectives*:** To provide diagnostic support to referred samples and outbreak investigations
Work done: The laboratory provided diagnostic support to clinical specimens referred from different states on viruses, including KFD, CCHF and Nipah (Table 2).

Table 2: Diagnostic support provided on clinical specimens (virus wise)

Total number of positives/cases (April 2020- March 2021)						
Tested for	Human	Monkey	Ticks	Cattles	Tiger	Grand Total (Positive/ tested)
KFD	23/491	1/17	0/381	-	-	24/889
SARS-CoV-2	8/230	-	-	-	0/3	8/233
DENV	1/36	-	-	-	-	1/36
CHIKV	0/37	-	-	-	-	0/37
ZIKV	0/36	-	-	-	-	0/36
Nipah	0/2	-	-	-	-	0/2
CCHF	2/36	-	0/110	0/77	-	2/223
Lassa Virus	0/1	-	-	-	-	0/1
Bunya Virus	0/1	-	-	-	-	0/1
SFTSV	0/1	-	-	-	-	0/1
CDV	0/0	-	-	-	0/3	0/3
RVF	0/8	-	-	-	-	0/8
Grand Total	34/879	1/17	0/491	0/77	0/6	35/1465

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Project 1: Computational drug repurposing approach for the development of therapeutics against Hepatitis E Virus

Investigators: Lole KS, Cherian SS & Mainkar P (IICT, Hyderabad)

Funding: ICMR (Extramural)

Duration: 2019-2022

Background: Hepatitis E virus (HEV) infection mostly follows a self-limited course; however in certain conditions antiviral therapy is required. Though ribavirin (RBV) and PEG-IFN show promising results in acute and chronic HEV patients, there are certain limitations. Hence new therapies are required. In the proposed study we plan to use two computational approaches for repurposing, first to target the host pathways/ molecules that are important for HEV replication and the second that directly targets viral proteins using structural bioinformatics approaches.

Objectives

1. To generate transcriptome data and identify signature gene profiles of genotype 1 HEV infection
2. Systems biology and structural bioinformatics approaches to shortlist drugs for repurposing and evaluation of antiviral activity of selected drugs against HEV

***Findings*:**

(i) HEV transcriptome analysis: Global transcriptome analysis of HEV infected cells was carried out. S10-3 cells were transfected with HEV-1 full genome (FG) replicon and HEV-1 GDD replicon (as control) and cells were harvested after 96 hr and total RNA was isolated. Transcriptome analysis was done and differentially expressed genes were identified. Connectivity Map (cMAP) analysis was undertaken to do drug repurposing predictions. This work is in progress.

(ii) Drug screening: Twenty-seven drugs shortlisted by both systems biology and structural biology approaches based on published transcriptome datasets were evaluated against HEV-1 using subgenomic replicon system in S10-3 cells. Maximum non-toxic concentration of drugs was used to evaluate inhibitory effect on HEV replication. The inhibitory activities of these drugs ranged between 0-62%, with VX-702 (40% inhibition), MLN2238 (42.8% inhibition), Artesunate (ART, 59% inhibition) and ribavirin (62% inhibition). The EC_{50} values for RBV and ART were 2.9±0.53µM and 19.51±4.03µM respectively Table 1.

Table 1: List of drugs with CC50 values and HEV inhibitory activities against HEV-1

Sr. No.	Drug	Type	CC_{50}	MNTD	% inhibition
1	Rosiglitazone	Anti-diabetic	54µM	0.97µM	14.3
2	Pioglitazone	Anti-diabetic	67.3µM	0.97µM	29
3	Raltegravir	HIV-1 integrase inhibitor	106µM	1.95µM	13.8
4	Elvitegravir	HIV-1 integrase inhibitor	1.9µM	0.20µM	3.7
5	Nelfinavir	HIV-1 protease inhibitor	19.7µM	0.24µM	35
6	Lopinavir	HIV protease inhibitor	17.6µM	0.48µM	20
7	Saquinavir	HIV-1 protease inhibitor	7.2µM	0.24µM	No inhibition
8	VX-702	Anti-inflammatory	31.6.5µM	0.24µM	40
9	Mirtazapine	Antidepressant	27.4µM	0.97µM	No inhibition
10	MLN2238	Anti-cancer	3.31µM	0.097µM	42.8
11	Clofibric acid	Anti-cancer	75.2nM	0.97nM	10.1
12	Entecavir	Nucleoside analogue (HBV)	43.2µM	0.24µM	3.5
13	Hydroxychloroquine	Antimalarial	3.1µM	0.048 µM	8.6

14	Chloroquine	Antimalarial	4.77 µM	0.06 µM	22%
15	3-Amino benzamide	(NAD(+) ADP-ribosyl-transferase) inhibitor	45.38µM	0.48µM	No inhibition
16	Danoprevir	NS3/4A HCV protease inhibitor	8.05nM	0.24nM	27
17	Fenretinide	Antineoplastic	18.76µM	0.24µM	No inhibition
18	Eicosatrienoic acid ethanolamide	Agonist binding to CB1 and CB2 receptors.	164.3µM	0.122µM	31
19	Rucaparib	PARP inhibitor	14.5µM	0.24µM	No inhibition
20	Isotretinoin	Vitamin A derivative	109µM	7.8µM	21.9
21	All trans retinoic acid	Acne and acute promyelocytic leukemia	55.85µM	0.97µM	Testing in progress
22	Ilaprazole	Proton pump inhibitor	31.23µM	0.24µM	Testing in progress
23	Tenatoprazole	Proton pump inhibitor	38.89µM	0.24µM	
24	Finofibrate	Treat abnormal blood lipid levels	141.8µM	0.48µM	
25	Deferiprone	Iron chelator	39.3µM	0.48µM	
26	Ribavirin	Panviral inhibitor	39 µM	3.12 µM	62
27	Artesunate	Antimalarial drug	189 µM	19.5 µM	59

HEV helicase modeling: HEV helicase 3-D structure was predicted using tomato mosaic virus (ToMV) helicase template which has 32% sequence identity with HEV helicase. It showed good quality structure with 89% residues lying in the most allowed region where as 9.1 and 1.1% residues in additionally allowed and generously allowed regions respectively. The NTP binding site of superfamily 1 helicase involves highly conserved Walker A motif (motif I) (976-GVPGSGKSRS-985). Docking of acarbose, miglitol, BOT-5. BOT-11, ART and primaquine showed that ART binds with high affinity (-7.4, kcal/mol). ART showed three hydrogen bond interactions with Walker A motif residues Gly 979, Lys 982, Ser 983, whereas it interacted with Arg 984 residue via van der waals interactions indicating that it could inhibit the enzyme (Fig 1).

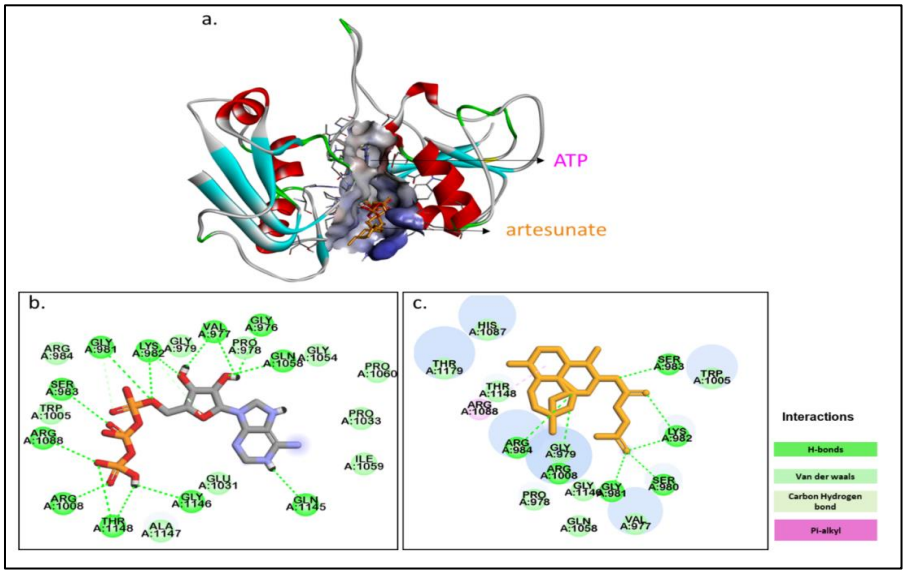


Fig 1: Docking interaction analysis of ATP and artesunate with HEV helicase domain

Project 2: Hepatitis E virus replication and cellular autophagy

Investigators: Lole KS, Srivastava M

Funding: DST WOSA-A project **Duration: 2018-2021**

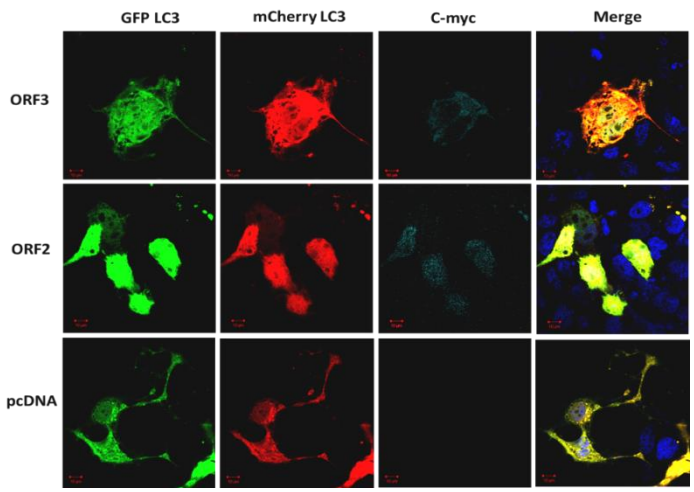
***Background*:** HEV is known to cause mostly self-limiting illness in humans and several aspects of HEV pathogenesis remain unexplored due to lack of efficient replication models. Autophagy is a conserved mechanism in eukaryotes to maintain cellular homeostasis with a crucial antiviral role. In response to viral infection, autophagy in host cells is activated by virus-encoded activators of autophagy-inducing signaling by cellular stresses, elicited by infection. Our earlier findings presented in last year's annual report documented dependence of HEV on early autophagy for efficient replication, likely via recruitment of autophagosomal membranes in establishing replication complexes**.**

Objectives:

1) To evaluate the role of autophagy in HEV replication
2) To know whether HEV replication requires autophagy machinery
3) To understand the mechanism of modulation of autophagy by HEV

***Findings*:** A step further in the earlier findings, we investigated the effect of HEV ORF2 (capsid protein) and ORF3 protein (a viroporin that contributes to the release of the virus from infected cells) on autophagy. For that, HEV ORF2 and ORF3 protein, both tagged with c-myc, were expressed in hepatoma cells to see the effect on autophagy. ORF3 displayed an intense accumulation of GFP-LC3 vesicles, similar to HEV infected cells, while ORF2 protein did not reveal any particular changes. The increased autophagic activity upon ORF3 protein expression was also confirmed by western blot analysis as accumulation of lipidated LC3II protein. In order to delineate whether, ORF3 expression also enhanced autophagosome fusion with lysosomes as observed during HEV infection, we explored autophagosome acidification and GFP degradation/quenching of the mRFP-GFP-LC3 reporter tandem construct in HEV ORF3 transfected cells. A complete overlay between autophagic vesicles (RFP) and HEV ORF3 expressing cells indicated that HEV ORF3 transfection induces autophagy (Fig 2). ORF2 did not reveal any fusion with acidified proteolytic lysosomes. These observations suggested important role of ORF3 protein in modulating autophagy during HEV infection.

A)



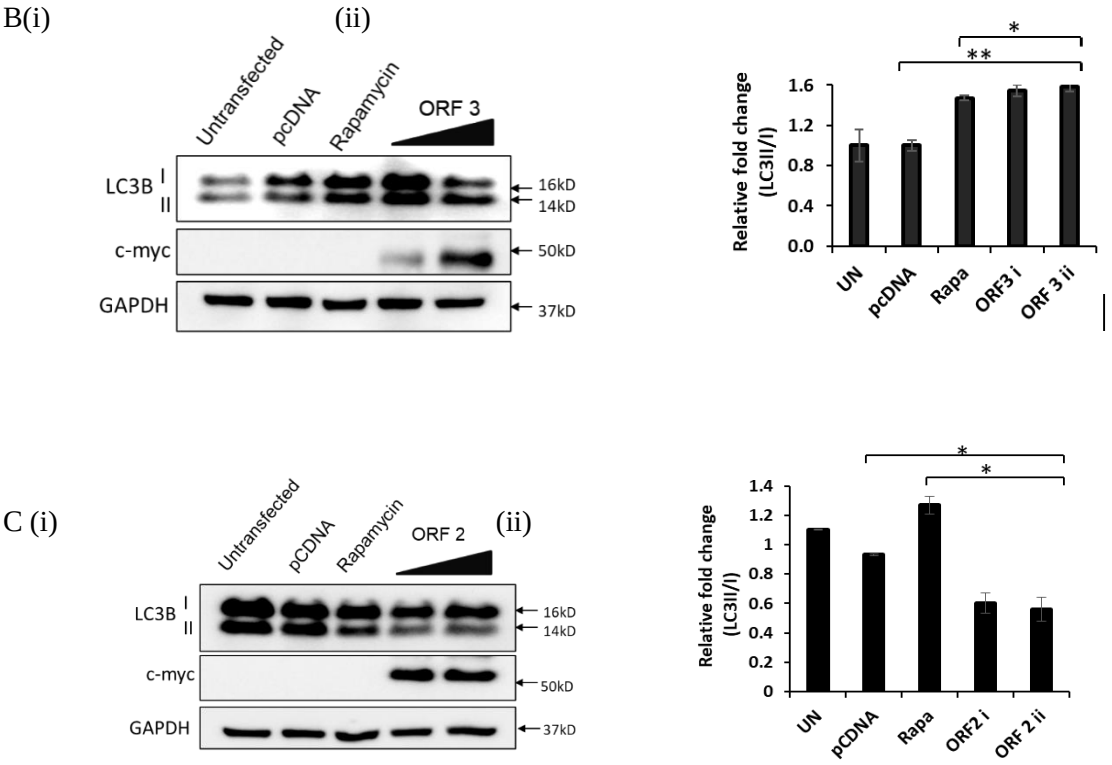


Fig 2: HEV ORF3 protein as key regulator for modulating complete autophagy. Immunofluorescence microscopy and colocalization, B) & C) Western blot of analysis of S10-3 cells transiently transfected with- ORF2 with c-myc tag or ORF3 with c-myc tag

Project 3: Assessment of risk factors for the development of Cardiovascular disease (CVD) in patients with active HCV infection

Investigators: Tomar SJ, Lole KS

Funding agency: ICMR (Extramural)

Duration: 2019-2022

***Background*:** HCV infection has been associated with increased risk of atherosclerosis, peripheral artery disease, myocardial injury, cerebro and cardiovascular events and increased mortality. Till date, most studies on cardiovascular risk and HCV were conducted on patients treated with IFN based regimens, which makes it impossible to differentiate whether the effects observed, are due to virus clearance or an effect of the IFNs themselves.

Aim: To assess risk factors of cardiovascular disease in patients with active HCV infection

Primary Objectives:

a) Assessment of various parameters as risk factors for CVD in patients with active HCV infection

b) To assess the association of metabolic syndrome and CVD risk

Secondary *Objectives*:

a) Association of different HCV genotypes to CVD risk

b) Association of Hepatitis C viral load with CVD risk

***Work done &Findings*:** Patient enrolment for was initiated on 1st March 2020 at NEIGRIHMS, Shillong and 27 treatment-naïve patients with active-HCV infection were enrolled till March 2021. Baseline clinical findings of active HCV patients and healthy participants are presented in Table 3. Active HCV patients had significant hypocholesterolaemia at baseline, and the HDL levels were much lower in comparison to healthy participants. The liver enzymes, aspartate aminotransferase (AST/SGOT) and alanine aminotransferase (ALT/SGPT) were significantly raised in HCV patients. There was no statistically significant difference seen in carotid-intima media thickness (CIMT), insulin resistance (HOMO-IR) and liver fibrosis indexes (FIB-4) values.

Table 3: Baseline clinical findings of patients with active HCV infection

Variables		Active HCV infection (n=12)			Healthy individuals at baseline (n=8)			p-value*
		Mean	SD	Median	Mean	SD	Median	
Lipid profile	Cholesterol	125.3	56.24	109.5	191.25	47.77	172	*0.01**
	HDL	31.9	10.4	31.5	47.2	11.1	43	*0.008**
Liver enzymes	AST	64.4	35.3	62.5	32.8	15.6	28	*0.007**
	ALT	77.16	43.8	67	34.3	16.1	30.5	*0.02**
PT		14.7	3.5	14.1	15.2	2.2	14.2	0.55
CIMT	RT	0.377	0.2	0.35	0.27	0.08	0.29	0.17
	LT	0.38	0.22	0.35	0.27	0.06	0.27	0.15
Fibroses indexes	APRI	1.05067	1.13443	0.59	0.41	0.21	0.35	*0.03**
	FIB-4	1.49	1.37	1.05	0.91	0.28	0.88	0.58
HOMA-IR		2.2	0.81	2.4	1.8	0.25	1.75	0.12

***p-value <0.05 = Statistically significant**

Table 4: Number of samples tested: April 2020 to March 2021

Sample tested for	Apr-20	May-20	Jun-20	Jul-20	Aug-20	Sep-20	Oct-20	Nov-20	Dec-20	Jan-21	Feb-21	Mar-21	TOTAL
HAV IgM	1	1	2	2	1	3	2	6	3	2	5	2	30
HEV IgM	1	1	2	2	1	3	3	5	3	2	6	2	31
HBsAg			1	1	2		2				1		7
HCV RNA				1			2						3
Anti-HBs											4		
Genotype											1		1
DCI sample	40	11	51	49	35	53	47	46	45	30	49	43	499
HBV DNA quant	2		4	2	3	3		7	8	6	10	5	50
PHC Savarde chiplun		6											6
Water sample (HAV&HEV RNA)	0	4	4	7	5	4	4	7	5	4	4	9	57
Covid-19 sample Navale hospital				30	41	0	15	22	41	21	23	21	214
Covid-19 transcriptome project													104
Total	44	23	64	94	88	66	75	93	105	65	103	82	902

INFLUENZA GROUP

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Komal	Staff Nurse	Deepak	Staff Nurse
Ashwani	Staff Nurse		

- Served as the National Apex laboratory for SARS-CoV-2 laboratory diagnosis
- Provided laboratory diagnosis services for COVID-19, influenza and other respiratory viruses.
- A total of 7301 clinical samples were tested using real time RT-PCR and 61 samples were found positive for influenza viruses. &93 samples were also tested for other respiratory viruses and found 12 samples positive.
- The Influenza A/H1N1/pdm09 circulating in 2019-20 showed a clade 3C.2a.1 genotype.
- Studies on prevalence of influenza in elderly showed incidence rate in ARI was 11.43 and 0.26 in ALRI per 1000 elderly per week.
- NIC activities on Global Influenza networking and WHO external quality assessment programs were carried out as mandated

Core Service Project: National Influenza Center activities
Investigators: Potdar V, Choushary ML, Sumeet B, Kaushal
Funding: Intramural
Duration: ongoing

Background: ICMR-NIV has developed and standardized a combo testing protocol to diagnose SARS CoV-2, Influenza A and influenza B viruses simultaneously and has been used since January 2021. A total of 7301 clinical samples were tested using real time RT-PCR and 61(0.83%) samples tested positive for influenza viruses [Influenza A/H3N2virus = 59 (0.8%), influenza A/H1N1pdm09=2 (0.02%).

***Work done*:** Representative influenza positive samples were processed for virus isolation in MDCK cell line (n=58) and yielded 32 H3N2 isolates. Phylogenetic analysis of HA gene (n=23) of influenza A/H3N2 virus showed circulating strains grouped in Clade 3C.2a1b; subclade 2a2. The newer delineated 2a2 subclade represented by A/Bangladesh/10006/2020 which share HA1 substitutions of Y159N, T160I (resulting in the loss of a glycosylation site), L164Q, G186D and D190N.These viruses are antigenically similar to A/Cambodia/e0826360/2020 (H3N2)-like virus which was 2021-22 northern hemisphere vaccine component (Fig 1). All the H3N2 viruses remained susceptible for Neuraminidase Inhibitor drug (Tamiflu).

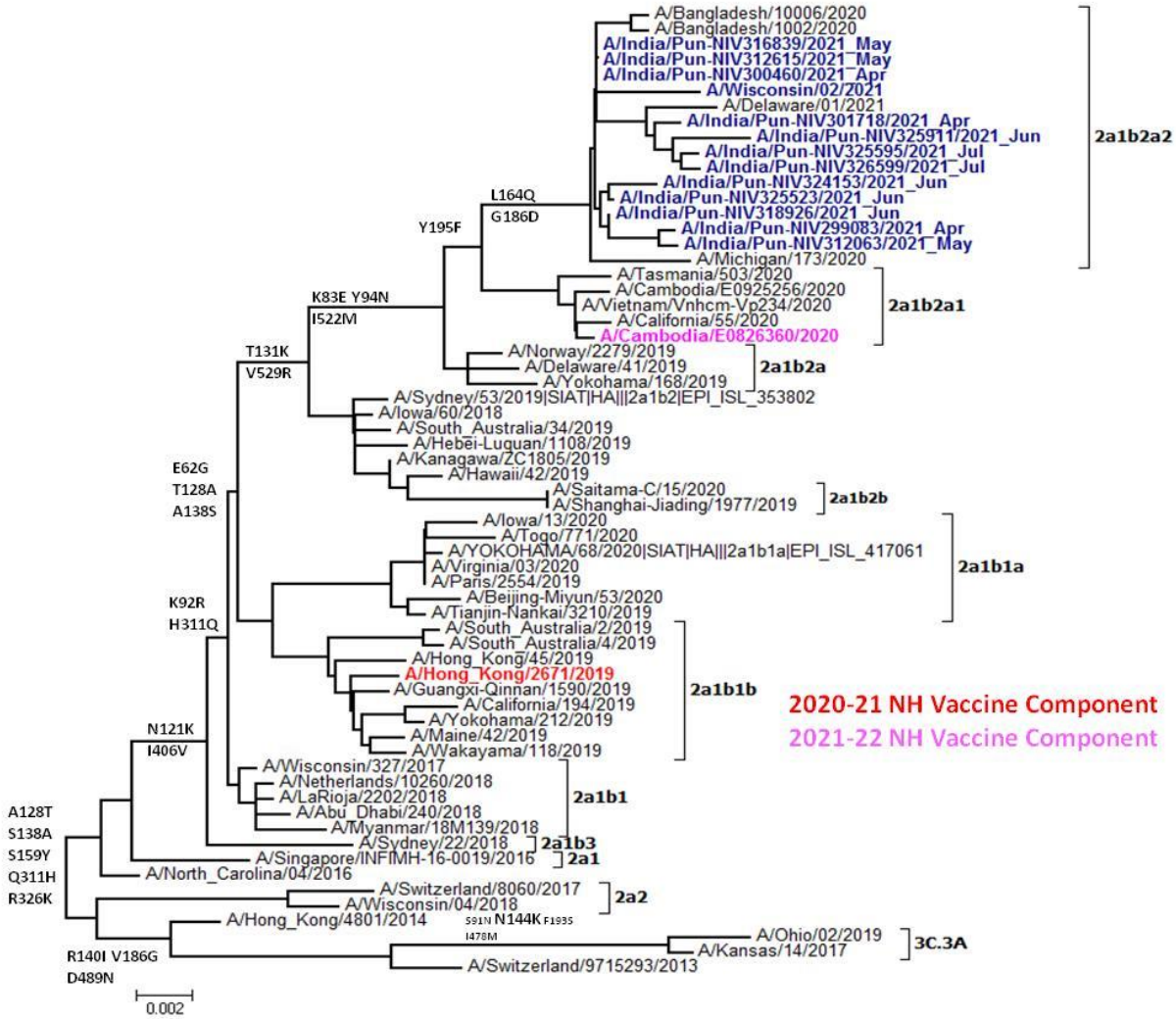

Fig 1: Phylogenetic analysis of HA gene of influenza A(H3N2) from the year 2020-21.

Project 2: Surveillance of respiratory viruses in Pune city
Investigators: Potdar VA, Choudhary ML & Bhardwaj SD
Funding Agency: Intramural
Duration 2020-2021

Background: Respiratory viral infections are a leading cause of disease and mortality and the severity can vary from mild upper airway infections to severe wheezing, bronchiolitis or pneumonia.
Objectives: To carry out epidemiological and virological surveillance for influenza and non-influenza respiratory viruses, *viz.*, RSV, hMPV, PIV, adenoviruses, rhinoviruses in Pune.
Work done: During this period, 2473 SARI patient's throat/nasal swab samples were tested for different respiratory viruses by duplex real time PCR. Weekly distribution of samples tested and positives were shown in Figure 2. Total 793 ILI cases from suspected COVID-19 referred samples were tested for influenza viruses and 12 samples found influenza positive (Figure 2).

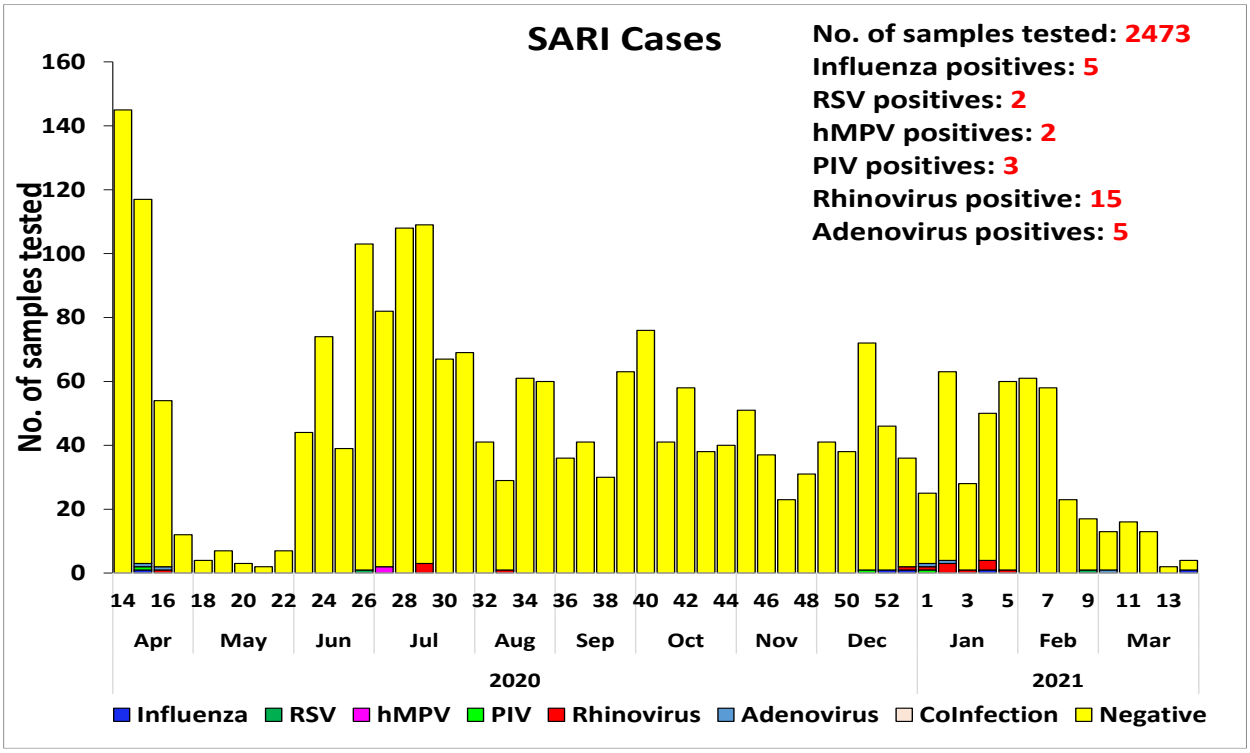

Fig 2: Weekly distribution of respiratory viruses in SARI cases from April 2020 to March 2021

Project 3: Strengthening/promoting evidence-based advocacy for influenza prevention and control in India
Investigators: Bhardwaj SD, Potdar VA
Funding Agency: CDC
Duration: 2018-2022
Background: Influenza studies in India to date have largely focused on children, and those conducted among older adults are limited in sample size and geographic spread. Hospital-based studies will likely underestimate the burden of illness among older adults as they are less likely to visit hospitals especially in low and middle-income countries, because of reduced mobility and other access-related issues.

Objectives: To determine the burden of illness for influenza and RSV among elderly (age ≥60 yr) people in India. Community-based surveillance platform provides the necessary population denominators for this purpose.

***Work done*:** Community-based surveillance is being conducted among an open cohort of 1046 elderly subjects to find the burden of illness for influenza and RSV in India. Trained project nurses conducted household surveillance five days a week to screen and enrol individuals for the presence of acute respiratory infection [ARI]. After 26 weeks of follow-up, 23452 [95.07%] house to house visits were conducted while due to COVID-19 restrictions, rest of the 26 weeks tele-surveillance was performed (Figure 3). The incidence rate was 11.43 in ARI and 0.26 in ALRI per 1000 elderly per week, however, none of the samples (n=50) tested positive for influenza or RSV.

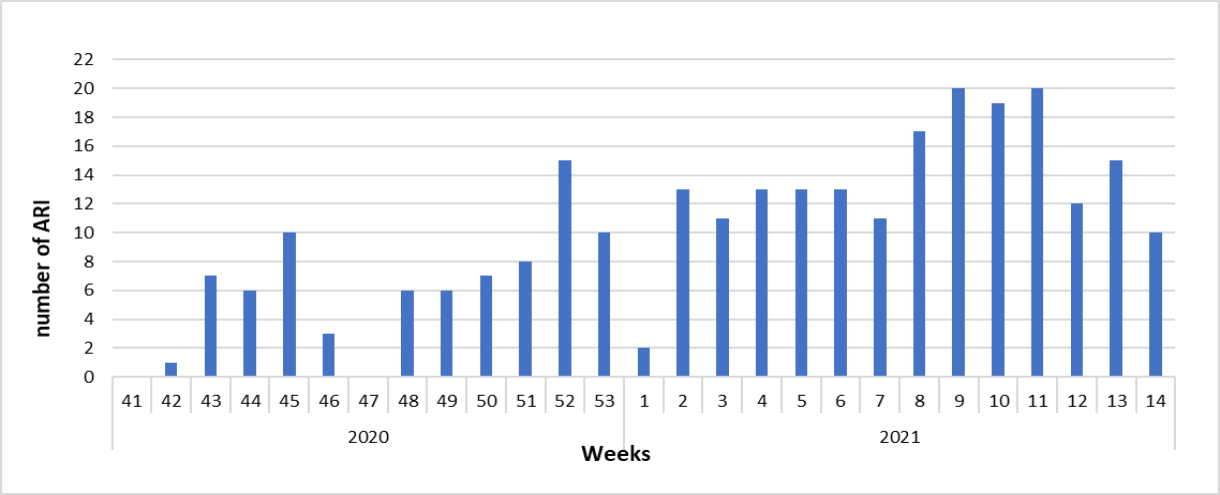

Figure 3. ARI distribution from April 2020 to March 2021 in the community cohort.

Project 4: Tracking community mortality due to respiratory syncytial virus in collaboration with University of Colorado and MAHAN Melghat

Investigators: Potdar VA.

Funding Agency: Bill & Melinda gates Foundation

Duration: 2020-2021

Background: RSV is a major cause of morbidity and mortality among children globally. There is insufficient data on RSV mortality in children below the age of 2 years in India.

Objective*:* To identify RSV associated mortality in infants/children below 2 years of age in Melghat, a tribal area in Maharashtra.

Work done: A total of 498 samples received from Melghat were tested for RSV and other respiratory viruses by qRT-PCR. RSV was detected in 37(7.4%) samples; PIV was detected in 24(4.8%) samples while Rhino and Adenovirus were detected in 30 (6%) and 7(1.4%) samples respectively.

Contribution to Global Influenza Network

Virological data for 15957 clinical samples including positives; 9 influenza A(H1N1)pdm09, 21 A(H3N2), 1 Influenza B, were submitted to Global Influenza Surveillance and Response System (FLUNETPLUS). Influenza isolates (n=19) (H1N1pdm09: 8, and H3N2: 11) were submitted to WHO CC, CDC, Atlanta.

WHO External Quality Assessment Programme (EQAP)

Panel number 19 (2020) for influenza A real time PCR from WHO, CHP Hong Kong was received; this contained 10 samples of A(H3), A(H5), A(H1)pdm09, influenza B and other Influenza A, the results were 100% concordant. We also participated in WHO external quality assessment system (EQAS) panel for detection of SARS-CoV-2 and have achieved 100% score.

NABL Accreditation: Real Time PCR test for influenza virus diagnosis has been assessed and accredited in accordance with the standard ISO/IEC17025:2005 in the discipline of biological testing by National Accreditation Board for Testing and Calibration Laboratories (NABL). Reassessment of the test has been conducted in June 2018 and certificate received with validity till Sep 2020.

DIAGNOSTIC VIROLOGY GROUP

Scientific Staff

Dr. Gajanan N Sapkal,	Scientist ‘E’ & Group Leader
Dr. Gururaj Rao Deshpande	Scientist ‘B’

Technical Staff

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Project Staff

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Mr. Chetan Patil	Technician C
Ms. Kajal Jarande	Technician C
Mrs. Roshni Patil	Technician C
Ms. Sanskruti Saka	Technician C
Mr. Prasad Gomade	Data Entry Operator

Core service project: Resource Centre for Virus Diagnostic research Laboratories (RCVRDL) at NIV, Pune

Investigators: Sapkal GN, Ullas PT, Deshpande GR, Potdar VA, Kaduskar O, Deshpande K, Gomade P, Phagiwala D, Patil R, Saka S.

Funding Agency: DHR/ICMR

Duration: Ongoing

***Background & Objectives*:** RCVRDL was created in response to the recommendations of the Virology Task Force monitoring the VRDL network in India, with an objective of providing training to different categories of staff in the existing and newly establishing VRDL network, conducting quality assurance (QA)/quality control (QC) programs as well as extending scientific and technical expertise.

Work done:

(i) Training:

- Conducted two day hands-on training program on laboratory diagnosis of COVID-19 by real-time RT-PCR for scientists and staff from National Center for Cell Sciences, Pune.
- Conducted a training program on viral diagnostics for a team of clinicians and laboratory staff from Molecular Diagnstics Laboratory, Sindhudurg Maharashtra on biosafety, biomedical waste management, ELISA and PCR.

(ii) Quality Control Program for VRDL

The RCVRDL continued to conduct Quality Control Programs of VRDLs in viral diagnostics. During the period, however, due to COVID-19 restrictions, only 04 VRDLs participated in the quarterly QA/QC program of serodiagnostic testing for 07 etiologies (Dengue, Chikungunya, Japanese encephalitis, Hepatitis A, B, Rubella, and Measles). A total of 168 samples were received and tested. The average concordance for all the participating centres for the year was found to be 100%.

(iii) External Quality Assurance (EQA) Program for VRDL

As part of continuous improvement program, prepared a plan, designed and performed experiments for executing EQA. Serum panel for detection of anti-dengue, chikungunya and Japanese encephalitis IgM by ELISA was distributed to 64 VRDLs across the country. The average concordance of test results was 98%. This reassured the quality of testing in pandemic situation for all the three parameters.

Project 1: Surveillance for Zika virus infections in India

Investigators: Sapkal GN, Yadav PD, Deshpande G, Ullas PT, Gunjikar RS, Shaman H, Patil C.

Funding agency: DHR/ICMR

Duration: 2019-22

Background: to bolster the National preparedness against Zika virus (ZIKV) disease, the RCVRDL has been assigned as the apex laboratory for coordinating ZIKV surveillance activities in India.

Objectives: Enhanced surveillance of Zika virus based on:

(i) Zika virus disease symptoms and case history in retrospective and prospective samples
(ii) Congenital Zika virus syndrome including microcephaly in infants

Work done: To accomplish continuous monitoring of ZIKV infection in humans in the country, supplied diagnostic reagents/kits to 35 network laboratories and shipped 10250 reactions. This ensured screening of 4200 serum samples however, all the samples tested Zika RT-PCR negative. Anti-Zika antibody screening has been initiated in dengue and chikungunya negative samples from selected VRDLs.

Project 2: ***Cohorts for Zika Epidemiology in India***
Investigators: Sapkal GN, Bhatnagar T, Malhotra B, Deshpande G, Ullas PT
Funding agency: ICMR
Duration: 3years 6 months
Background: A prospective cohort study was planned to understand the incidence and extent of fetal abnormalities as well as maternal clinical presentations among pregnant Indian women carrier of ZIKV and their uninfected counterparts. This prospective study was initiated within 6 months after the Zika outbreak in Jaipur in 2018.
Objective*:* To estimate the risk of adverse fetal outcome in ZIKV infected women compared to uninfected pregnant women.
Findings
Pregnant women cohort 1

Enrolment and follow-up were completed. Five deliveries were recorded in cohort 1.

Pregnant women cohort 2

Out of the 119 enrolled pregnant women, 104 and 90 were followed-up in the 1st and 2nd visits, respectively. In the second year, out of 119 tested samples from enrolment, two women (1.6%) tested positive for Zika anti IgG and anti IgM by $PRNT_{90}$. One sample was also positive for chikungunya whereas the other was positive for dengue, Japanese encephalitis (JE) and Chikungunya. Nine percent of women showed positivity for dengue in the enrolment and follow-up groups. Across enrolment and follow-up, 9-20% was positive for chikungunya, 9-17% for dengue and 10-18% for rubella. Cytomegalovirus and varicella zoster were deteted in 2% and 1% respectively while one each was positive for Herpes Simplex Virus -2 and toxoplasma.

	Enrolment (N=119)	**Follow up 1(N=62)**	**Follow up 2 (N=11)**	**Follow up 3 (N=8)**
Zika (PRNT)	2 (1.7)	2 (3.2)	1 (9.01)	3(37)
Dengue	11 (9.2)	6 (9.6)	1 (9.01)	0
Chikungunya	24 (20.2)	7 (11.3)	2 (18)	0
Japanese encephalitis	14 (11.7)	11 (17.7)	1 (9.01)	0
Rubella	13 (10.9)	4 (6.4)	2 (18)	0
Herpes Simplex Virus-2 (HSV-2)	2 (1.7)	0	0	0
Cytomegalovirus	3 (2.5)	2 (3.2)	0	0
Varicella zoster	2 (1.7)	0	0	0
Toxoplasma	2 (1.7)	0	0	0

Project 3: Impact of measles and rubella vaccination campaign on population immunity in India (IMRVI study)

Investigators: Sapkal GN, Tandale BV, Deshpande G, Ullas PT
Co-investigators: N Gupta, Sangal L, Murhekar M, Ahmad M, Hayford, Shete A, Moss W, Lessler J, Metcalf J, Ferrari M K.
Funding agency: ICMR (Extramural)/Johns Hopkins University

Duration: 2017-2020

Background: A community-based, cross-sectional sero-survey was undertaken among different age groups to estimate the seroprevalence to measles and rubella viruses in districts with MRHRUs and other priority areas. Also, a facility-based sero-survey from participants in a similar population (e.g., a representative convenience or easy access sample such as children at the outpatient department) will be assessed as control population.

Objectives*:*

a. To estimate age-specific population immunity to measles and rubella viruses within a specified precision of 10% within three age strata (children 9 months to 4 years and 5 to 14 years of age, and women 15 to 49 years of age) in India using serological surveys.
b. To compare accuracy, precision and cost of estimating the age-specific measles and rubella population immunity using convenience samples from health care facilities vs community-based serosurveys.

Work done*:*

A total of 1633 samples from pre and post campaign sites were retested for anti-measles IgG antibody. NIBSC 3rd International Standard for anti-measles (NIBSC code: 97/648) was procured for troubleshooting of results from new batches of Euroimmun measles IgG ELISA kits and linear equation was derived to normalize the difference obtained between kit lots. The derived equation was applied to Punjab and Assam post campaign survey samples.

Project 4: Congenital Rubella Syndrome (CRS) Surveillance in India

Investigators: Sapkal GN, Viswanathan R, Patil R, Murhekar M (ICMR-NIE Chennai)

Funding Agency: ICMR

Duration: 05 years

Background: In view of the proposed introduction of rubella vaccine in the national immunization program in the country, surveillance for CRS was established to provide a baseline estimate of disease burden and also to help monitor the impact and progress made by rubella vaccination. Based on the guidelines outlined in the strategic plan for Measles and Rubella elimination in Southeast Asia region, six sentinel sites were established in India. Periodic sero-surveys among pregnant women attending selected antenatal clinics in areas where MMR vaccine is in use, is also envisaged as part of the strategy.

Objectives:

(i) To establish a facility-based surveillance for CRS in selected medical Colleges/hospitals in different parts of country to monitor the time trends of the disease.
(ii) To conduct periodic serological surveys to monitor the rubella sero-surveillance among pregnant women over the time.

Findings

Quality Control testing of 292 serum samples received from 9 sentinel sites showed concordance at 91.81 and 95.61 for anti-rubella IgM and IgG antibodies respectively. Of the 226 throat swabs received from suspected rubella patients, 103 tested Negative in diagnostic rubella RT-PCR.

Project 5: Development of serodiagnostic assays for Nipah Virus

Investigators: Sapkal GN, Yadav P, Deshpande GR, Srivatsav R

Funding Agency: DHR

Duration: 2018-21

***Background*:** Nipah virus (NiV) activity was detected in the country since 2018 after a gap of 11 years and the Kozhikode outbreak was massive with case fatality rate of 89% and created waves of threat and panic

across the country. Accurate diagnosis is critical for providing appropriate care in infectious diseases and to break the chain of transmission. Recent studies by ICMR-NIV, Pune have confirmed the role of the *Pteropus* bats in the transmission of NiV.

Objectives:

1. To develop anti-NiV IgG antibody detection ELISA for screening human, bat and swine serum samples using characterized monoclonal and polyclonal antibody anti-Nipah Human IgM antibody detection.

Findings: Generation of Hybridoma

Spleen cells from NiV immunized mice were fused with myeloma (SP2/O) cells and the hybrids were cultured in DMEM with 10% FBS supplemented with 1X HAT medium for 3 weeks. After 3 weeks of HAT selection procedure, hybridomas were supplemented with 1X HT in DMEM with 10% FBS for 3 subsequent weeks. After this period, 15 hybridoma clones were selected.

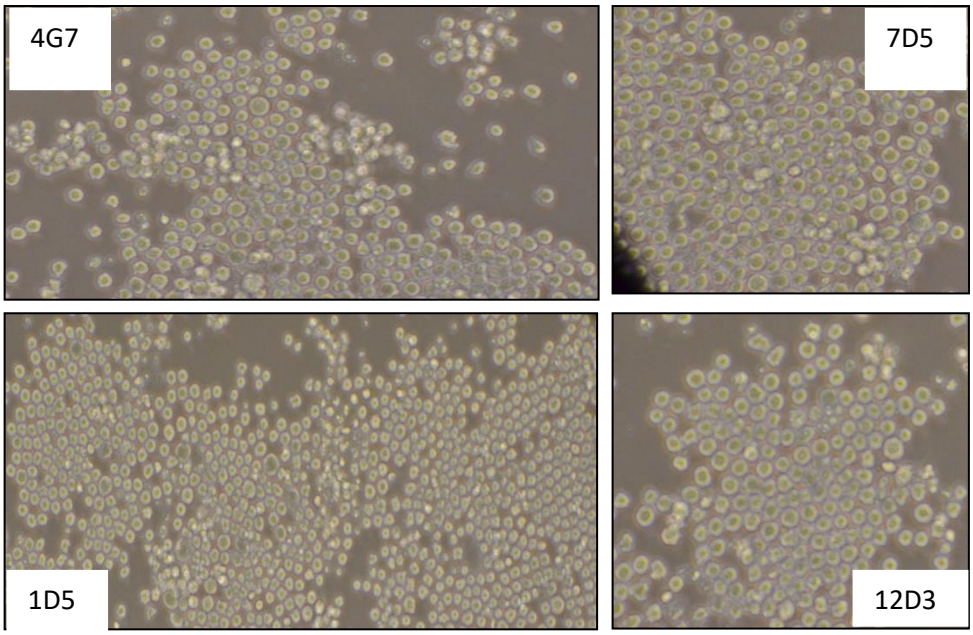

Fig 1: Microscopic images of Nipah hybrid clones (20x) generated during the study

POLIO VIROLOGY GROUP

(Former Avian Influenza Group)

Scientific Staff

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Dr. Shailesh D. Pawar	Scientist E (Nodal Officer for Polio Essential facility-PEF)

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Dr. Vaishali S Tatte	Senior Technical Officer-1
Mr. Sachin S. Keng	Technical Officer-B
Mr. Dinesh K. Singh	Senior Technician-2
Mr. Rameshwar P Khedekar	Technician-3
Mr. Javvaji P. N. Babu	Technician-2
Mr. Ratnadeep More	Technician-1
Ms. Vaishnavee Bagde	Technician-1

Research Scholar

Ms. Rewati Kasbe	Ph.D. Fellow (UGC)

Project Staff

Ms. Deeksha S. Tare	SRF (ICMR)
Ms. Jyoti Chamale	JRF (DST)

Core service Project: ***Operation and maintenance of High Containment Laboratory***
Investigators: *Mullick J, Mathapati BS, Khare AB, Singh DK, Keng S, Babu JPN*
Funding Agency: Intramural
Duration: Ongoing
The High Containment laboratory (BSL-3) is a specialized laboratory designed to work on high risk pathogens, providing protection to workers and the community. Under the current COVID-19 pandemic situation, experiments on validation of ozone, negative ion and UV based technologies for inactivation of SARS CoV-2 from surfaces of the personal protective equipment were conducted. The studies were done at the animal BSL-3 laboratory as the BSL-3 laboratory has been designated as Polio Essential Facility (PEF) and are under consideration for modification as per Global action plan III (GAPIII). Though PEF was shut down for modification as per the GAP III guidelines, surge of SARS CoV-2 and the pressing needs of the country to evaluate antivirals against SARS CoV-2 virus, the designated PEF was used to conduct antiviral testing of potential antiviral compounds as per the instructions of ICMR.

Project 1: Development of Polio Essential Facility in line with the Global Action Plan III at ICMR-NIV, Pune to support work on Polio
Investigators: *Pawar SD, Mullick J, Mathapati B, Khare AB, Kode SS, Tatte VS, Keng SS, Singh DK, Khedekar RP, Babu JPN, More R, Bagde V*
Funding agency**:** Intramural.
Duration**:** 2019-2022

The competent authority has decided to develop a Poliovirus Essential Facility (PEF) at the ICMR-NIV, Pune and designated Dr. Shailesh D Pawar as the 'Nodal Officer' by Director, ICMR-NIV and ICMR HQ. Due to COVID-19 pandemic restrictions, facility work and training could not progress as per the timeline. However, preparation of risk assessments, executive procedures, policies, lists, etc in addition to regular PEF core committee meetings to assess the progress in documentation and assessment of the bottlenecks were done. Highlights of the activities are as below.

- Visit of Lt. Col. Ambrish Kumar, ARDE with regard to CCTV ~~advice~~.
- Reviewing of the inventory of poliovirus material stored at the designated PEF was conducted biannually.
- Documents were also prepared as asked by the GCC—CWG for assessing the CP application.
- PEF External Expert Engineering Committee Meeting was held online on 17th December 2020 and visit to evaluate the PEF took place on 28th & 29th Dec 2020.
- Biorisk management Committee (PEF) meeting for discussing and implementation of the identified jobs within the time-frame, was held on 20th January 2021
- Technical evaluation of equipment required in PEF, opening of BID, etc. were completed.
- Compilation and preparation of Master List of records for PEF.

List of facility and documentation work was completed. Various PEF committees formed had meetings to move ahead.

Project 2: ***Gene pool analysis of highly pathogenic H5N1 and low pathogenic H9N2 avian influenza viruses isolated from India***
Investigators: *Pawar SD, Tare DS (ICMR-SRF)*
Funding: *ICMR, SRF* grant to Deeksha Tare (VIR/Fellowship/1/2008-ECD-I) starting 17th August 2018
Project duration: August 2018 to August 2021

Background: Low pathogenic avian influenza (LPAI) H9N2 viruses are also cause human infections and have pandemic potential. LPAI-H9N2 viruses are prevalent in poultry in India and the first human case of LPAI-H9N2 in India was reported in 2019. There is no data on the gene pool analysis and genetic characterization of LPAI-H9N2 viruses from India.

Objectives*:*

(i) Gene pool analysis of avian influenza viruses isolated from India
(ii) Full genome sequencing and characterization of LPAI and HPAI viruses isolated at ICMR-NIV

Findings*:* Phylogenetic analysis sholwed LPAI-H9N2 viruses isolated from India in 2015 and 2017 formed a separate cluster for all genes along with the human LPAI-H9N2 virus and found related to viruses from Bangladesh, Pakistan and Iran. The internal genes bore resemblance to HPAI-H7N3 and H5N1 viruses. Substitutions associated with increased virulence, mammalian adaptation and high polymerase activity were observed. These findings suggest that there are independent introductions and continuous evolution of LPAI-H9N2 viruses towards mammalian adaptation posing a potential risk, underscoring the need of rigorous surveillance.

NABL (National Accreditation Board for Testing and Calibration Laboratories) Accreditation of human Influenza, Avian Influenza, Maximum Containment Laboratories and Engineering Support Group as per ISO/IEC 17025:2017.

As a WHO approved research and diagnostic centre, ICMR-NIV is maintaining the Quality standards as per the ISO/IEC 17025:2017 guidelines since 2016.

Salient features

- Conducted internal audits of Human influenza, MCL, AI, Engineering Support and Diagnostic Virology Groups as per ISO/IEC 17025:2017 guidelines, May 2020, Sept 2020.
- Conducted two Management Review Meetings with the Top Management, 21/5/2020, 18/9/2021.
- Revised the Laboratory Quality and Executive Procedure Manuals, as per ISO/IEC17025:2017 guidelines.
- Conducted meetings in connection with the forthcoming NABL transition audit of the laboratories at ICMR-NIV, Pune, 6 Aug 2020.
- Organized and executed NABL Transition audit of ICMR-NIV as per ISO/IEC 17025:2017 guidelines, 27/8/2020.
- NABL granted continuation of accreditation to ICMR-NIV as per ISO/IEC 17025:2017.

VIRUS REGISTRY AND VIRUS REPOSITORY

Scientific Staff

Dr. Sunil R. Vaidya, Scientist-E

Technical staff

1. Mrs. Neelakshi S. Kumbhar, Sr. Technician-3
2. Mr. Madhukar B. Kamble, Sr. Technician-1
3. Mrs. Divya R. Bhattad, Technician-C
4. Mr. Sarang S. Kamble, Lab Assistant-1
5. Mr. Roben P. George, Lab Assistant-1 (Transferred to NARI on 25th March 2021)
6. Mr. Raju M. Rahurkar, Lab Assistant-1
7. Mrs. Surekha S. Mohol, Lab Assistant-1

Project staff

1. Ms. Payal Kelkar, M. Sc. Virology student

Service project: ***Outbreak Investigations/ Diagnostic services***
Investigator: Vaidya SR
During the reporting period, laboratory diagnostic support for measles and varicella were provided to local hospitals and Primary Health Centers (PHC). Of the 35 sera/CSF, samples referred by local hospitals and PHCs for measles virus (MeV) IgM diagnosis, two were found positive for measles virus specific IgM antibodies whereas all five specimens referred from Akola district (MS) were found positive for VZV IgM antibodies. Laboratory reports were sent to the concerned official for undertaking necessary action.

Project 1: Genetic and antigenic characterization of measles, mumps and rubella virus isolates
Investigator: Vaidya SR
Funding: Intamural
Complete genome sequencing of measles virus isolates from India: As part of the project**,** 15 MeV isolates obtained from Maharashtra, Uttarakhand, Odisha, Gujarat, Tamil Nadu and Union territory of Dadra and Nagar Haveli during 2006-2017 sequenced during the year (Table 1). Twelve isolates belonged to D8 genotype while 03 belonged to D4. All 15 MeV isolates showed standard genome length except two D4 isolates (15900 nucleotides) with 96.54 to 97.04 % match to MeV Edmonston vaccine strain. Of the 3-D4 isolates, Pune (2014) and Jamnagar (2014) isolates were clustered in D4.1 and Perambalur (2007) in D4.2 sub-lineage. All D8 isolates were sub-clustered into D8.1 and D8.2 (Figure 1). Krishanagiri (2006) and Villupuram (2006) isolates are found to join as outermost node of the D8 lineage. All 15 MeVs showed Q315 mutation in a loop epitope of H-protein but not in previously reported 43-MeVs. A seven-nucleotide insert of 'CCCCCCC' in M-gene and deletion of one nucleotide in F-gene was evident in 2-D4 isolates. This has been reported earlier from Italy, USA,

Table 1: Details of 15 MeV isolates during the year.

MeV isolate	Isolation source	Age #	Sex	MeV isolate (GenBank ID)	Year of isolation	Place, State/ UTs
1	TS	4	F	MVi/Pune.IND/10.13/1 (MW916919) D8	2013	Pune, Maharashtra
2	TS	3	F	MVi/Haridwar.IND/06.14/3 (MW916922) D8	2014	Haridwar, Uttarakhand
3	TS	15	F	MVi/Sundargarh.IND/10.14 (MW916921) D8	2014	Sambhalpur, Odisha
4	TS	11	M	MVi/Pune.IND/16.14 (MW916928) D4	2014	Pune, Maharashtra
5	TS	10	M	MVi/DadraandNagarHaveli.IND/18.15 (MW916924) D8	2015	Silvassa, Dadra and Nagar Haveli
6	Urine	2.6	M	MVi/Jamnagar.IND/18.15 (MW916925) D8	2015	Jamnagar, Gujarat
7	Urine	7	F	MVi/Jamnagar.IND/18.15/2 (MW916926) D8	2015	Jamnagar, Gujarat
8	Urine	5	F	MVi/Jamnagar.IND/23.15 (MW916920) D8	2015	Jamnagar, Gujarat
9	Urine	6	M	MVi/Daman.IND/7.15 (MW916923) D8	2015	Daman and Diu

10	TS	4	M	MVi/Anand.IND/33.15/2 (MW916927) D8	2015	Anand, Gujarat
11	Urine	5	F	MVi/Jamnagar.IND/4.17/1 (MW916929) D4	2017	Jamnagar, Gujarat
12	TS	4	M	MVi/Krishnagiri.Ind/12.06 (MW916930) D8	2006	Krishnagiri, Tamil Nadu
13	TS	16	M	MVi/Villupuram.Ind/13.06 (MW916931) D8	2006	Villupuram, Tamil Nadu
14	TS	4	F	MVi/Villupuram.Ind/03.07 (MW916932) D8	2007	Villupuram, Tamil Nadu
15	Urine	7	M	MVi/Perambalur.Ind/17.07 (MW916933) D4	2007	Perambalur, Tamil Nadu

[#]Age in years, TS- Throat swab; TCF- tissue culture fluid.

Spain, Croatia, Netherlands, UK, Canada and India. The nucleotide divergence was found to be higher for M-F (D4; 6.2%, D8; 3%) compared to H (D4; 1.5%, D8; 1%) and N (D4; 1.4%, D8; 1.8%) genes. Present study reconfirms the prevalence of two lineages of D4 and three lineages of D8 in India and warrants complete genome sequencing to track transmission of MeVs.

Measles M/F gene sequencing on isolates and clinical specimens: During the reporting period, 41 M/F gene sequences were generated from clinical specimens received from Gujarat (17), Maharashtra (12), Tamil Nadu (4), Chhattisgarh (2), Uttarakhand (2) Odisha (1), Rajasthan (1), and Union Territories of Dadra and Nagar Haveli (1) and Daman and Diu (1) during 2008 and 2017. Multiple sequence alignments of three separate datasets *viz*., N-450, M/F intergenic region and H gene were carried out independently for all the MeV isolates. Phylogenetic trees were reconstructed using maximum likelihood methods as implemented in IQ-TREE with 100 bootstrap replicates. Trees were visualized using an iTOL viewer. Mean nucleotide diversity for each of these datasets was carried out using Nei and Kumar's diversity metrics as implemented in MEGA X. Based on the diversity metrics and percent conserved residues (Table 2), we observed that M-F intergenic region is a better marker for genotyping of MeV as compared to N450 as well as H-gene.

Table 2: Nucleotide diversity in N, M/F and H genes of Measles virus.

Sequence dataset	% Conserved nucleotides	Mean nucleotide diversity
N-gene (N450)	76	0.04
M/F intergenic region	40	0.08
H-gene	81	0.02

Genetic and antigenic characterization of rubella virus isolates: Rubella is a contagious disease caused by Rubella virus (RuV) that manifests as fever with skin-rashes in children and adults along with complications in pregnant women. WHO-SEAR has set a target for Rubella elimination by 2023. This is the first report of antigenic characterization and genome sequencing of nine RuVs sampled during 1992, 2007-9, and 2015-17 from four Indian states. Comparative analysis of Indian RuVs (2B) with that of global isolates and vaccine strain RA 27/3 (1a) revealed that the observed mutations in structural proteins have no major impact on the 3D structure, function and antigenicity. Indian RuVs formed three major clusters (Pune-1992, Kannur-2009 and Chitradurg-2007) in genome-based phylogeny. Neutralizing antibody titers in a panel of serum samples (non-measles cases) were significantly higher to the vaccine strain compared to a wild-type 2B isolate (Kannur) with concordance of 91.9%, thereby substantiating the use of current vaccines.

Project 2: Measurement of virus specific IgM, IgG and neutralizing antibody levels in suspected Measles and Rubella cases

Investigator: Vaidya SR

Comparative serological results in fever with skin rash cases: Screening of 300 sera samples referred for measles and rubella diagnosis revealed 42% and 17.3% positive for measles IgM and rubella IgM antibodies respectively. Measles virus specific IgG antibodies were detected in 82.6% samples, whereas 97.3% samples showed neutralizing antibodies. Rubella virus specific IgG antibodies were detected in 59.3% samples while 69% samples showed neutralizing antibodies. As expected, a higher number of cases with fever and rash were confirmed as measles compared to rubella. Interestingly, a large number of suspected cases showed presence of neutralizing and total IgG antibodies for both the viruses. Additional data/ statistical analysis are in progress.

COVI-19 support

During COVID-19 pandemic, Virus Registry and Virus Repository (VRVR) staff (n=2) was involved in supply of kits/ reagents, packing and dispatch to various laboratories across the country between April 2020 to October 2020. Altogether, 57 lacs SARS CoV-2 real time RT-PCR reactions/ kits were supplied to various states and union territories. In addition, one staff was involved in molecular and VTM kit evaluation activities. For supporting centralized depot activities for the state of Karnataka, Dr. Vaidya was transferred to NIV Bengaluru unit for a period of three months (May-2020 to July-2020).

Virus Registry Activity

- During the reporting period, various clinical specimens (n=1485) were referred to NIV Pune through Virus Registry for the diagnosis of dengue (n=561), Chikungunya (n=331), Kyasanur Forest Disease (398), Crimean Congo Haemorrhagic fever (n=1), Japanese encephalitis-Chandipura/ Cytomegalovirus/Varicella (n=104), Hepatitis (n=50) and Measles/Varicella (n=40) viruses.
- Request for procurement of prototype viruses from various institutes/ organizations were received and processed as per the standard protocol of NIV Pune. Dengue serotype-2 (NIV strain: 803347) and serotype-4 (NIV strain: 611319) and Chikungunya (NIV strain: 61573) strains were supplied to ICMR-Virus Control Research Centre, Puducherry. Herpes Simplex Virus-1 (NIV strain: 0116209) passaged in BHK-21 cells was supplied to ICMR-National Institute for Research in Reproductive Health, Mumbai.
- As per ICMR headquarters initiative, involved in the collaborative project title ‘Establishment of a network of ICMR-COVID-19 biorepositories in India’. The ICMR sanctioned this project.

NIV Field Units

BENGALURU UNIT

Scientific staff

1.	Dr. Ashok M	Scientist-B & Officer-In-Charge

Technical staff

2	Mr. Madhava Rao	Technical Officer-A
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10	Mr Srinivas Vilasagaram	Technical Assistant
11	Mr. R.Basavaraju	Assistant
12	Kum. J. Jayajyothi	Assistant
13	Mr. Arjun Jogangari	MTS (Maintenance)
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18	Mis. Mala D	Lab Technician-C
19	Mr. Kiran Kumar	Lab Technician-C
20	Mr. Madhu	Lab Technician-C
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22	Mis. Krishnaveni	Lab Technician-C
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30	Mrs Bhuvaneshwari	Technical Assistant
31	Mr Jagan	Lab Technician-C
32	Mrs Hemalatha	Lab Technician-C

Project 1: AFP Surveillance (WHO-SEAR Polio Lab Network in the WHO's Global Eradication of Poliomyelitis Programme)

(i) Surveillance of Acute Flaccid Paralysis (AFP) cases from Karnataka, Kerala and Bihar
(ii) Intratypic differentiation of poliovirus isolates from AFP cases received from National Polio laboratories across the country.
Investigators: Ashok M, Rao M
Funding Agency: World Health Organization
Duration: Service project (since 1997)

***Background*:** Acute flaccid paralysis surveillance for global eradication of poliomyelitis programme was initiated by Govt. of India in collaboration with WHO/WHO-SEARO/WHO-NPSP. India has one specialized and seven national laboratories. NIV-BU is one of the National Polio Laboratories (NPL) and is playing an important role in polio eradication by laboratory investigation of AFP samples from Karnataka, Kerala, Uttar Pradesh and Bihar since 1997.

Objectives: Surveillance for polio virus in referred samples and interruption of Polio virus transmission
Findings: During the year, received 3184 samples from Bihar (n=2004, 63%), Karnataka (n=804, 25%) and Kerala (n=364, 11.5%). NPEV (RD cell line positives) accounted for 18% (n=568) while L20B cell line positives accounted for 3.2% (n=104) and all were positive for Sabin like 1 & 3. Reports of 95.63% samples were communicated within 14 days of receipt (Table 1).

Table 1. Summary of AFP samples investigated by NIV BU during April 2020 to March 2021

Region	**No. cases**	**No. Sample**	**L20 B**	**NPE V**	**ITD**				**VDPV**		
					S1	**S3**	**S1 + S3**	**NPEV PCR**	**S1**	**S3**	**S1 + S3**
Karnataka	402	804	18	115	0	14	4	0	0	14	4
Kerala	179	364	5	6	2	3	0	0	2	3	0
Bihar	1011	2004	81	447	24	30	28	1	24	30	28
Others	6	12	0	0	0	0	0	0	0	0	0
Total	**1598**	**3184**	**104**	**568**	**26**	**47**	**32**	**1**	**26**	**47**	**32**

NPEV-Non Polio Entero Viruses; S1-Sabin like 1; S3-Sabin like 3; VDPV – Vaccine Derived Polio Virus

Project 2: ***Environmental surveillance of sewage samples from Bangalore city as a part of WHO-SEAR Polio Lab Network in the WHO.***
Investigators: Ashok M, Prema
Funding Agency: World Health Organization
Duration: Since January 2019

***Background**:* Environmental surveillance of Polio virus in sewage samples is a part of Global Eradication of Poliomyelitis Programme. In Bangalore city, four sewage sites have been identified for this purpose.
Objectives: To identify Polio virus in sewage plants in Bangalore city.
***Findings**:* ICMR-NIV-BU received 120 sewage samples from four sewage zones of Bangalore city and 74% (n=89) samples tested positive in RD cell line while 20% (n=31) tested positive in L20B cell line. Among the L20B positive samples, 28 were positive for Sabin-like 1 & 3, while two samples were NEV and one tested positive for NPEV by PCR (Table 2).

Table 2. Summary of EV samples investigated by NIV BU

Site name	No. Sample	L20B	NPEV	ITD				VDPV		
				S1	S3	S1 + S3	NPEV PCR	S1	S3	S1 + S3
V Valley	30	10	20	2	6	2	0	2	6	2
HBL	30	05	25	1	2	2	0	1	2	2
KCV	30	07	23	1	1	3	1	1	1	3
RCL	30	09	21	3	5	0	1	3	5	0
Total	**120**	**31**	**89**	**7**	**14**	**7**	**2**	**7**	**14**	**7**

NPEV-Non Polio Entero Viruses; S1-Sabin like 1; S3-Sabin like 3; VDPV – Vaccine Derived Polio Virus

Project 3: Surveillance of Measles in Karnataka and Kerala States as part of WHO-SEAR Measles Laboratory Network in the WHO's Global Measles Elimination Programme.

***Investigators**:* Ashok M, Manjunath MJ, Vilasagaram S
***Funding Agency*:** World Health Organization
***Duration**:* **S**ervice project (since 2006)

***Background*:** India has the largest number of measles & Rubella cases at the global level despite both being vaccine preventable diseases. Hence, WHO-SEAR Measles laboratory network (NML) and MeaslesNetIndia network was established and is being expanded in a phased manner. NIV- Bangalore unit is one of the 19 functional WHO-SEAR national laboratories in India.
Objectives: To eliminate Measles & to control Rubella virus from India
Findings: During the reporting period, 736 serum samples received from different parts of Karnataka was screened that revealed 32 and 62 samples positive for Measles and Rubella IgM antibodies respectively. Screening of 198 throat swab samples for Measles and Rubella RNA yielded one positive sample for Measles virus (D8 genotype).

Project 4: National Vector Borne Disease Control Program (NVBDCP) for human and mosquito samples

(i) Surveillance of dengue, chikungunya and Japanese encephalitis cases in Bangalore city, urban, rural and neighbouring areas under NVBDCP.

***Investigators**:* Ashok M, Manjunath MJ, Vilasagaram S
***Funding Agency*:** NVBDCP
***Duration**:* Service project (since 2010)

***Background**:* NVBDCP is an umbrella programme for prevention and control of vector borne diseases in India and the NIV-BU covers Japanese encephalitis, dengue, & chikungunya surveillance. Transmission of vector borne diseases depends on prevalence of infective vectors and human-vector contact, which is further influenced by various factors *viz.*, climate; sleeping habits of humans, vector density etc. The unit is also one of the 32 sentinel surveillance centers for dengue & chikungunya identified in Karnataka.
Objectives: Serological investigation of dengue & chikungunya infected cases from urban and rural areas of Bangalore district.
***Findings**:* During the reporting year, 233 samples were received from Bangalore and nearby districts. Screeing of the samples showed 40 and 32 samples positive for anti-dengue and anti-CHIKV IgM antibodies. Dual infection of both the viruses was detected in 04 samples.

(ii) National Vector Borne Disease Control Program (NVBDCP) for mosquito samples

Surveillance of dengue, chikungunya and Japanese encephalitis virus in mosquito samples from Bangalore city, urban, rural and neighbouring areas under NVBDCP.

As part of the arboviral surveillance in Bangalore and adjoining districts, Bangalore unit is also identified as state laboratory to test mosquito samples for detection of arbo viruses.
Conventional PCR investigation of dengue, chikungunya and JE viruses in mosquito samples collected from urban and rural areas of Bangalore district.During the year, 348 mosquito pools received from various districts of Karnataka state was tested for JE, dengue and chikungunya virus. Screening of 159 *Aedes* pools by conventional PCR revealed 11 pools positive for dengue virus. Chikungunya was totally absent. JEV RNA was detected in one pool out of 189 *Culex* mosquito pools tested.

Project 5: Congenital Rubella Syndrome (CRS) Surveillance

Rubella is a vaccine preventable disease. In India, reliable estimates of CRS burden are not available and WHO has recommended options for assessing the disease burden as (A) establishing nationwide CRS surveillance to estimate the disease (B) investigating rubella outbreaks by time, place and person and (C) conducting sero-surveys to document population immunity. The CRS surveillance focuses on identifying infants 0-11 months of age with CRS attending health facilities and testing these infants for rubella infection.To establish a facility-based surveillance for CRS in selected medical colleges/hospitals in different parts of country to monitor the time trends of the disease. NIV-BU received 74 samples fulfilling case definition for CRS from IGICH hospital. Tests revelated 02 samples positive for IgM and 10 for IgG antibodies to Rubella virus. Thirty two throat swabs were also tested for Rubella RNA by conventional PCR but with negative results.

Project 6: Laboratory investigation of severe acute respiratory infection (SARI) cases

Investigators: Ashok M, Manjunath MJ
Funding Agency: Intramural
Duration: Ongoing project (Since September 2017)

***Background*:** SARI is defined primarily by clinical, radiological and/or histopathological evidence of pulmonary parenchymal disease (e.g., pneumonia, pneumonitis, or Acute Respiratory Distress Syndrome [ARDS]), typically associated with hospitalization, intensive care unit management and/or other severity marker (such as death). There are numerous pathogens that cause SARI, including but not limited to novel influenza viruses and other respiratory viruses.
Objectives: To investigate SARI cases using real time RT-PCR for 13 viruses
Findings: 1481 samples of SARI were received for Influenza A PCR testing. Influenza A RNA was detected in 20 samples and all of them belonged to H3N2 subtype.

Virus Research Diagnostic Laboratory Network projects

Department of Health Research (DHR) and ICMR have established Virus Research and Diagnostic Laboratory Network (VRDLN) to strengthen the laboratory capacity in the country for providing timely diagnosis of disease outbreaks. VRDLs, which follow a uniform protocol for laboratory testing, for various viral aetiologies *i.e*., (hepatitis: hepatitis A, B, C & E); arboviruses: JE, West Nile, dengue, chikungunya, Chandipura, Kyasanur Forest Disease; respiratory viruses (influenza, parainfluenza, RSV, adenovirus,

rhinovirus); fever with rash (measles, rubella, varicella zoster, mumps and parvovirus B 19); herpesvirus family (EB virus, herpes simplex virus and cytomegalovirus); enteric viruses (rotavirus, enteric adenoviruses, norovirus and astrovirus).

The main objectives of the program are **(**1) To create infrastructure for timely diagnosis of viruses and other agents, causing significant morbidity at public health level and specifically agents causing epidemics and/or potential agents. (2) To develop capacity for identification of novel and unknown viruses and other organisms, emerging and re-emerging viral strains and develop diagnostic kits. Findings of projects that were in collaboration with VRDLN network are: **(**i) Monitoring of dengue virus and detection of serotypes circulating in India, genotype and lineages utilizing viral research & diagnostic laboratories network.

One hundred and sixty four samples (NS1 positive by ELISA) received from 12 districts of the state were processed for dengue serotyping by real time PCR. Studies revealed predominance of dengue serotype 2 (n=78). Dengue 1 & 3 represented two each. Presence of dual serotypes was also detected; seven with dual serotypes of DEN 2&3 while one with DEN 1 & 2.

Testing for Scrub Typus for Acute Encephalitis Syndrome (AES) and Fever Rash Syndrome (FRS)

A total of 59 samples fulfilling criteria for Scrub Typus was tested for Scrub typhus IgM antibodies and 43 found positive.

Table: Number of samples tested

Sl No	Viruses	Total samples	Sample Type	Virus Isolation	Serology Positives	Molecular Positives
1	SARS CoV-2	320942	Throat/Nasal swab	-	-	18903
2	SARS CoV-2	1861	Serum	-	361 IgG	-
3	Polio	3184	Stool	NPEV-586 L20B-104	-	104 for Sabin Like 1&3
4	Polio	120	Sewage	NPEV-89 L20B-31	-	28 for Sabin Like 1&3
5	Measles/Rubella	736	Serum	-	32 Measles IgM 62 Rubella IgM	-
6	Measles/Rubella	198	Throat swab	-	-	One D8 Measles genotype
7	Dengue/Chikungunya	233	Serum	-	40 Dengue IgM 32 Chikungunya IgM 04 Dengue & Chikungunya IgM	-
8	Rubella	74	Serum	-	2 Rubella IgM 10 Rubella IgG	-
9	Influenza A & B	1481	Throat swab	-	-	20 Influenza A H3N2
10	Dengue serotyping	164	Serum	-	-	DEN 2= 78;DEN 1=2; DEN 3 - 2
11	Scrub Typhus	59	Serum	-	49 IgM	-
12	Dengue/Chikungunya/ JE	348	Mosquito	-	-	11 – Dengue 01 – JE

NIV KERALA UNIT

List of Staff

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Mr. Govindsing Patil	Staff Car Driver (OG)

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Ms. Moomina V. M.	Technician-II
Mrs.Sandra V P	Contract Technician
Ms.Nancy Jhon	Data entry operator
Mr.Johny Joseph	Data entry operator
Mr.Jerlit PC	Data entry operator

Project 1: Establishment of a network of laboratories for managing epidemics and natural calamities

Investigators: Sugunan AP, Anukumar B

Funding agency: DHR

Duration Project: 2019-24

Background: The project is in response to DHR's timely initiative on setting up of State Level Virus Research and Diagnostic Laboratory (VRDL) at ICMR-National Institute of Virology in Kerala state to strengthen the existing national facilities in surveillance, outbreak investigations and research on medically important viruses.

Objectives: To establish a state level diagnostic virology laboratory including sequencing facility to investigate viral diseases of regional and national importance.

Findings: The reporting period was dedicated mainly for COVID -19 testing and screened >2 lakh samples. Training was imparted to 29 staff members of various Medical institutes in Kerala and Lakshadeep on RT-PCR diagnosis of SARS-CoV-2. ICMR and Kerala state government have designated NIV Kerala facility as an external quality control laboratory to validate antigen, RT-PCR, RNA, and VTM kits. For quality assurance, the unit is tagged to 107 laboratories and received 1030 samples to test. In addition, three RNA kits, 05 LAMP PCR kits, 24 RT-PCR kits, 03 antibody detection kits, and 06 antigen detection kits were validated as per request from state government. In addition to COVID-19 testing, 1296 samples were also screened for other pathogens. The most prevalent pathogen found was Leptospira. Details of other pathogens detected are presented in Table 1.

Table 1: Number of samples screened for different viruses using various diagnostic tools

Sl. No	Virus	Conventional PCR (positive)	Real time PCR (positive)	IgM ELISA (positive)
1	JE		0/1	1/25 /
2	Dengue	3/57		0/4
3	West Nile		0/4	
5	VZV	0/3		
6	EBV		0/16	
7	HSV 1 & 2	5/247		
8	HCV	1/4		
9	Entero virus		7/194	
10	Influenza A &B		0/310	
11	CMV		4/31	
12	Adeno		3/42	
13	Nipah (Tru-naat)		0/15	
14	Zika		0/1	
15	Lepto		45/261	4/23
16	Scrub Typhus		0/2	0/20
17	KFD		9/40	
TOTAL		9/311	68/917	5/72
	SARS-CoV2		40153/218644	-

Project 2: Development and Efficiency evaluation of tick repellent from plant extract: A scientific and social intervention on tribal health against ticks and tick-borne diseases in Kyasanur forest disease endemic area of Kerala.

Investigators: Balasubramanian R, Sahina S

Funding: DST (Women Scientist Project), New Delhi. Duration: 2019-22

***Background*:** Tick-borne diseases produce significant morbidity and mortality to humans and livestock. Since no effective vaccines or drugs are available for tick borne infections especially viral infections, avoiding/preventing tick bits using repellents is the best option. Medicinal plants with latent effects on different life stages of ticks are considered a viable and preferred alternative.

Objectives: To evaluate cidal and repellent bioactivity of *Tagetes erecta* (flower and leaf) and *Lawsonia inermis* (leaf and seeds) as a tick repellent.

Findings: Among the five solvents (water, ethanol, methanol, acetone and chloroform) used for extraction of *T.erecta*, ethanol and methanol extracts showed highest bioactivity against *Rhipicephalus sanguinius ticks*. Ethanol extract of *T.erecta* flower had the highest ovicidal activity (86.1%) followed by leaf extract (75%) at 25mg/mL concentration. Methanol and ethanol extracts of *T.erecta* leaf showed highest larval repellency (83%) at 2.5 mg/mL concentration. Significant (R^2= 0.97, P-value = 0.001) tick repellency (> 90%) was found in both methanol and ethanol extracts of flower at 2mg/mL concentration.

Project 3: Clinical and epidemiological study of Lyme disease: a multi-centric taskforce study in India.

Investigators: Sugunan AP, Balasubramanian R

Funding: ICMR

Duration: 2020-23.

Background: Lyme disease is a tick borne multi system inflammatory zoonotic disease posing a major public health threat. However, no data is available on the local distribution of the etiological agent in Indian subcontinent. Hence, standardized approach for human surveillance is needed to understand the spread of Lyme disease in India and also to predict hotspots.

Objective: A prospective observational multicenteric study indenting to collect information on Lyme disease from seven states, in correlation with ticks and vector incrimination.

***Findings*:** Procured permission from State Forest Department for rodent trapping and tick collection along the Wayand forest area. Work could not be initiated due to Covid 19 restrictions.

Project 4: Response of urban health service systems to road traffic injuries:

Investigators: Babu RG, Shafi MA, Abhijith AK

Funding Agency: ICMR, New Delhi

Duration: 2019-2021

Background:

The study focused to examine to what extent the road traffic injuries are rendered in primary, secondary and tertiary care centers by the urban health care system. Mapping of existing mechanisms and gaps in services within the health system is therefore needed.

Objectives:

To map the range of urban health care systems and their capacities within the government sector for road traffic injuries and to interpret the challenges faced by persons with RTIs while accessing post- crash emergency care within the golden hour.

Findings: Urban health care system in Alappuzha is well organized throughout the district; however road traffic injuries are largely catered by Alappuzha Medical College irrespective of severity of injuries, leading overburdening the tertiary care system due to lack of adequate staff and equipment in secondary care centers. Due to the overburden of the tertiary centre, victims had to seek health care from private institutions elsewhere. Downgrading of Advanced life saving ambulance to Basic life saving ambulance under public private partnership has restricted the service as a means of transportation. Both service providing staff and accident victims found unsatisfied with the change. The number of deaths per month over the years is 31.5, 29.6, 34, 31.08, and 35.6 respectively during 2015 to 2019. Most of the participants (Victims/relatives) in the study opined that the service of the tertiary health centre is unsatisfactory and trauma care is generally being provided by medical students (PG).

Project 5: Public trust in vaccine: A qualitative study on the determinants of acceptance and hesitancy towards JE vaccines in various Blocks in Alappuzha District.
Investigators: Babu RG, John RP, Sarma K
Funding Agency: ICMR
Project Duration: 2020 -2021

Background: The Mathrubhumi Daily, on 21st July 2016, reported that more than 400 children in Alappuzha District are not immunized or only partially immunized for Japanese encephalitis virus. However, the official report of May 2020, from District Medical Officer, Alappuzha shows 296 non-immunized/partially immunized children below the age of five. Among these, 20 children are non-immunized specifically with JE Vaccine while 276 are partially immunized in general.
Objectives: **T**o analyze and understand the underlying determinants and decision pathways of vaccine hesitancy and also to explore how the peer-to-peer communication influences vaccine hesitancy and perspective of stakeholders towards vaccination hesitancy as a public health challenge.
Findings/Current Status**:** Qualitative data was collected from households who are not interested to ensure that their children are vaccinated, using Key Informant Interview, In-depth Interview and Focus Group Discussion. After the literature review, initial work for primary data collection was deligated to 1862 ASHA workers in 16 health blocks of Alappuzha district, comprising 71 village Panchayats and 4 Municipalities. Data collection began in Ambalapuzha health block which has the highest number of non-immuninused/partially immunized cases. A total of 40 in-depth interviews of parents and eight key informant interviews were conducted in which 2 non-immunized cases and 75 partially immunized cases were identified. After data analysis, major themes emerged are the influence of other stream of medicines, internet and social media, fear of AEFI, history of AEFI, lack of trust, low perception of contracting vaccine preventable diseases, allegations related to fertility issues, contrary/ confusing information, gender dominance, influence of health care provider, culture and religious beliefs etc.

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Ms. Gauri Pawar	Lab Technician (COVID 19 Project)
Mr. Akash Bandal	MTS (COVID 19 Project)
Mrs. Darahsna Vichare	Clerical Assistant (contract)
Mr. Shrawan Kegde	Data Entry Operator (Contract)
Ms. Prajakta Kegade	Data Entry Operator (Contract)
Mr. Niraj Amrutkar	Laboratory Attendant (contract)
Mr. Pratik Kamble	MTS (Contract)
Mr. Yogesh Kadu	Driver (Contract)
Mr.Sandesh Palav	Driver (Contract)
Mr. Arvind Shedge	Security Guard (Contract)
Mr.Sandeep Vichare	Security Guard (Contract)

Core Service Project 1: National Polio Surveillance Project (NPSP), India
Investigators: Sharma D, Nalavade U, Pawar SD

***Funding agency*:** Extramural (funded by National Polio Surveillance Project, WHO, India)
Duration: Ongoing (since 1997)

Background: The last case of wild poliovirus was detected in India in January 2011and WHO has certified India as wild poliovirus free in 2014. The surveillance system has therefore become more stringent as there is always a risk of importation of wild poliovirus from neighboring polio-endemic countries, *i.e*., Pakistan and Afghanistan.

Objectives: To carry out rigorous surveillance of polioviruses until global eradication is achieved.
Findings: During April 2020 to March 2021, 3970 stool specimens were received from AFP cases reported in Maharashtra, Madhya Pradesh and Goa. A total of 81 Sabin-like (SL) polioviruses (P1SL=39; P3SL= 54; P1+P3SL= 17 and NPEV= 4) were isolated from the 3970 specimens. Under environmental surveillance, 92 sewage specimens/concentrates were tested at NIVMU as per the WHO protocol and isolated 42 polioviruses. All the isolates were found to be Sabin-like poliovirus type 1 and type3 and non-polio enteroviruses (P1SL-9, P3SL-26, P1SL+P3SL-8 and NPEV-45). Wild or circulating vaccine derived poliovirus was not detected during this period in India. Also PV2, currently under containment was not detected in India from AFP cases or sewage.

Core Service Project 2: Measles and Rubella Surveillance

Investigators: Sharma D, Nalavade U, Pawar SD
Funding agency: Extramural (National Polio Surveillance Project, WHO, India)
Duration: Ongoing (since 2016)

Background: The WHO Measles and Rubella network in India comprises 19 laboratories and NIVMU is the WHO reference laboratory for sequencing. The measles and rubella surveillance programme has the goal of elimination of measles and rubella by 2024. Under this surveillance activity, NIVMU receives serum specimens as well as urine/throat swabs from suspected cases of measles and rubella from Maharashtra, Madhya Pradesh and Goa. The serum specimens are tested using Measles and Rubella IgM ELISA whereas urine/throat swabs are tested using conventional RT-PCR.

Objectives: **S**urveillance of suspected samples for measles/ rubella detection.

Findings: Screening of 1244 serum samples for antibodies against measles and rubella viruses revealed presence of anti-measles antibodies in 49 (3.94%) samples while 91(7.32%) tested positive for Rubella antibodies. 1.37% and 1.61% of the remaining specimens were equivocal for Measles and Rubella respectively while 86.01% tested negative for both. Testing of 1801 urine/throat swabs received from Maharashtra, Madhya Pradesh and Goa using RT-PCR yielded 123 (D8 genotype) specimens positive for measles virus while none tested positive for rubella. Being a reference laboratory for sequencing, NIVMU received a total of 295 PCR products from WHO MR network labs of which 120 measles PCR products

were genotyped successfully (D8:119 and B3:1). Of the 04 rubella positive PCR products, none could be successfully genotyped and the work is in progress.

Project 1: Strengthening laboratory quality management systems including EQA of DHR / ICMR VRDL laboratories for confirmation of outbreaks including measles and rubella

Investigators: Pawar SD, Sharma D, Nalavade U
Funding agency: Department of Health Research
Duration:2018-2021

Background: NIVMU, which is a WHO reference laboratory for measles and rubella sequencing, was selected as the nodal laboratory for capacity building of VRDLs. This includes providing training and technical guidance/support to these laboratories to attain proficiency for diagnosis of measles and rubella.
Objectives*:* To provide training and technical quidance and to provide External Quality Assurance System (EQAS) for the VRDLs along with on-site assessment to make them WHO proficient laboratory for measles and rubella diagnosis to expand the WHO MR network.
Findings*:* Under this project, six selected ICMR-VRDLs were provided trainings in 2018 and 2019 for serology and molecular diagnosis of measles and rubella including molecular analysis of generated sequences. This was followed by successful testing of unknown panels, EQAS panels from WHO and on-site review by WHO of these VRDLs which led to their integration into the WHO MR network.
Additional nine VRDLs were trained in August 2019 followed by provision of practice panels for serology and molecular diagnosis. Due to the COVID-19 pandemic and country-wide lockdown there was a gap in continuing with the integration process. With relaxation in lockdown, these VRDLs were provided with unknown serology and molecular panels which were successfully tested. Currently these VRDLs have now initiated testing of field samples collected from suspected cases for diagnosis of measles and rubella. Once they successfully complete testing of EQAS panels and on-site review by WHO, these can be integrated into the WHO MR network.

Project 2: *In-vivo* potency evaluation for the hexavalent and IPV trivalent vaccine formulation containing IPV type 1, 2 and 3 viruses
Investigators: Pawar SD, Lingala R, Sharma DK
Funding: Extramural- Contract research, funded by Indian Immunologicals Ltd, Hyderabad
Background: The contract research project in collaboration with the IIL, Hyderabad was undertaken.
Objective: *In-vivo* potency evaluation for the hexavalent and IPV trivalent vaccine formulation containing IPV type 1, 2 and 3 viruses.
Findings: Potency of the hexavalent and IPV trivalent vaccine formulation was assessed and found that antibodies against Sabin-like type 1 and type 3 were present in immunized animal indicating their immunogenic potential.

Project 3: Study on Polio and Non-Polio enterovirus infections in children with Primary Immunodeficiency at multiple medical institutes across India

Investigators: Mohanty M, Madkaikar M, Mohammad A, Varose S, Fenandes M, Sawant U, Pawar SD & the study site investigators

Funding Agency: Extramural (WHO)
Project Duration: 2 years (2019-2021)
Background: As a potential reservoir for neurovirulent VDPV strains, children with Primary Immunodeficiency (PIDs) represent a global risk to unimmunized contacts and to the Global Polio Eradication Initiative. In the absence of routine screening of patients with PIDs for poliovirus infection and excretion, India faces the risk of re-establishment of poliovirus transmission.
Objectives: **(i)** To screen patients with primary immunodeficiency across the selected medical institutes in India for poliovirus and non-polio enterovirus excretion and identify any long term excreters among them. (ii) To characterize the virus isolates and to correlate virus excretion with host immunological parameters.
Findings: A total number of 304 stool samples of 121 patients of Primary Immunodeficiency (PIDs) with humoral, combined and other PIDs received from six collaborating hospitals across India from Dec 2019 to March 2021 were assessed and followed up for enterovirus excretion. From 121 PID patients 10 were SCIDs, 14 were CVIDs, 13 XLAs and 84 other PIDs. Stool specimens of 18 patients (14.87%) were tested positive for enteroviruses.

Project 4: Seroprevalence of Enterovirus 71 antibody among Indian children
Investigators: Mohanty M, Varose S, Rane S, Tandale BV, Pawar SD
Funding agency: Intramural
Duration: 2019- 2021
***Background*:** Circulation of multiple genotypes of EV71 causing HFMD has been reported in India but no data is available on the seroprevalence of EV71 in Indian children. Keeping in mind the severity of the outbreaks caused by EV71 all over the world, a study was initiated to investigate the seroprevalence of EV71 antibodies in Indian children to explore the circulation of EV71 in India.
Objective: To estimate sero-positivity and GMT of children with age group of 1-5 years to all four EV71genotypes found in India and compare the prevalence.
***Findings*:** Seropositivity between EV71 genotypes A, D, G, and C was compared between age groups from 1-5 years of age. The percent seropositivity for age 1-5 years significantly increased in all the EV71 genotypes with comparatively higher seropositivity to EV71-G genotype (Fig 1). Within the genotype G, statistically significant difference was observed between age groups 2-5 years, 3-5 years and 4-5 years respectively ($p \leq 0.05$). The Geometric mean titre for all the EV71 genotypes increased from 3 years of age which decreased above the age of 4 years for EV71 G and C genotypes. Interestingly, no significant difference could be found in seropositivity to the three Indian genotypes in the age group of 1-5 years. The data indicates circulation of all four EV71 genotypes in India with antibody prevalence to all genotypes, with comparatively higher seropositivity to EV71-G genotype.

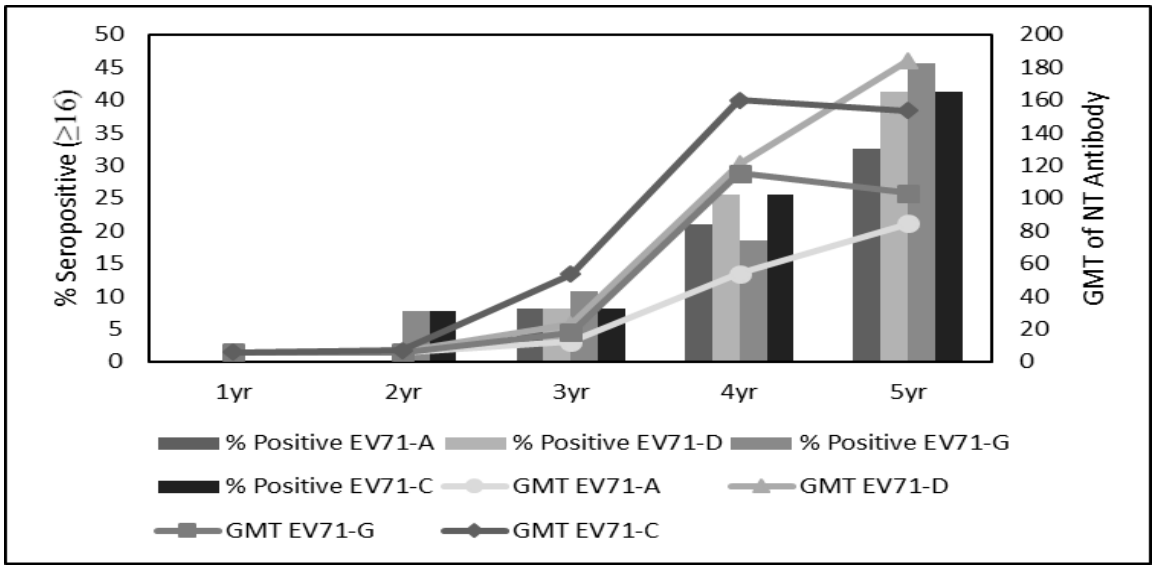


Figure 1: % Seropositivity and GMT between EV71 genotypes A, D, G, and C

Project 5: RT-LAMP Assay for detection of human β -Actin housekeeping gene
Investigators: Nandi SS, Lambe U, Sawant S, Gohil T, Deshpande J
Funding agency: Intramural
Duration: 2020-2021

Background: Housekeeping genes of host genome or normal microflora genomes are widely used as internal controls. Housekeeping genes are majorly active constitutive genes that are required for survival. These genes are expressed in all cells of an organism under normal and patho-physiological conditions. Internal control gene amplification indicates quality of sample, tells about quality of DNA/RNA extraction and inhibition of amplification due to inhibitors.
Objectives: **T**o develop an RT-LAMP assay for the detection of human β-Actin housekeeping gene.
Findings**:** A colorimetric RT-LAMP assay to be used as an internal control (IC) was developed. RT-LAMP primers were designed for the β-Actin gene. This study involves primers and a method for detecting IC gene (Beta actin) using a colorimetric reverse transcription loop-mediated isothermal amplification (RT-LAMP) assay. The IC helps as an indicator for quality check of sample collection, nucleic acid extraction and quality of reaction (Figure 1). The β-Actin internal control primers designed in-house for RT-LAMP assay were found to be working efficiently. This has been validated by 34 samples which showed positive results with RT-LAMP assay. This IC can be used in combination with various other RT-LAMP based diagnostic assays.

Sr. No.	Reaction Name	Beta Actin gene	Image	Interpretation
1	No Template Control	-		Pink colour indicates no amplification
2	Positive Control	+		Yellow colour indicates amplification
3	Sample 1	+		Yellow colour indicates amplification Sample quality good
4	Sample 2	-		Pink colour indicates no amplification Sample quality poor

Fig 1: Interpretation of RT-LAMP assay for detection of Housekeeping gene

Project 6: A Point of Care Device, Method and Kit involving Club Cell protein 16 as a marker for Silicosis/ Silico-Tuberculosis
Investigators: Nandi SS, Lambe U, Sawant S, Deshpande J
Funding agency: ICMR-NIV & ICMR-NIOH.
Duration: 2 years (2019-2021)

Background*:* Club cell protein 16 (CC16) is the most abundant protein in broncho-alveolar secretions. Many chronic pulmonary inflammatory diseases, *i.e*., anthraco-silicosis, chronic obstructive pulmonary disease (COPD), asthma etc., cause depletion of CC16. Chronic silicosis, is an irreversible occupational ailment of the respiratory system caused by invasion of lung tissue (parenchyma) with dust containing crystalline silica or silicon dioxide. Evidences suggest significant reduction of CC16 in chronically silica dust-exposed workers. Normal chest radiology and lung function tests indicates that serum CC16 could be an early asymptomatic detection tool for silicosis among silica-exposed population.
Objectives: To develop a point of care, semi-quantitative lateral flow device based on gold nanoparticles for screening of occupational silica dust exposed workers for early detection of silicosis.
Findings: In this current study, we describe a Point of Care assay that can be particularly employed for semi-quantitative estimation of CC16 in human serum samples. This assay can be used periodically to assess CC16 levels among workers with a history of silica dust exposure and can be considered as a proxy bio-marker and screening tool for early detection of silicosis. Performance evaluation of the assay was done by testing 104 serum samples in parallel with lateral flow assay and commercially available ELISA (Fig 2).

Rapid LAMP assay for detection of *Corynebacterium diphtheriae*
Investigators: Nandi SS, Lambe U, Sonali Sawant, Deshpande J
Funding agency: Intramural. This project is carried out in collaboration between ICMR-NIV Mumbai Unit and ICMR-NIE, Chennai
Duration: 2020-2022
Background: Diphtheria is an acute, highly infectious, and potentially lethal disease caused by diphtheria toxin-producing bacterial strain of *Corynebacterium diphtheriae.* Increasingly more cases are

being reported from adults, which, till recently was known to be primarily a childhood disease. Diphtheria is usually diagnosed based on a patient's clinical presentation. Laboratory detection of *Corynebacterium diphtheriae* in the throat swab samples require culture and isolation or PCR based tests, which is expensive, requires expertise and can be performed only in high-end laboratories.

Objectives: to develop an RT-LAMP assay for detection of toxigenic genes for differentiation between toxigenic and nontoxigenic species of *C. diphtheriae*.

Findings: For detection of *Corynebacterium diphtheriae*, two genes have been targeted by performing bioinformatics analysis. It is established that *tox* gene encoding diphtheria toxin is present only in the pathogenic strains of *C. diphtheria* and *dtxR* gene encoding global regulator is a species-specific gene. The *C. diphtheriae* species can be detected by using species specific gene *dtxR* (Fig 3).

Sr. No.	Figure	Description	Sr. No.	Figure	Description
1		**Negative control** **Observation:** **Control line**: One band observed **Test line**: No band observed	2		**CC16: 0 to 6 ng/ml (Suspected moderate to advance silicosis)** **Observation:** **Control line**: One band observed **Test line**: Single band observed
3		**CC16: 6.1 to 9 ng/ml (Suspected early Silicosis)** **Observation:** **Control line**: One band observed **Test line**: Two bands observed	4		CC16: 9ng/ml and above (Healthy or early silicosis, not detectable by X-ray) **Observation:** **Control line**: One band observed **Test line**: Three bands observed

Fig 2: Interpretation of semi-quantitative assay for detection of silicosis.

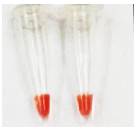
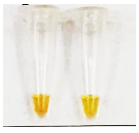
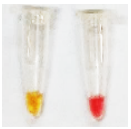
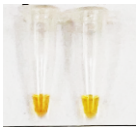
Reaction mixture color	Observation	Reaction mixture color	Observation
dTxR *Tox* 	NTC (Negative control) Both *dTxR* and *Tox* gene reaction tubes are pink	*dTxR* *Tox* 	PTC (Positive control) Both *dTxR* and *Tox* gene reaction tubes are yellow
dTxR *Tox* 	*dTxR* gene reaction tube is yellow *Tox* gene reaction tubes is pink. The test is positive for *Corynebacterium diphtheriae* but non toxigenic.	*dTxR* *Tox* 	Both dTxR and *Tox* gene reaction tubes are yellow The test is positive for *Corynebacterium diphtheriae* and toxigenic.

Fig 3: Interpretation of LAMP assay for detection of *Corynebacterium diphtheria*

Project 7: Assay for detection of epidemiologically important SARS-CoV-2 genetic variants

Investigators: Nandi SS, Lambe U, Sawant S, Deshpande J

Funding agency: Intramural

Duration: 2021-23

***Background*:** Emergence of genetic variants of COVID-19 across the globe with increased transmissibility, increased morbidity and mortality and decreased susceptibility to antiviral drugs has

necessitated the constitution of Genomics Consortium (INSACOG) to study the variants by genomic sequencing of samples from Pan-Inda. Due to the tedious process of whole sequencing analysis, it becomes difficult to sequence a larger number of samplesand hence an easier technology is the need of the hour.

Objectives: To design and develop assay(s) for screening of clinical samples for identification of epidemiologically important genetic variants of SARS-CoV-2.

Findings*:* SNP primers along with RT-PCR primers were designed to specifically amplify the Spike protein gene of SARS-CoV-2. The amplification of these specific regions using multiplex reverse transcriptase polymerase chain reaction was performed. The amplified DNA was later subjected to multiplex reaction to identify mutations using SNaPshot reagent in Genetic analyzer. This was followed by analysis of results in GeneMapper software. This assay is modifiable and updatable in case of occurrence of new variants (VOC/VOI) based on the need.

Project 8: CD155/PVR knockout cell strains from human rhabdomyosarcoma cell line (RD) for use in polio and non-poliovirus diagnostics and research.

Investigators: Nandi SS, Sawant S, Gohil T, Deshpande J

Funding agency: Extramural (DHR)

Duration: 2018-2021

***Background*:** Achieving poliovirus containment concomitant with certification of global polio eradication is the highest priority of the Global Polio Eradication Initiative. WHO GAP III recommends laboratories wanting to culture viruses from potentially infectious materials in poliovirus permissive cell lines have to establish bio-safety/bio-risk management systems and obtain certification by National Containment Authority.

Objectives: to develop a human RD cell lines with CD155-PVR knockout or deletion and to check the growth of non-polio enterovirus and polio virus in newly generated RD cell strains.

Findings: The CD155/PVR knockout RD cell line developed using CRISPR/Cas9 technology renders resistance to poliovirus growth though susceptible to non-polio enteroviruses. Poliovirus non-permissive RD cells did not express CD155-specific surface immunofluorescence. A selected clone (RD-SJ40) was field tested against 626 stool samples of acute flaccid paralysis (AFP) cases by three National Polio Laboratories. The cells did not support growth of poliovirus from positive stool samples. All NPEV types isolated in parental RD cells were also isolated in RD-SJ40 (Fig 4). Thus, RD-SJ40 cells are safe for NPEV isolation from poliovirus PIM without derogating GAPIII containment requirements. It is envisaged that the WHO Global Polio Laboratory Network will also support use of CD155 knockout RD cells for enterovirus work/ enteric virus in all the network laboratories all over the World. RD-SJ40 cell line is deposited at ATCC, USA for patent purpose.

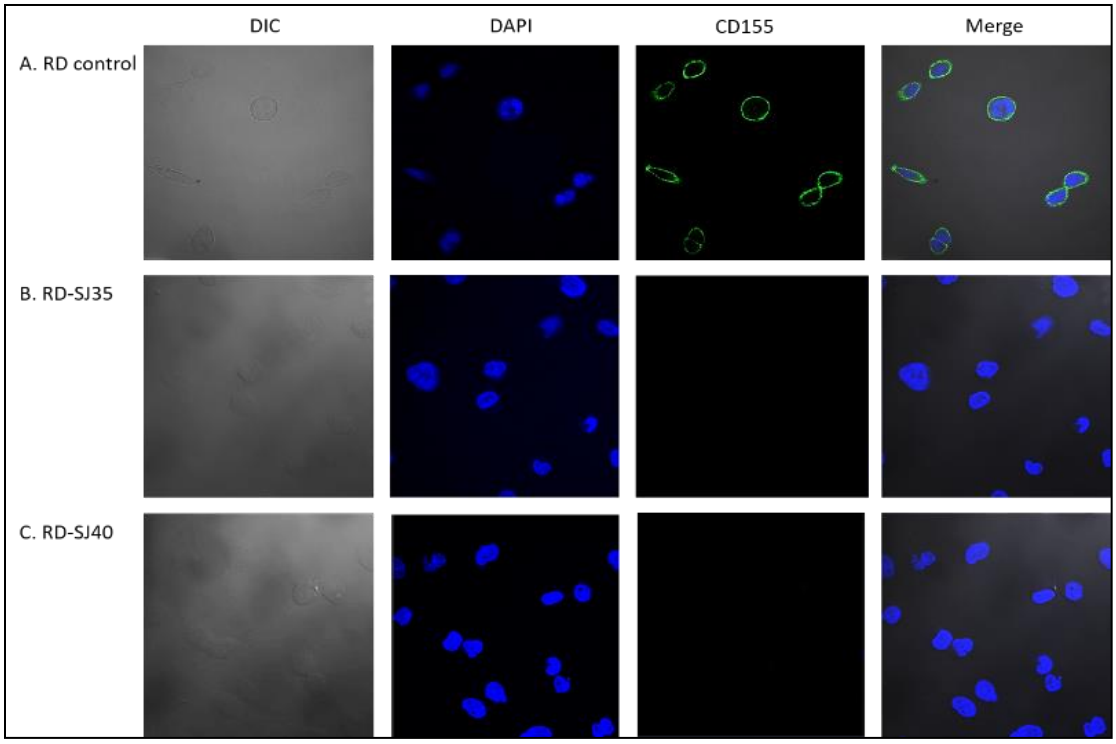


Figure 4: The new knock out cell line

Project : Studies on the role of different cell surface receptors of Enterovirus A71
Investigators: Nandi SS, Lambe U, Deshpande J
Funding: Extramural (ICMR).
Duration: 2019-2022
***Background*:** EV-A71 utilizes SCARB2, PSGL-1 and Fibronectin (FN) receptors for virus attachment and internalization. EV-A71 genotypes D and G appear to be confined to India only. There are no reports of outbreaks of HFMD, encephalitis/ meningitis or AFP associated with EV-A71, may be due to low virulence (naturally attenuated) of the Indian genotypes. During the study, identification and over expression of the most preferred receptor by Indian strains will be attempted for high titer yield which can be utilized for antigen production, vaccine development etc.
Objectives: (1) To dysfunction of EV-A71 receptors sequentially and in combinations to identify most preferred receptors by different genotypes of EV-A71. (2) To study the effect of over expression of the preferred cell surface receptor on per cell yield of EV-A71.
Findings: Sequence analysis of all the three receptors through literature search was performed. The PAM (protospacer adjacent motifs) sites were identified from the structurally important regions of the receptors. Five CRISPR-Cas9 constructs with integrated sgRNAs were used for SCARB2 and PSGL1 gene knockout of HEK-293T cells. For SCARB2, 2400 wells were seeded with single cell suspension and obtained 114 colonies showing healthy cell growth. Similarly, for PSGL1, 2400 wells were seeded with single cell suspension that yielded 68 colonies. The screening of knockout of PSGL1 and SCARB2 genes has been performed by performing real-time PCR based expression analysis.

Project 9: Investigation of host genetic susceptibility markers to Enterovirus A71 infection in Indian population.

Investigators: Nandi SS, Sawant S, Gohil T, Deshpande J
Funding agency: Intramural
Duration: 2018-2021
***Background*:** EV71 infection is endemic to India, however, no major outbeaks associated with the virus is reported from the counry may be due to low virulence of circulating strains. To explore mutations in several genes, a multiplex SNP assay has been designed to understand genetic contribution to susceptibility to EV71 infection in Indian population.
***Objectives*:** to develop multiplex single nucleotide polymorphism assay for investigation of host genetic risk factors in different genes by Enterovirus 71 infection, and to carry out population based surveys of SNPs in genes contributing to EV71 susceptibility.
***Findings*:** The Single Nucleotide Polymorphism (SNP) multiplexed assay was developed targeting 15 genetic markers from 12 genes against EVA-71 infection and validated at the Central Railway Hospital, Kalyan, Govt. of India. Data for 100 blood samples have been generated and polymorphisms observed for each of the 12 selected genes were analyzed and the frequency of the individual alleles was calculated. Further work is in progress.

Project 10. Outbreak investigation of highly pathogenic avian influenza (HPAI) H5N1 virus in India
Investigators: Pawar SD
***Background &Objective*:** India reported widespread outbreaks of HPAI H5N1 and H5N8 viruses in birds in parts of India in 2020-2021, during COVID-19 pandemic period. As a part of the Central Investigation Team, the outbreaks of HPAI H5N1 and H5N8 viruses were investigated in Kerala, Gujarat and Maharashtra states. Various epicenters were visited, mortality in birds, sanitation, prevention and control measures, human-animal interface aspects were assessed.
***Findings*:** All specimens from humans who were in close contact with infected birds were negative, indicating absence of human infections of AI.

Application of frozen and stored glutaraldehyde-fixed Turkey red blood cells for hemagglutination and hemagglutination inhibition assays for detection and identification of influenza viruses
Investigators: Pawar SD, Kode SS, Tare DS, Mullick J
***Background*:** Hemagglutination (HA) and hemagglutination inhibition (HI) assays are conventionally used for detection and identification of influenza viruses, using red blood cells (RBCs) from mammalian and avian sources. However, there could be limitations for availability of fresh RBCs due to situations such as pandemics, outbreaks in avian species, lack of animal facilities, animal ethics concerns; or resource-constrained laboratories. Turkey RBCs (tRBCs) are widely used for HA and HI assays. ***Objective*: To e**xplore the possibility of use of glutaraldehyde-fixed tRBCs stored at -80 ºC for HA and HI assays.
***Findings*:** There was no significant difference ($p > 0.05$) between mean HA and HI titers using fresh and glutaraldehyde-fixed turkey RBCs. In addition, the HA and HI titers using fixed tRBCs before and after storing at -80 ºC were equivalent, indicating suitability of the fixed and stored RBCs. This is the first report of the use of fixed and stored tRBCs for HA and HI assays of influenza viruses, highlighting their applicability as a ready-to-use reagent for laboratory diagnosis of influenza.
NABL (National Accreditation Board for Testing and Calibration Laboratories) Accreditation of human Influenza, Avian Influenza, Maximum Containment Laboratories & Engineering Support Group as per ISO/IEC 17025:2017

***Background*:** The Quality Management system was established at ICMR-NIV in 2015-19 and the ICMR-NIV is maintaining the QMS.

***Objective*:** Quality management system as per ISO/IEC 17025:2017 guidelines.

***Findings*:** As part of the Quality Management system, conducted internal audits of Human influenza, MCL, AI, Engineering Support and Diagnostic Virology Groups as per ISO/IEC 17025:2017 guidelines. Conducted two Management Review Meetings with Top Management. Revised the Laboratory Quality and Executive Procedure Manuals, as per ISO/IEC17025:2017 guidelines. Conducted meetings in connection with the forthcoming NABL transition audit of the laboratories at ICMR-NIV, Pune. Organized and executed NABL Transition audit of ICMR-NIV as per ISO/IEC 17025:2017, 27/8/2020. *The NABL granted continuation of accreditation to ICMR-NIV as per ISO/IEC 17025:2017.*

Number of samples tested

Virus	Samples	Number of cases	Number of samples
Polio Virus	Stool	2001	3970
	Isolates received from other labs	3	4
	Isolates (for Banking)	162	162
	Sewage	92	90
	Sewage Isolates received from other labs	34	44
Measles/ Rubella	Serum	1244	1244
	Urine/ Throat	1801	1801
COVID-19	Respiratory specimens	42117	42117

Section 4: Other activities

ACADEMIC CELL

List of Staff

Dr. Kavita S. Lole	Scientist F & Academic Cell In-charge
Dr. V S. Ghole	Co-ordinator Academic Cell (Retd Professor, Pune University)
Dr. Tejaswini M. Deshmukh	Scientist C

Technical Staff

Mrs. Swati Bohodkar	Sr. Technician 3
Mr. Hemant R. Band	Sr. Technician 1
Ms. Anuradha Vaidyanathan	Technician C

Project staff

Mrs. Neethi Jayaram	Technical Officer
Ms. Manisha T. Dudhmal	Technical Assistant (DHR)

M. Sc. Virology Program:

M. Sc. Virology post-graduate program was started by the ICMR-National Institute of Virology in June 2005 and is affiliated to the Savitribai Phule Pune University (SPPU, formerly Pune University, Pune) through the Institute of Bioinformatics & Biotechnology (IBB, SPPU, Pune).

Fifteenth batch of M.Sc Virology passed out during the year. Twenty two students were enrolled for the batch all the students passed our in flying colours. Six students procured 'O' grade wahile four secured 'A' grade. **Mr Acirup sanyal** has topped the batch and the Gold medal for thebest performer was presented by Prof. Priya Abraham, The Director, ICMR-NIV in the presence of faculties and students virtually (Fig 1). Mr. Jose Antony Jenish R scored the second highest marks. Score details of other students is depicted in Table 1.

Gold medal presented to Mr Sanyal by Prof: Priya Abraham, the Director, ICMR- NIV, Pune in a virtual ceremony held on 08-10-2021.

Achievers of M.Sc. Virology, 15th Batch:

1. Mr. Shubham Dutta secured admission for Ph. D. program at the Vaccine and Infectious Disease Organization, University of Saskatchewan under Dr. Yan Zhou.
2. Mr. Vaishnav Wagh, batch 2019-21 worked as a SPPU student volunteer in the deployment of Point of care Rapid Antigen Detection test (RAT) for Covid-19 in Pune City under joint endeavor of PMC, IISER and SPPU. He was awarded the Gaurav Puraskar in August 2020 by the Guardian Minister of Pune for completing more than 4500 RAT without any monetary benefit.
3. M. Sc Virology Students have qualified various competitive examinations *viz*., GATE (9), CSIR-LS (1), CSIR-JRF (1), ICMR-JRF (1), TIFR (3), DBT-JRF(1), IELTS(2), TOEFL(1) in the past year.
4. Two students from 2019-21 batch have been awarded the Teach for India Fellowship, January 2021.

Details of the program are available on-

1. https://icmr.nic.in/institutes
2. www.niv.co.in

Ph. D. Program:

As a premier research institute, ICMR-NIV supports the prospective students to improve their academic qualification by conducting research at this institute towards achieving a Ph. D. degree. ICMR-NIV provides the best possible guidance and research exposure to students to get access to reputed international universities for PDF and faculty positions. ICMR-NIV faculty is recognized to guide students in various disciplines, viz., Biotechnology, Basic Medical science, Biochemistry, Microbiology and Zoology. Fifteen faculty members are registered guides of University of Pune.

During the year, four NIV staff were awarded Ph. D degree by the university. Several students are pursuing Ph. degree under various disciplines.

ICMR-NIV conducted online interviews for admission into Ph. D. program 2021-22 through independent advertisement, shortlisted 86 candidates for the academic program.

LIBRARY & INFORMATION SERVICES

Scientific Staff

Dr. M.D. Gokhale	Scientist "D" & Library In charge

Technical Staff

Mrs. Vandana Chandere	Senior Technical Officer (2)
Mr. Vishal R. Mali	Senior Technical Officer (1)
Mrs. Ekta Jain	Library & Information Assistant
Mrs. Reshma A. Rade	Library & Information Assistant

Library Apprentices

Ms. Akshada Bhalerao	(Till 31/10/2019)
Mrs. Madhusmita Dash	(Till 31/10/2019)
Mrs. Prabin Das	(From 01/11/2019 to 31/12/2019)
Mrs. Pallavi A. Parshuramar	From 01/11/2019 to 31/03/2020)

The mission of any library is to provide the right information to the right user at the right time and ICMR-NIV Library continues to cater to the needs of ICMR- NIV scientists and student in the best possible way. It has rich collection of 19729 books, 3796 bound volumes of journals, 1967 copies of annual reports, 268 theses, 222 dissertations, and a huge collection of reprints and other pamphlets related to virology in addition to 1498 of audio visual materials (Fig 1).

ICMR-NIV Library is well equipped with modern facilities to support biomedical research. Barcode technology has implemented for all books & bound volumes for smooth circulation and stock verification purposes. The library is fully computerized and installed LIBSYS 4.0 (Rel 6.0) Software. Check-out and Check-in of books are carried through Barcode Scanner with automatic email system for transaction of books, renewals, overdue of books etc.

The library renders different services *viz*., citation analysis for scientists, reference and literature search, document delivery service, CAS, SDI, reprography, binding and lamination etc. It recently introduced flashing of recent news items related to virology published in newspapers and send to scientists for ready reference a daily basis. Anti-plagiarism checking is another important service provided to scientists and students through Turnitin Ithenticate, which is mandatory before submission of theses and manuscripts. Library imparts orientation and information literacy programs to M.Sc. virology students, research scholars and newly joined staff. The Library also conducts trainings, seminars, conferences on various topics for its users. Online e-journals are accessed through IP in NIV, MCC Campus and the three field units Bangalore Kerala and Mumbai. Library has its own Web OPAC to retrieve online library collection effectively.

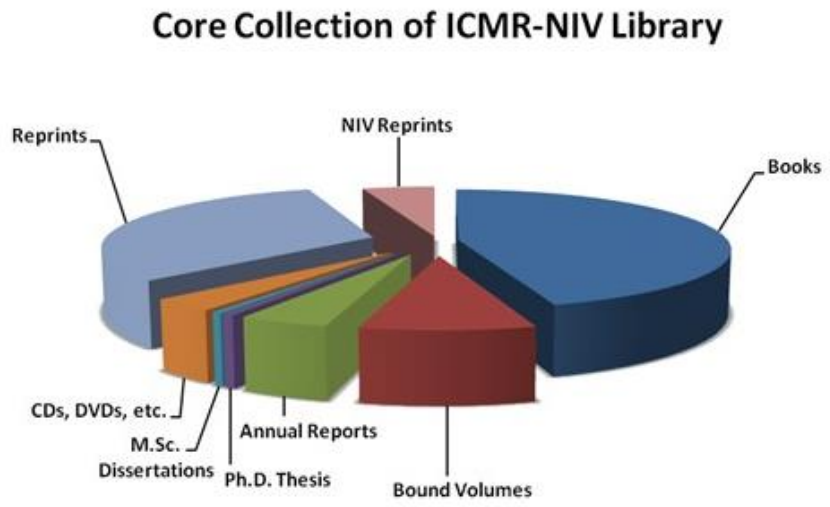


Fig. 1: Total Collection of the ICMR-NIV Library

Library Support during COVID-19:

COVID - 19 presented unique challenges to education and research institutes in the procurement and dissemination of scientific literature. ICMR-NIV Library initiated services such as providing published literature as well as newspaper reports to scientists on daily and weekly basis and displayed print copies of reprints of papers published by NIV Scientists on the Notice Board of Library on a regular basis.

Routine activities carried out during the year:

- Renewal of journals, magazines and newspapers.
- Citation Analysis of publications for scientists.

- Maintained and updated bibliographic details of NIV research articles, hyperlinked with full text on NIV webpage.
- Updated and maintained in-house NIV Scientific Publications from 1953-2021.
- Updated list of NIV holdings (Books and Bound Volume of Journals from 1953-2021
- Carried out Stock Verification of Library documents for the period 2020-21.
- Purchased Turnitin Ithenticate Software for the plagiarism detection of thesis, dissertations and Manuscripts.
- Scanning of in-house publications of ICMR-NIV for preservation of publications.

The following consortiums based access for e-journals is managed by ICMR-NIV library:

- J-Gate Plus database for access for 4176 journal titles with access to full text, abstract and on Document Delivery Request (DDR) from other ICMR libraries for One month on trial basis.
- ERMED Consortium through National Medical Library, an IP based consortium for access to 239+ e-journals from five publishers *i.e.*, BMJ Publishing Group, Cambridge University Press, Lippincott Williams & Wilkins, Oxford University Press, Wiley Blackwell.

Details of books/journals added to NIV Library during the year

Description		**Quantity**
Books	Purchased/Gifts/Gratis	101
	Bound Volumes	-
Journals	Print (Subscribed)	13
	Print (Gratis)	25
	ICMR Consortium	4
	J Gate	4176 (One Month Trial Basis)
	ERMED Consortium	239+
	Loose Issues	86
Ph.D. Theses		6
Others; CDs, Microfilms, Floppies		93

Other services provided to scientists:

1.	Newspaper Clippings	5076
2.	Reference Service	643
3.	Inter Library Loan Received and Sent	5
4.	Photocopy Service	3433
5.	Binding (Thermal & Spiral)	1
6.	Lamination	1
7.	Citation Analysis done	9
8.	Anti-Plagiarism Service	15
9.	Organized Demo on Turnitin Ithenticate Software for NIV Scientists to check plagiarism and generate reports on 16th Feb 2020.	

ENGINEERING SUPPORT GROUP

List of staff

Sr. No.	Name of Staff	Designation
Staff at NIV, Pune		
1.	Mr. A.B.Khare	HOD, Sr. Tech. Officer-3 (ES)
2.	Mr.A.R. Sable	Sr. Tech. Officer-2 (ES)
3.	Mr. D.R. Kumbhar	Sr. Tech. Officer-1 (ES)
4.	Mr. A.K.Kasar	Tech. Officer- A (ES)
5.	Mr. G.K. Bagul	Sr. Technician-2 (ES)
6.	Mr. V.J. Bhosale	Sr. Technician-2 (ES)
7.	Mr. A.J. Suresh	TA (ES)
8.	Mr. N.S. Dhawale	Sr.Technician-1 (ES)
9.	Mr. V.D. Jagtap	Sr.Technician-1 (ES)
10.	Mr. Y.M. Taru	Sr.Technician-1 (ES)
11.	Mr. B.S. Shelar	Laboratory Asst.- (ES)
12.	Mr. A.B. Kelkar	Sr.Technician-1 (ES)
13.	Mr. S.S. Utale	Laboratory Asst.- (ES)
14.	Mr. S.S. Holkar	Sr. Technician-1 (ES)
15.	Mr. R. S. Gadhave	Sr. Technician-1 (ES)
16.	Mr. D. V. Sutar	Technician-B
17.	Mr. D.K. Jagtap	Laboratory Asst. (ES)
18.	Mr. M.V. Gadhave	Technician-A (ES)
19.	Mr. A.M. Pawar	Technician-3 (ES)
20.	Mr. A.N.Kale	Technician-A (ES)
21.	Mr. S.R.Jagtap	Technician-A (ES)
22.	Mr. Md Nazim Akhtar	Technician-1 (ES)
23.	Ms. Komal Jangid	Technician-1 (ES)
Apprentice at NIV, Pune		
24.	Miss. Pratiksha Shete	Electrician
25.	Mr. Alwyn Kamble	Electrician
26.	Mr. Tushar Surve	Electrician
27.	Mr. Parag Nandanvar	Electrician
28.	Mr. Rushikesh Devkar	Electrician
29.	Mr. Santosh Jagtap	Electrician
30.	Mr. Sachin Chorge	Electrician
31.	Mr. Pavan Chaudhari	Electrician
32.	Mr.Aditya Deshmukh	Ref & ACM
33.	Mr.Amit Shaikh	Ref & ACM
34.	Mr.Kersing Changal	Carpenter
35.	Mr. Aniket Pachngane	Plumber
Staff at Pashan campus		
36.	Mr. M. S. Mohite	Technical Assistant (ES)

37.	Mrs.P.C. Lokhande	Technical Assistant (ES)
38.	Mr. S.D.Pote	Sr.Technician (1)(ES)
39.	Mr. S. N. Surbhaiya	Sr.Technician (1)(ES)
40.	Mr. S.D.Bathe	Sr.Technician (1)(ES)
41.	Mr. G.R.Ghogare	Technician-B (ES)
42.	Mr. V.T.Ishte	Technician (2)(ES)
43.	Mr. I.R.Dedunda	Technician-A (ES)
44.	Mr. Mritunjay Singh	Technician-1 (ES)
45.	Mr. Nand Kumar	Technician-1 (ES)
46.	Mr. Niteesh Kumar Yadav	Technician-1 (ES)
47.	Mr. Vishal Ashok Gaikwad	Technician-1 (ES)
48.	Mr. Govind Sharan Meena	Technician-1 (ES)
Apprentice at Pashan campus		
49.	Mr. Amol Surwade	Carpenter
50.	Mr. Gaurav Jadhav	Plumber

Engineering Support group works 24*7 for operation and maintenance of various electrical, HVAC, mechanical installations. Carrying out routine / preventive / breakdown maintenance works. Regular servicing, overhauling of machines and equipments were under taken to extend their performance and life.

Work carried out by Engineering Support

Pune Unit:-

- Carried out major repairing of AC plant at Basement.
- Repairing of -80^{o}C freezer of HI department has been carried out with replacement of compressor and modification in second stage system.
- Waterproofing of terrace of staff quarter building no- 35 & 36
- Replacement of HT metering kiosk completed.
- Old copper cable replaced with new fiber optic connected for PRI line.
- Provision of New water proof plywood for newly made MS meter boxes at Staff quarter Yamunanagar Nigadi, Pune.
- Energy Meter rewiring work was carried out with PVC channel, 4 Sq.mm Copper wires of all four buildings at Staff quarter Yamunanagar.
- DRF Division wooden wall mounting Filing cabinet with lamination and installation at the concerned division has been done.
- Provision of chemical Earthings for New building Electrical panels and distribution boards has been done by PWD contractor.
- 800 KVA both Transformer oil filtration work has been completed.
- HEPA Filter replacement work of Influenza Lab. HVAC system has been done and the Integrity testing of HEPA also has been done.
- Oil leakage work of Transformer No. 2 has been completed before filtration.
- Provision of stand using with Acrylic sheet for placing of movable cameras for SAC meeting.
- 12.5 HP Open well water pump installation and modification in the existing piping work has been done.
- Drainage choke up problem solved at Yamunanagar Staff qtr. 7 Jan 2021.

- Faulty wires/cables of street lights in NIV Campus replaced with 1.5 sq.mm armoured copper cable to avoid frequent breakdown in the rainy session.
- Telephone MDF box renovation work has been completed at NIV, Telephone system.
- Projector cable laying work has been carried out in-house at Director's meeting hall.
- RCC Structure completed for Chiller plant is in progress – CPWD, Pune.
- Air Handling Unit of Human Influenza division has been repaired in Room No.110 loft at first floor.

Pashan unit:-

- Testing of Corona Killer and ION generator machines on request Indotec Industrial solutions Pvt Ltd and Chakr innovation Ltd.
- Repairing of lyophilizer, Ice flake machine, Repairing of -80 freezer, Animal scanning machine and refrigerated centrifuge of BSL4 was carried out indigenously
- Breakdown of HT panel mains VCB at substation no-1: To avoid the consumption of diesel VCB of substation no-2 shifted in place of mains VCB and charged the supply. Necessary repairing work of faulty VCB also carried out immediately and reinstall within 1 hour. Servicing of all VCB of HT Panels and fixed gaskets to HT panel doors
- Attended breakdown of cold room and freezer room at DVG: Repairing work and Gas charging work.
- Emergency Exit panel in BSL3 fallen: Temporary arrangement has been done.
- Carry out Internal audits for NABL accreditation for testing of Biosafety cabinet.
- Oil filtration of Transformers.
- Measurement of Earth resistance of all earth pits of substations and Updation of the information on name plates. Provision of new Earthing for BSL3/PEF electrical panels
- Internal audit of substation no- 1 & 2 and Fire alarm system at main building.
- Calibration of equipments of engineering department under NABL scope.
- Attended the fault of DG no-4: The start command continuously goes to starter even after DG getting started due to which the starter can get faulty. The AMC agency suggested to replace the AMF panel as the parts are very old and not available for which approx cost of Rs.3-4 lakhs is required. Hence checked the fault and found the timer giving the command after every 8 seconds. Hence change the program of the timer and resolve the issue without any cost and now DG is working fine.
- Attended Breakdown of underground water line at play ground on 23.10.20.
- Alignment of chiller pump no-5 with digital laser alignment meter carried out
- Repairing work of autoclave of DVG (replacement of contactor and setting of pressure) carried out.
- Alignment of chiller pump no-5 with digital laser alignment meter carried out.
- Replacement of Change over switch in LT panel room substation no-2.
- Jointing work of OFC of BSL4 to resume the network connectivity.
- Internal audit of Autoclave at PEF.
- Temporary supply provision to MDP-3 as the underground cable was failed.
- Integrity test of HEPA plenum of BSL3 animal side AHU
- Carried out underground Cable jointing work of MDP-3 behind BSL3 (outsourced).
- Replacement of compressor of chiller plant of NIV Mumbai unit.
- In house Construction of foundation for street light poles on development area near boy's hostel. Erection Street light poles on development area. Laying of underground cable, termination work in control panel etc.
- Survey of the campus for CCTV surveillance system

- Conversion of DOL starter to Star Delta starters for newly installed chiller water pumps of PEF Ac plant.

❖ **Emergency Attended:**

- Breakdown of various lab equipment due to high voltage in April-20: CO2 incubators: 7, BOD incubator-1 -80 freezers-6, Deep freezer-1 and refrigerator-1 were affected. Repaired all equipments except one refrigerator and two Co2 incubators immediately within 6 hours.
- Emergency breakdown of lighting DB in room no-5 carried out necessary repairing work
- Breakdown of HT panel mains VCB at substation no-1: To avoid the consumption of diesel VCB of substation no-2 shifted in place of mains VCB and charged the supply. Necessary repairing work of faulty VCB also carried out immediately and reinstall within 1 hour.
- Attended Breakdown of cold room at DVG on 18th Sept 20: Repairing i.e. replacement of compressor, vacuum and gas charging work carried out.
- Attended Breakdown of underground water line at play ground on 23.10.20.
- Breakdown of underground water line at NIV, Pune Campus in Sept 2020.
- Breakdown of -80 freezer in BSL3 lab on 16.09.20: Vacated the -80 freezer at Hepatitis department and Shifted to BSL3.

❖ **Capital works:**

- Account settlement of BSL4 with HSCC and refund of amount Rs.13, 72, 87,190/- has been transferred to ICMR through RTGS on 10.11.2020.
- Obtained the cheque of refund of Rs. 16.04 lakhs from PWD towards cancellation of the work of Epoxy flooring and leakages of toilet ducts of boy's hostel. This amount has been transferred to ICMR.
- Renovation of Type-A, Type- B and Type V staff quarter at Pashan.
- Development of the area near boys and girls hostel, OHT, staff quarter, AC plant and substation no-2 has been initiated by CPWD.
- Replacement of compressors, chiller, and chilled water pump and related worn out piping of HVAC system, Pune.
- Replacement of 400 KVA DG set with 750 KVA DG set with AMF Panel, Pune.
- Replacement of AHU & ducting system, Energy efficient LED ,fans & LT panel in Dengue & Chikungunya group, Pune.
- Geese Tank relocation work.
- Construction of civil structure work for chiller plant installation.
- Demarcation of Land allotted for SCOH, Nagpur, through private agency.
- Construction of compound wall at SCOH, Nagpur.
- Quarterly process report of capital work sent to ICMR for June, Sept, Dec and March
- Requirement of Capital funds for 2020-21 sent to ICMR.
- Details of land and buildings sent to ICMR.
- Follow up with PWD for settlement of accounts of completed works.

ICMR- NIV ADMINISTRATION

***Senior Administrative Officer*:** Mr. Anil S. Gaikwad

Accounts Officer Mrs. V. V. Shendye

Administrative Officers:

Mrs. Shibi Jacob	Mrs. Amruta S Bakare	Mrs. A. B. Palkar
Administrative Officer (DDO)	Administrative Officer	Administrative Officer (Stores)

Senior private secretary: Mrs. R K Amale

Section officers:

Mrs. A. A. Bapat (Bills)	Mr. S R Vasam (Accounts)
Mrs. S. S. Pathak (Purchase)	Mrs. S. P. Mulay (Stock Room)
Mrs. A. R. Nair (Establishment)	Mrs. R. S. Moghe (Pension & Project)
Mr. D. V. Muneshwar (NIV Mumbai)	Mrs. P B Aher (Accounts)
Mr. P N Jadhav (Pashan-Admin)	Mrs. Jayajyothi (Bangalore)

Private Secretary Mr. J R Kumbhare

Accounts officer (Jr.Gr.): Mrs. P S Joshi

Assistants

Mrs. D. D. Marathe	Mrs. S. M. Bhave (PA)	Ms. Shakila Choudhari (PA)
Ms. MJA Shaikh	Mr. Y C Pote	

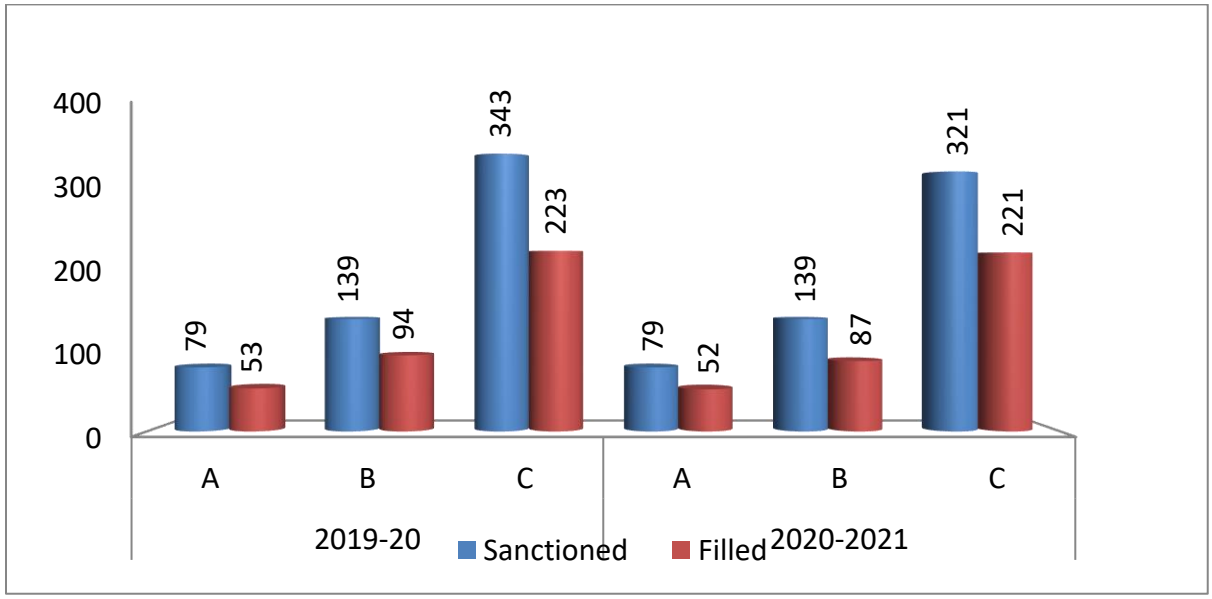
Upper division clerk

Mrs. T. T. Yadav	Mr. P. N. Chabukswar	Mr. A. E. Matkar
Mrs. D. N. Gujar	Mrs. Mangala Gangadharan	Mr. M. S. Malvankar
Mrs. M. L. Rupnar	Mrs. M. R. Kannalu	Mrs. S. B. Chakole
Mr. Prashant D. Patil	Mr. Prem P. Khandagale	Ms. Madhuri S. Tandan
Mr. Amol S. Lohbande	Ms. Prajakta A. Bapat	Mrs. Sadhana Veer
Mr. R. R. Jaiswal	Mr. P. B. Santhoshkumar	Mrs. Roshan B. Patel
Mr. Ajay S. Wable	Mr. Imran Jagirdar	Mrs. Ashwini Dudhane
Ms. Y. C. Bhandare		

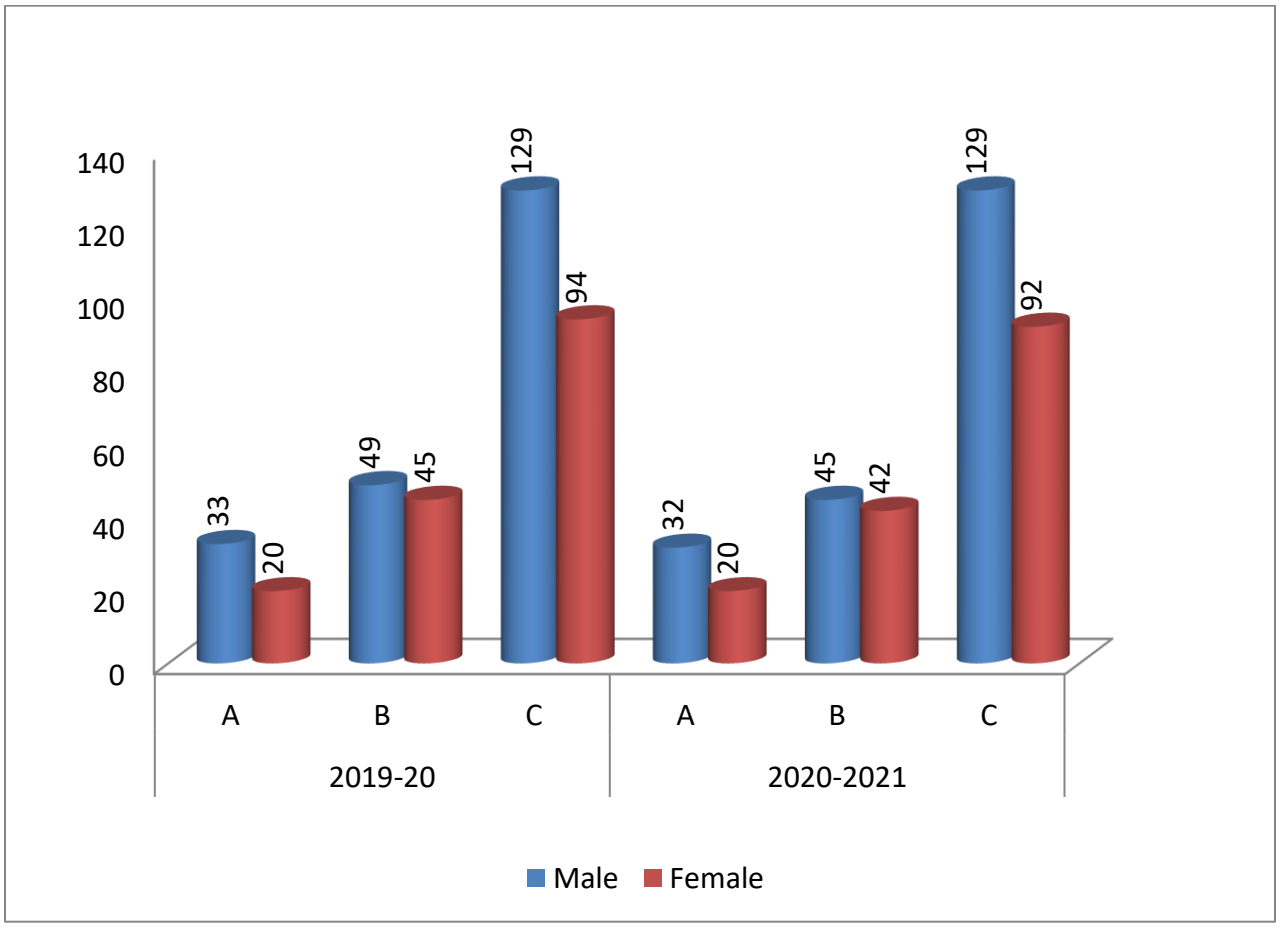
DIRECTOR'S OFFICE

Mrs. A. V. Shendrikar (Consultant) Mrs. R V Bachal, STO-2
Mr. Rohit Pawar (MTS)

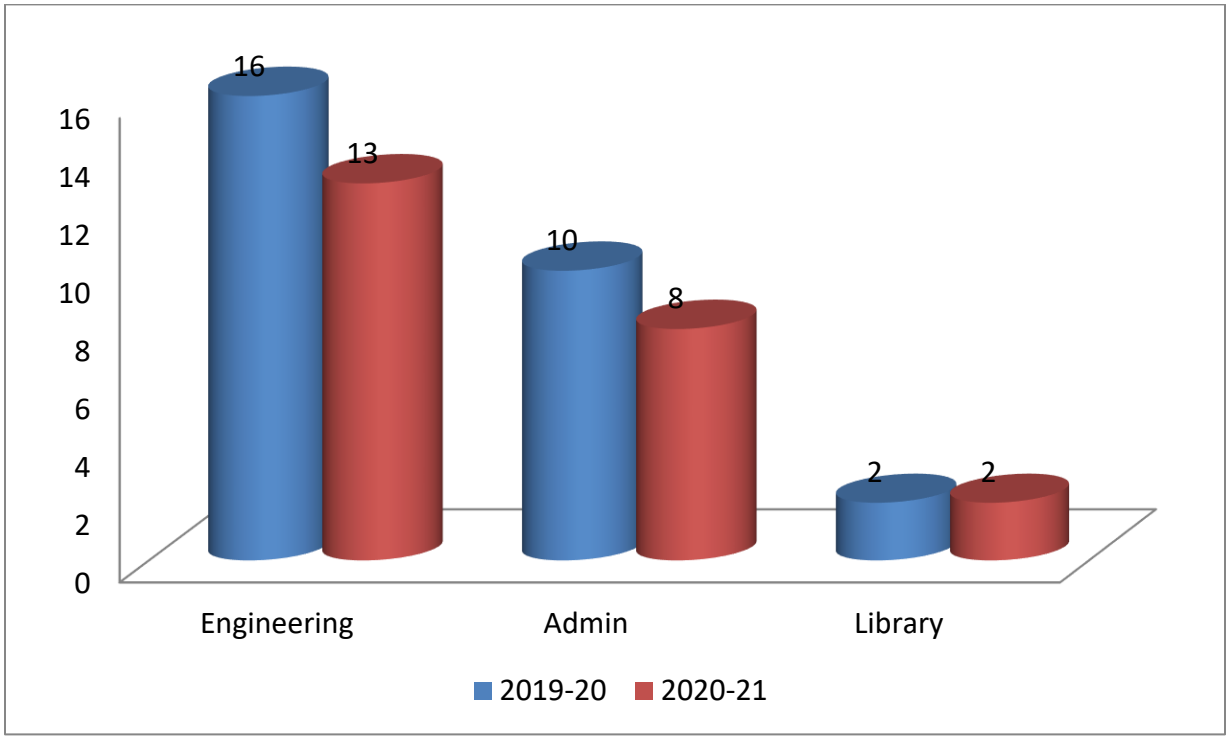
Group-wise Staff Strength



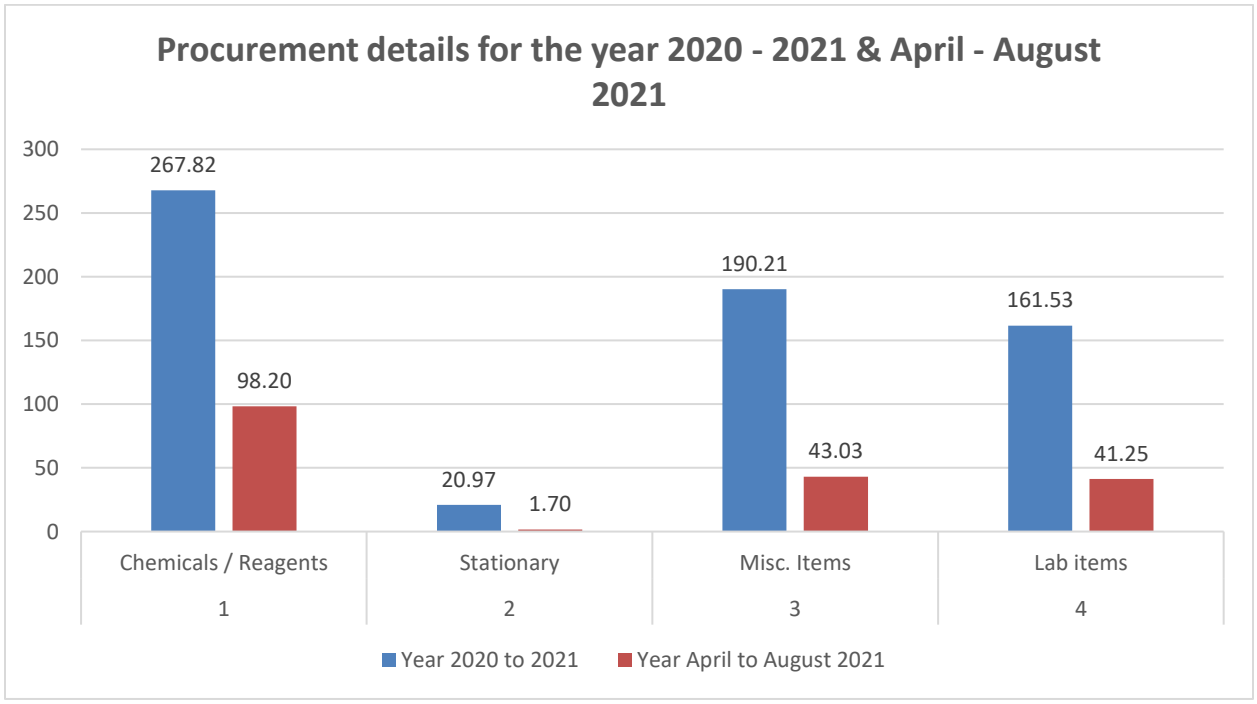
Group-wise Gender Distribution

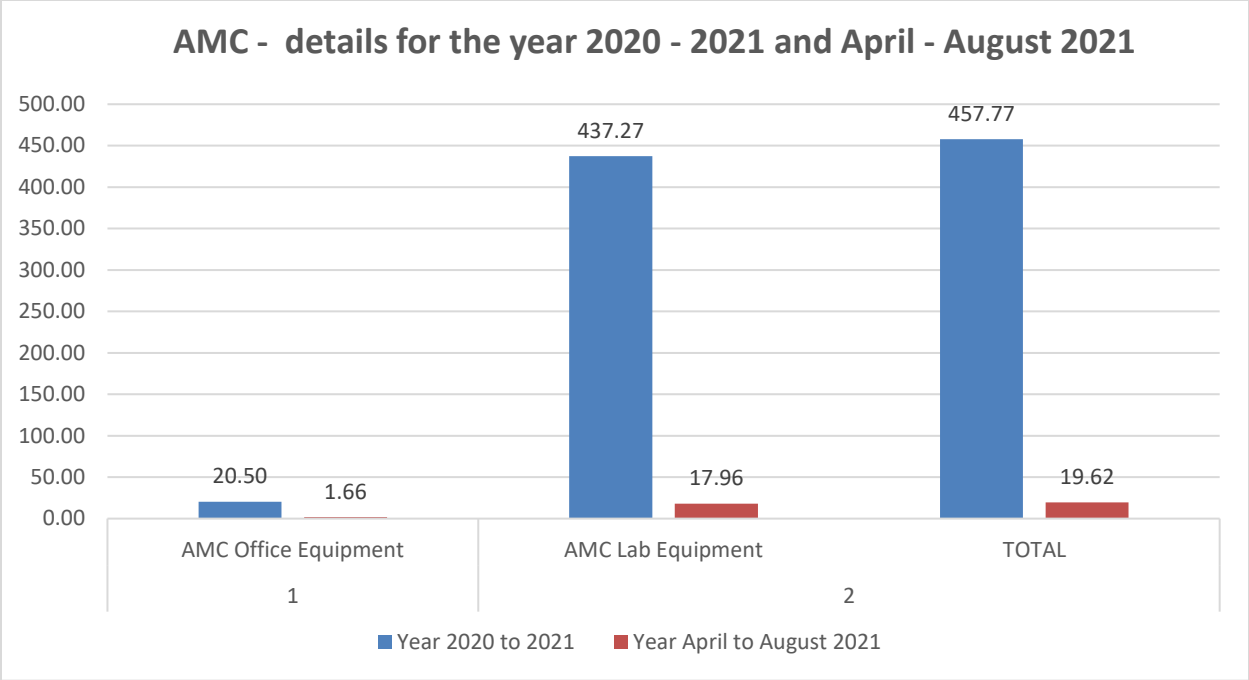


Apprentices Engaged

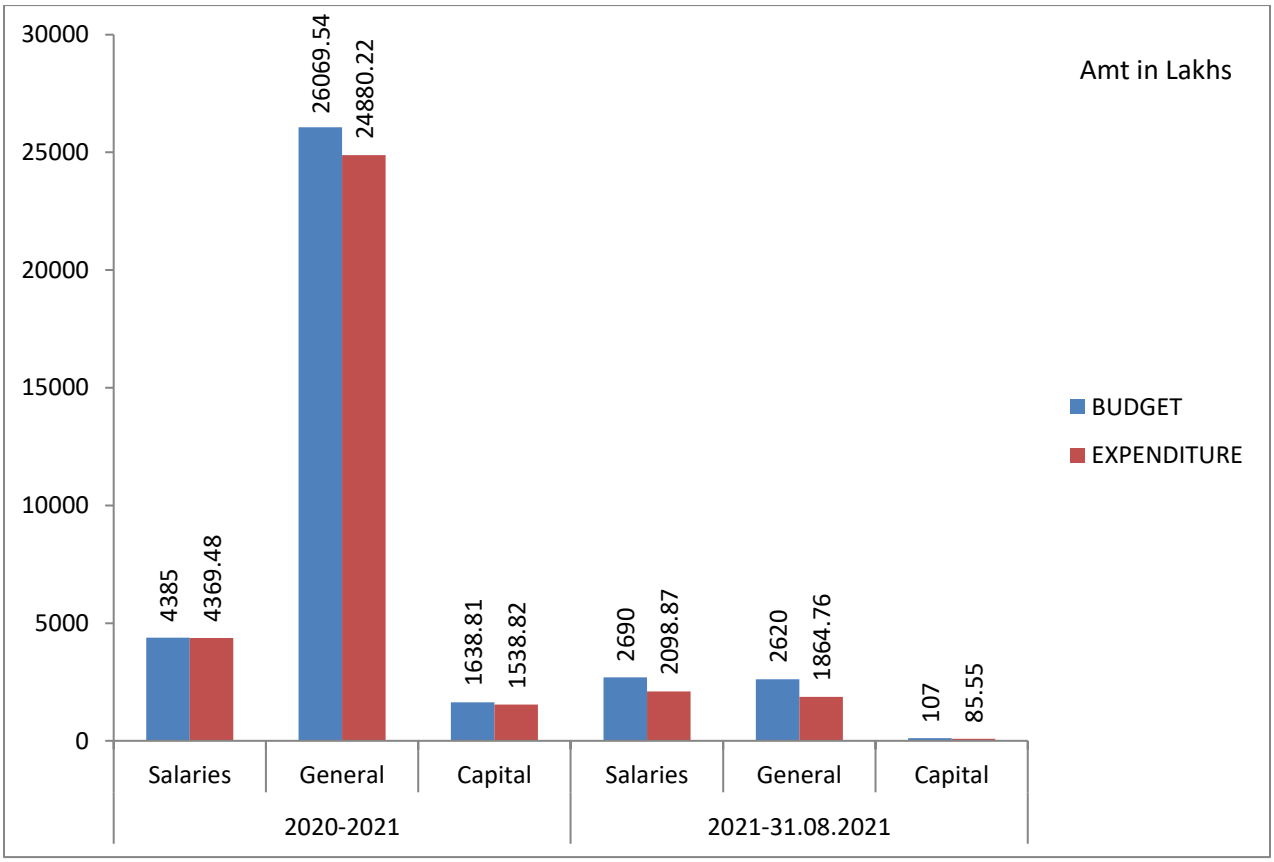


Details of Supplies





BUDGET ICMR-NIV PUNE (GRANT-IN-AIDS)



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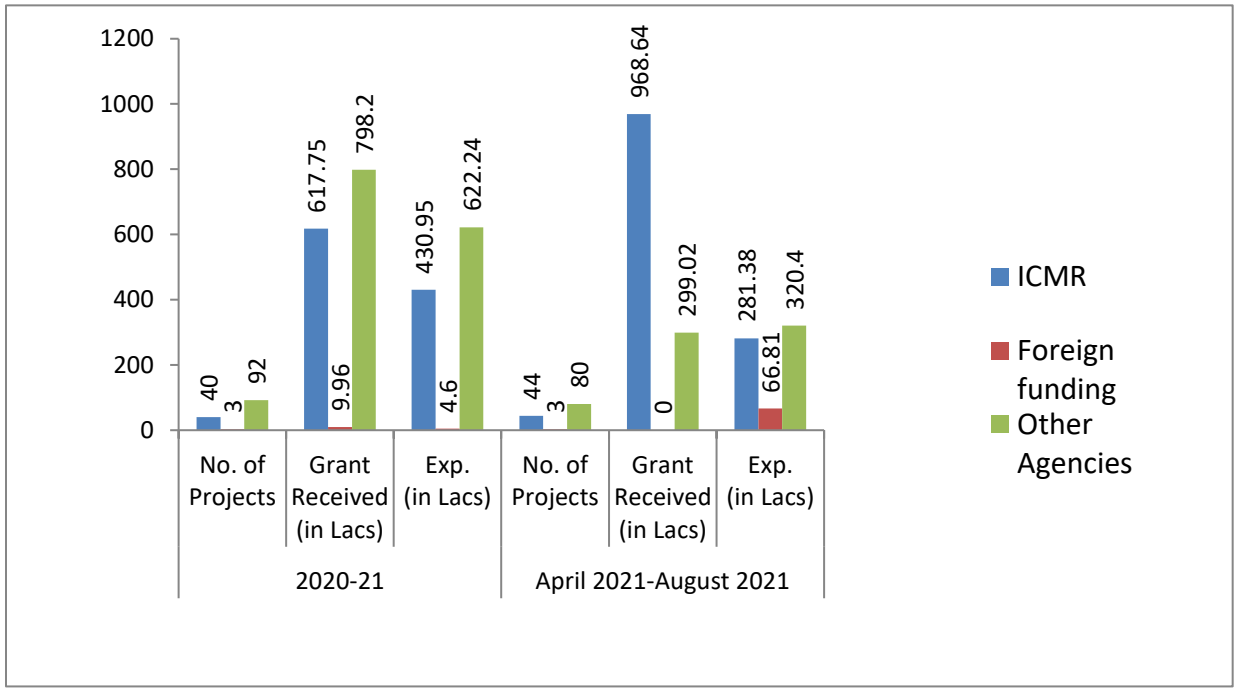
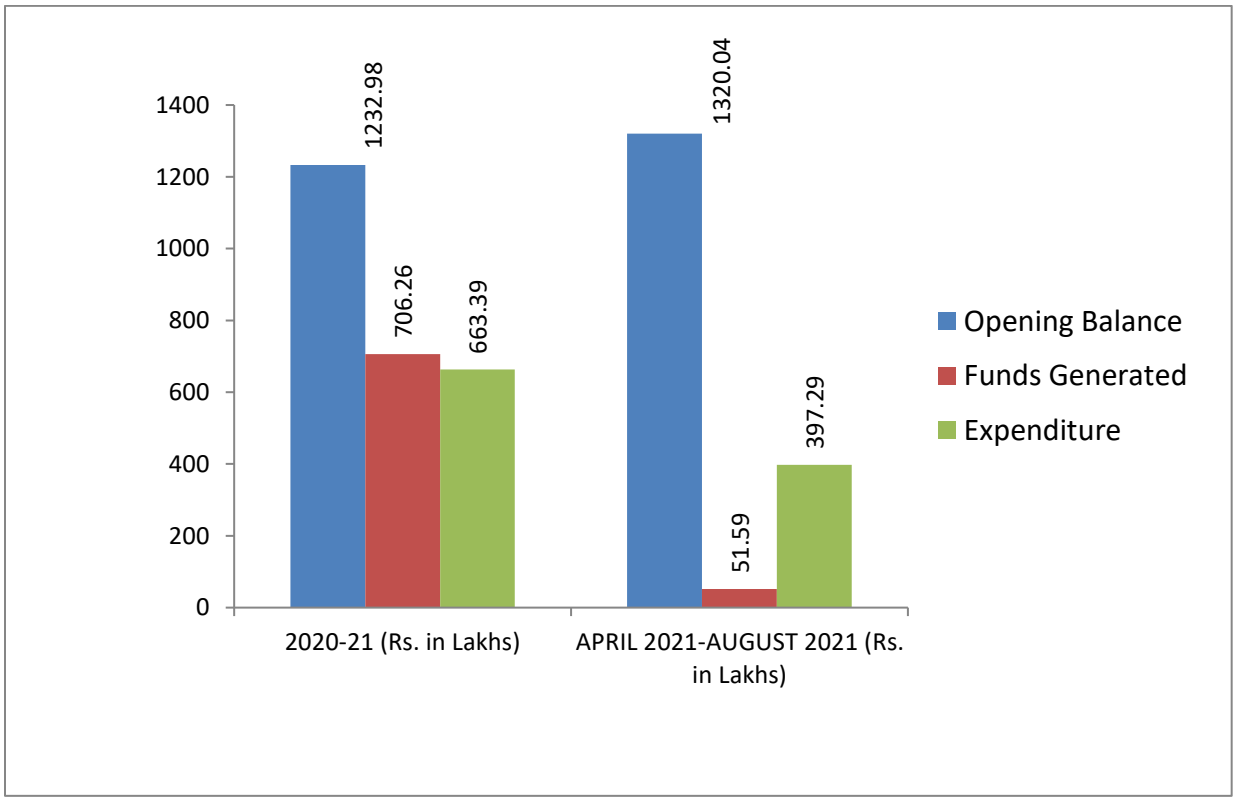
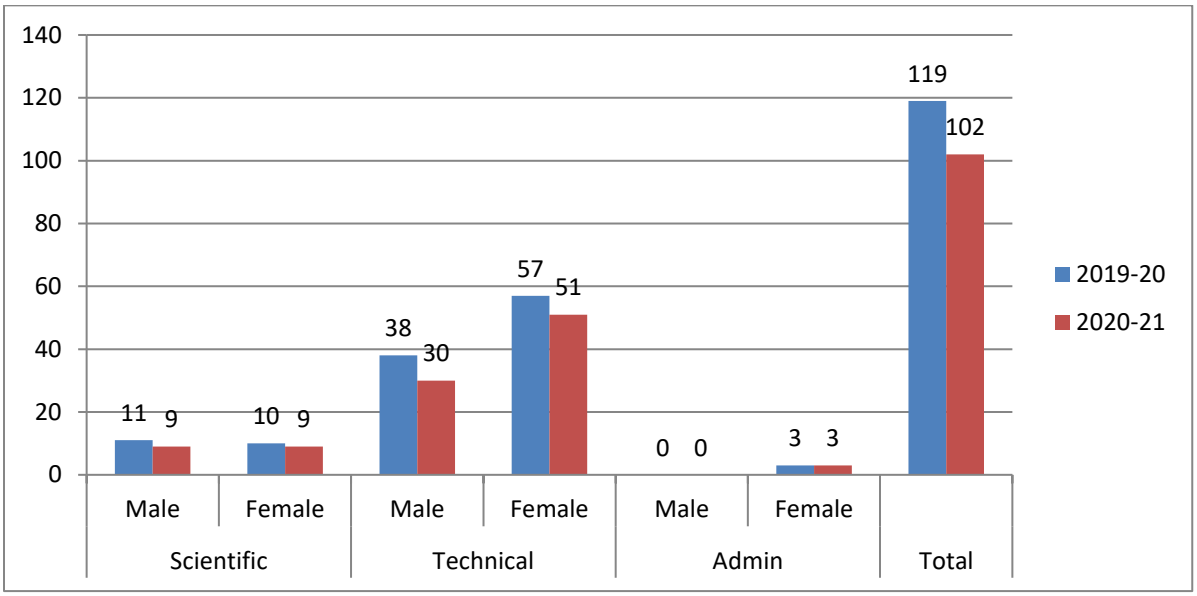
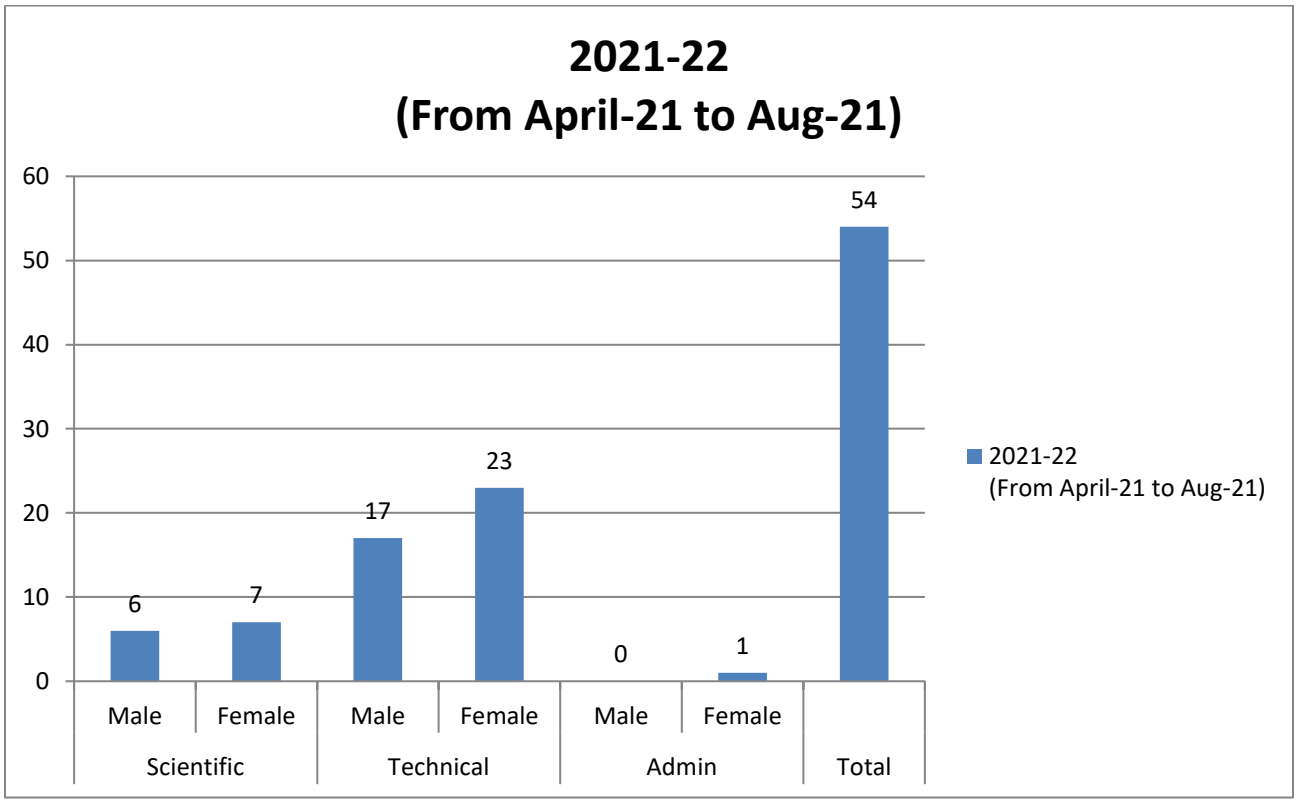


ICMR-NIV GENERATED FUNDS :



Project Staff Cadre wise Gender Distribution:





List of Papers Published by ICMR-NIV Scientists

April 2020- March 2021

Total Papers: 101

Published Papers: 96

Book Chapters: 05

Total Impact Factor: 398.701

Average Impact Factor: 4.862

SN	Particulars	Impact Factor
1.	Abraham P, Cherian S, Potdar V. Genetic characterization of SARS-CoV-2 & implications for epidemiology, diagnostics & vaccines in India. ***Indian J Med Res***. 2020 Jul & Aug;152(1 & 2):12-15. doi: 10.4103/ijmr.IJMR_3667_20.PMID: 32896836	2.375
2.	Agarwal A, Mukherjee A, Kumar G, Chatterjee P, Bhatnagar T, Malhotra P; PLACID Trial Collaborators (Kaushal H, Yadav PD, Sapkal G, Abraham P). Convalescent plasma in the management of moderate covid-19 in adults in India: open label phase II multicentre randomised controlled trial (PLACID Trial) ***BMJ.*** 2020 Oct 22;371:m3939. doi: 10.1136/bmj.m4232. PMID: 33144278	39.890
3.	Agrawal P, Sharma S, Pal P, Ojha H, Mullick J, Sahu A. The imitation game: a viral strategy to subvert the complement system. ***FEBS letters***. 2020 Aug;594(16):2518-42. doi.org/10.1002/1873-3468.13856. doi: 10.1002/1873-3468.13856. PMID: 32506518.	4.124
4.	Alagarasu K, Choudhary ML, Lole KS, Abraham P, Potdar V, NIC Team. Evaluation of RdRp & ORF-1b-nsp14-based real-time RT-PCR assays for confirmation of SARS-CoV-2 infection: An observational study. ***Indian J Med Res.*** 2020 May;151(5):483-5. doi: 10.4103/ijmr.IJMR_1256_20. PMID: 32474555	2.375
5.	Alagarasu K, Kakade MB, Bachal RV, Bote M, Parashar D, Shah PS. Use of whole blood over plasma enhances the detection of dengue virus RNA: possible utility in dengue vaccine trials. ***Arch Virol***. 2021 Feb;166(2):587-91. doi: 10.1007/s00705-020-04892-0. PMID: 33245437.	2.574
6.	Alagarasu K, Potdar VA, Vipat V, Hundekar S, Gunjikar R, Choudhary ML, Abraham P, Lole KS. Utility of a modified heat inactivation method for direct detection of SARS-CoV-2 by RT-qPCR in viral transport medium bypassing RNA extraction: A preliminary study. ***Indian J Med Res.*** 2020 Jul & Aug;152(1 & 2):108-10. doi: 10.4103/ijmr.IJMR_3121_20. PMID: 32952145	2.375
7.	Anukumar B, Mun AB. Ribavirin inhibits the Chandipura virus replication in Vero cells. ***J Med Virol.*** 2020 Dec;92:2969-75. doi: 10.1002/jmv.26184. PMID: 32543712.	2.327
8.	Atre NM, Khedkar DD. A Review on Herbal Remedies for Sexually Transmitted Infections (STIs) from Melghat Region of Maharashtra State, India. ***European J Med Plants.*** 2020 Sept;31(14):1-17. DOI: 10.9734/EJMP/2020/v31i1430313	NA
9.	Bagepally BS, Chaikledkaew U, Gurav YK, Anothaisintawee T, Youngkong S, Chaiyakunapruk N, McEvoy M, Attia J, Thakkinstian A. Glucagon-like peptide 1 agonists for treatment of patients with type 2 diabetes who fail metformin monotherapy: systematic	3.388

	review and meta-analysis of economic evaluation studies. ***BMJ Open Diabetes Res Care***. 2020 Jul;8(1):e001020. doi: 10.1136/bmjdrc-2019-001020. PMID: 32690574	
10.	Balakrishnan A, Malik N. Chandipura Virus' Oncolytic Potential inExperimentally Induced Tumor in Mice. ***Intervirology.*** 2021 Jan;64:48-51. doi: 10.1159/000512299. PMID: 33321508.	1.763
11.	Basawarajappa SG, Rangaiah A, Padukone S, Yadav PD, Gupta N, Shankar SM. Performance evaluation of Truenat™ Beta CoV &Truenat™ SARS-CoV-2 point-of-care assays for coronavirus disease 2019. ***Indian J Med Res.*** 2021 Jan-Feb;153(1-2):144-50. doi: 10.4103/ijmr.IJMR_2363_20. PMID: 33818471.	2.375
12.	Basu A, Sandhu H. International Conventions & One Health***. Indian J Med Res***. 2021 Mar;153(3):253-5. doi: 10.4103/ijmr.IJMR_644_21. PMID: 33906986.	2.375
13.	Bhatia R, Abraham P. Lessons learnt during the first 100 days of COVID-19 pandemic in India. ***Indian J Med Res.*** 2020 May;151(5):387-91. doi: 10.4103/ijmr.IJMR_1925_20. PMID: 3261190	2.375
14.	Bhatia R, Abraham P. The enigmatic COVID-19 pandemic. ***Indian J Med Res***. 2020 Jul & Aug;152(1 & 2):1-5. doi: 10.4103/ijmr.IJMR_3639_20. PMID: 32893843	2.375
15.	Bhukya PL, C VK, Lole KS. Transcriptome analysis of hepatoma cells transfected with Basal Core Promoter (BCP) and Pre-Core (PC) mutant hepatitis B virus full genome construct. ***J Gen Virol***. 2021 Mar;102(3). doi: 10.1099/jgv.0.001568. PMID: 33595430	3.891
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राजभाषा रिपोर्ट

संस्थान, भारत सरकार के राजभाषा विभाग द्वारा जारी नियमोंका एवं भारतीय आयुर्विज्ञान अनुसंधान परिषद, नई दिल्ली के आदेशों का अनुपालन करने के लिए सदैव कार्यरत हैं ।

(3) की उपधारा 3 की धारा 1973 राजभाषा अधिनियम का अनुपालन करते हुए संस्थान के अधिकतम दस्तावेज़ द्विभाषी हिंदी और अंग्रेजी -भाषा में अग्रेषण किया जाता हैं ।

(4)10 के नियम 1976 राजभाषा नियमके अनुसार संस्थान के कर्मचारीयोंको हिंदी का कार्यसाधक ज्ञान प्राप्त करने हेतु हिंदी शिक्षण योजना तथा अनुवाद प्रशिक्षण केंद्र(मुंबई) द्वारा आयोजित प्रशिक्षण वर्गों में नामित किया जा रहा है । इस साल हिंदी शिक्षण योजना तथा अनुवाद प्रशिक्षण केंद्र(मुंबई(द्वारा आयोजित परीक्षाओं में उत्तीर्ण कर्मचारिओं की संख्य निम्नवत है :

हिंदी टंकण – हिंदी शब्द संसाधन /2 और अनुवाद प्रशिक्षण केंद्र, मुंबई द्वारा आयोजित प्रारंभिक अनुवाद प्रमाणपत्र प्रशिक्षण -2

नगर राजभाषा कार्यान्वयन समिति द्वारा आयोजित की जा रही तिमाही बैठकों में संस्थान के अधिकारी उपस्थित रहते हैं । परिषद् द्वारा प्राप्त निर्देश तथा संस्थान की राजभाषा कार्यान्वयन समिति द्वारा लिए गए निर्णयानुसार संस्थान में हिंदी सप्ताह मनाया गया । संस्थान में आतंकवाद विरोध दिन, सतर्कता जागरूकता सप्ताह, कौमी एकता सप्ताह, स्वच्छ भारत अभियान सप्ताह के उपलक्ष्य में हिंदी भाषा में शपथ ग्रहण संपन्न करवाते हुए व्याख्यान, संगोष्ठी तथा विभिन्न प्रतियोगिताओं का आयोजन कर मनाया गया ।