ICMR-NATIONAL INSTITUTE OF VIROLOGY, PUNE

MADE IN INDIA
TECHNOLOGIES

The ICMR-National Institute of Virology has completed 70 years of its existence. This handbook on "Made in India Technologies" by ICMR-NIV on the occasion of Azadi ka AmritMahotsav (March 2021-August 2022) is a comprehensive compilation of technologies developed in-house by ICMR-NIV scientists over the period of its functioning.

Sixty years ago scientists from ICMR-NIV successfully isolated the Kyasanur Forest Disease (KFD) virus from the Kyasanur forest of Karnataka state. The first indigenous technology developed at the then Virus Research Centre (VRC) was the KFD vaccine and the same was transferred to the Karnataka government. In the last decade, the technology for the preparation of inactivated Japanese Encephalitis (JE) vaccine was transferred to biotech industry. During the currently ongoing COVID-19 pandemic, ICMR-NIV scientists isolated the severe acute respiratory syndrome coronavirus-2 (SARS CoV-2) and the same was transferred to a manufacturing industry for the development of the indigenous vaccine for COVID-19. Apart from vaccine development, ICMR-NIV has also contributed in developing molecular and serological diagnostic kits. In the recent past, ICMR-NIV has developed several indigenous diagnostic kits for the viruses of public health importance. Some of these are being used in communicable disease control programs launched by National Vector Borne Disease Control Programme (NVBDCP) and Department of Health Research (DHR). Diagnostic kits were developed for the diseases which include JE, dengue, chikungunya, Crimean Congo Hemorrhagic Fever (CCHF), nipah virus encepalitis, hepatitis A & E, viral diarrhoea, bronchiolitis/ pneumonia, measles and Chandipura encephalitis.

The establishment of the bio-safety level 4 (BSL-4) containment facility, first of its kind in Asia, made it possible to isolate the SARS-CoV-2 virus not only to develop a vaccine candidate, but also to develop diagnostic kits for it. It is worth mentioning that the SARS CoV-2 RT-PCR diagnostic kit developed at ICMR-NIV facilitated diagnosis of Covid-19 across the country and indeed led the nation to rapidly follow the 'TTT' protocol of 'Trace, Test and Treat'. The indigenous human SARS CoV-2 IgG ELISA (Kavach) technology
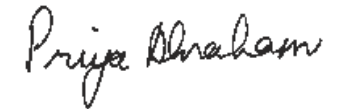
was transferred following an expression of interest to seven biotech companies.

I am confident that ICMR-NIV scientists would continue to develop newer technologies which will provide accurate and affordable options for our country in the times to come. I am sure that this handbook would be helpful not only to the younger scientists but also to the clinicians and healthcare personnel. I congratulate the scientists and staff of ICMR–NIV who have contributed to the development of the technologies and also the authors of this handbook for taking painstaking efforts to publish this handbook on the occasion of Azadi ka AmritMahotsav The handbook is indeed in sync with the Mahotsav which is dedicated to the people of India who have not only been instrumental in bringing India thus far in its scientific evolutionary journey but also echoes Honourable Prime Minister, Shri Narendra Modi's vision of Atmanirbhar Bharat (self-reliance).

I am thankful to the reviewers including senior scientists, Dr. P. S. Sathe, Dr. S. D. Chitambar, Dr. P. S. Shah and my senior colleagues, Dr. K. S. Lole, and Dr. A. B. Sudeep for their invaluable inputs in critically reviewing and providing insightful suggestions to finalize the write ups. The vital role played by the ICMR headquarters for their constant support in facilitating the transfer of technologies, is deeply appreciated. The efforts of Dr. Sarah Cherian and Dr Kanchankumar Patil in editing and coordinating the timely compilation of this book are much acknowledged.

Prof. Priya Abraham
Director, ICMR-NIV, Pune

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Serological Assays

1
Sheep / Goat CCHF IgG ELISA

a. **Name of the test / assay:** Sheep / Goat CCHF IgG ELISA

b. **Authors:** Dr. DT Mourya, Dr. PD Yadav and Dr. PS Sathe

c. **Background:**

Crimean-Congo hemorrhagic fever (CCHF) is a highly infectious tickborne disease caused by a virus CCHF virus (CCHFV) that belongs to family Nairoviridae genus *Orthonairovirus*. The disease is widespread in various countries in Africa, Asia, southeastern Europe and Eurasia. Cases have been documented recently in India. The virus is transmitted to humans by tick (*Hyalomma spp*) bite. CCHFV circulates in an enzootic tick-vertebrate-tick cycle. Ticks feed on domestic animals like cattle, sheep, goat and thus these animals play an important role in amplification and spread of the virus. Cattle, sheep or goat develop viremia lasting up to two weeks without any clinical signs or symptoms, followed by seroconversion. Presence of CCHFVs specific IgG antibodies in these animals would; therefore, be the first indication of virus circulation in a given region and is thus a valuable tool for determining the endemic status.

There is no commercial ELISA kit for detection of anti-CCHF IgG antibodies in sheep or goat. Anti-CCHF IgG animal screening ELISA test developed by CDC; USA is not available commercially. NIV has developed a safe, reliable, sensitive screening ELISA to monitor CCHFV specific IgG antibodies in sheep / goat that can be used even in remote health care facilities.

d. **Principle of the test:** IgG ELISA

IgG antibodies from serum of sheep/goat bind to the CCHFV (antigen) coated on to the solid surface (ELISA wells) but do not bind to the Normal Cell Slurry (Control antigen). In the next step, Biotin labeled anti-sheep IgG antibodies (Fc specific) is added that bind to Sheep IgG. Following incubation, the step is followed by addition of Avidin-HRP. After incubation, the wells are washed to remove any unbound Avidin-HRP. Subsequently, chromogenic substrate (TMB/H_2O_2) is added, the reaction is stopped by 1N H_2SO_4. Optical density is measured at 450 nm.

e. **Performance characteristics:**

One hundred and fifty-one sera samples; 55 CCHF IgG positive and 96 CCHF IgG negative, by CCHF animal IgG ELISA kit (CDC, Atlanta, USA) were used for validation (sensitivity and specificity) of Sheep / Goat CCHF IgG ELISA (NIV, Pune).

		CCHF IgG Positive or Negative by reference kit from CDC, USA.	
		Positive (n=55)	Negative (n=96)
Sheep / Goat CCHF IgG ELISA (NIV)	Positive	35	0
	Negative	20	96

Sensitivity	63.6%
Specificity	100%
Positive Predictive Value	100%
Negative Predictive Value	82.8%

Intra and inter assay variability (%CV) was 4% and 5% respectively.

f.   External Quality Assurance:

Three National level laboratories (NIHSAD-Bhopal, Microbiology department, IISc-Bangalore, NIRTH-Jabalpur) were provided with 20 coded serum samples (3 positive & 17 negative) and assay reagents. Results from all three laboratories showed 100% concordance with the reference results.

g.   Intended use:

Sheep/Goat CCHF IgG ELISA kit is intended for qualitative detection of IgG antibodies in the sheep/goat serum samples (Emerg Infect Dis. 2015;21(10):1837-1839).

h.   Translational Status:

Technology of the Sheep / Goat CCHF IgG ELISA has been transferred to biotech industry for the purpose of commercialization.

I.   Impact:

This assay was used for the nationwide cross sectional serosuvey of CCHF among sheep/goat population in India. Seropositivity levels found in these animals suggested prevalence of CCHFV infection among livestock across the country.

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2
Cattle CCHF IgG ELISA

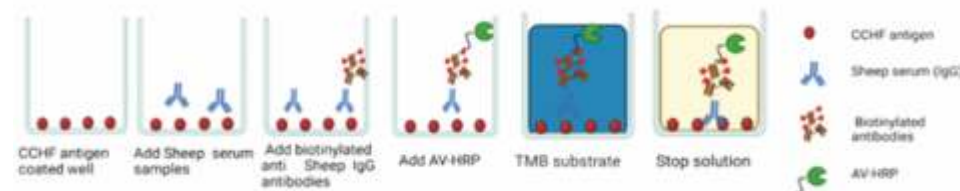**a.   Name of the test / assay:** Cattle CCHF IgG ELISA.

b.   Authors: Dr. DT Mourya, Dr. PD Yadav and Dr. PS Sathe

c.   Background:

CCHF causes severe illness in humans and has a case-fatality rate of up to 80%. The disease is widespread in various countries in Africa, Asia, southeastern Europe, and Eurasia, and cases have been documented in India. The virus is transmitted to humans by tick (*Hyalomma spp*) bite. The ticks are usually found on cattle, buffalo, goats, and sheep and thus these animals play an important role in amplification and spread of the virus. Viremia in cattle lasts for two weeks without any clinical signs or symptoms, followed by seroconversion. Presence of CCHFV specific IgG antibodies in cattle would; therefore, indicate virus circulation in a given region. There is no commercial ELISA kit for detection of anti-CCHF IgG antibodies in cattle and the one developed by CDC, USA is not available commercially. Hence, NIV has developed a safe, sensitive screening ELISA to monitor CCHFV specific IgG antibodies in cattle that can be used even in remote health care facilities.

d.   Principle of the test:

This is a qualitative ELISA for the detection of anti CCHF IgG antibodies in cattle. Cattle serum specimens are added to ELISA wells coated with

inactivated CCHFV antigen as well as to the control wells coated with uninfected vero cell antigen. After incubation and washing, cattle antibodies targeting CCHFV remain bound to the antigen whereas non-specific antibodies are removed during washing step. In the next step, biotin labeled anti-bovine IgG antibodies are added that bind to cattle IgG antibodies. Following incubation this step is followed by addition of Avidin-HRP. After incubation, the wells are washed to remove the unbound Avidin-HRP. Subsequently, chromogenic substrate (TMB/H$_2$O$_2$) is added, the reaction is stopped by 1N H$_2$SO$_4$. The optical density is measured at 450 nm.

e. Performance characteristics:

One hundred and twelve sera samples (36 CCHF IgG antibody positive and 76 CCHF IgG antibody negative by CCHF animal IgG ELISA kit (CDC, Atlanta, USA) were used for validation (sensitivity and specificity) of cattle CCHF IgG ELISA (NIV, Pune).

f. External Quality Assurance:

Four national level laboratories (NIHSAD-Bhopal, BJMC -Ahmadabad, Microbiology Dept, IISc-Bangalore, NIRTH-Jabalpur) were given panel of 20 (4 positive and 16 negative) coded samples & assay reagents. Results from all four laboratories showed 100% concordance with ICMR-NIV Pune.

		Cattle CCHF IgG Positive or Negative by Reference test CDC, USA.	
		Positive (n=36)	Negative (n=76)
Cattle CCHF IgG ELISA (NIV)	Positive	29	03
	Negative	07	73

Sensitivity	80.56%
Specificity	96.05%
Positive Predictive Value	90.63%
Negative Predictive Value	91.25%

Intra and inter assay variability (%CV) was 2% and 5% respectively.

g. Intended use:

Assessment of CCHF prevalence in livestock and screening of ticks and nymphs for the presence of CCHFV would give a better understanding for identifying the CCHF high-risk areas (*Emerg Infect Dis. 2015;21(10):1837-1839*). It can be used at clinical set up, public health centers and veterinary hospitals.

h. Translational Status:

Technology of the Cattle CCHF IgG ELISA has been transferred to biotech industry for the purpose of commercialization.

I. Impact:

This assay was used for the nationwide cross sectional serosuvey of CCHF among cattle population in India. Seropositivity levels found in these animals suggested prevalence of CCHFV infection among livestock across the country.

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3
Human Crimean Congo Hemorrhagic Fever (CCHF) IgM ELISA

a.   **Name of the test / assay:** Human CCHF IgM ELISA.

b.   **Authors:** Dr. PD Yadav, Dr. AM Shete and Dr. DT Mourya

c.   **Background:**

Crimean-Congo hemorrhagic fever (CCHF) is a highly infectious tickborne disease caused by ssRNA virus (CCHFV) that belongs to the family Nairoviridae genus *Orthonairovirus*. The disease is widespread in various countries in Africa, Asia, south-eastern Europe and Eurasia. Human cases have been documented from India.

The virus is transmitted to humans by the bite of infected ticks of Hyalomma spp or by contact with blood, body fluids or tissues of viraemic animals when they are slaughtered or related procedures or by consumption of infected meat. In addition, nosocomial infections are frequently reported. The pathogen has very low human-to-human transmission. Symptoms of CCHF infection in humans include haemorrhagic fever, flu-like symptoms, kidney failure, and hepatomegaly with high mortality rates. Human fatal cases have been reported from India in 2011. There is neither a vaccine nor an antiviral and therefore treatment is mainly supportive.

Human clinical sample during CCHF viraemic phase poses a high biohazard risk and can only be handled in BSL - 4 laboratories. The virus specific IgM antibodies appear in circulation 2-5 days post onset and therefore can be handled in district health laboratories in the endemic area.

There is only one commercial ELISA kit (Vector Best, Russia) for detection of Human CCHF IgM antibodies & is very expensive for developing countries. ELISA for screening CCHF virus specific Human IgM antibodies; developed by CDC, USA is not commercially available. Considering the need of a cost effective, sensitive and specific assay for detection of CCHFV specific IgM in humans, the present test was standardized.

d.   **Principle of the test:**

CCHF IgM Positive Control, CCHF IgM Negative Control and diluted serum samples are added to the earmarked wells coated with anti-human IgM antibodies. After incubation the wells are washed and inactivated CCHF antigen is added to each well. After incubation and washing step, biotin labelled anti CCHF antibodies (probe) is added to each well. After incubation and washing this is followed by addition of Streptavidin–HRP. Subsequently after washing, chromogenic substrate (TMB/H_2O_2) is added. An acidic stopping solution is then added and the enzymatic turnover of the substrate is determined by absorbance measurement at 450 nanometres.
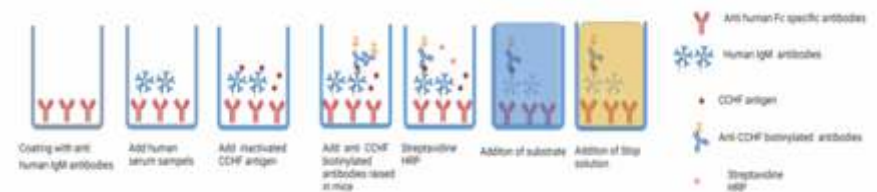
e.   **Performance characteristics:**

One hundred and fifty-eight sera samples (23 CCHF IgM positive and 135 CCHF IgM negative by the commercial kit (Vector Best) were used for validation (sensitivity and specificity) of the human CCHF IgM ELISA (NIV, Pune).

		CCHF IgM Positive or Negative confirmed by Vector Best (Reference kit)	
		Positive (n=36)	Negative (n=76)
Human CCHF IgM ELISA (NIV)	Positive	21	2
	Negative	02	133

Sensitivity	91.3%
Specificity	98.5%
Positive Predictive Value	91.3%
Negative Predictive Value	98.5%

The Human CCHF IgM ELISA is specific for detection of anti CCHF IgM antibody in human serum and showed no cross reactivity with Dengue (n=20), JE (n=20), Chandipura (n=20), RVF (n=2), Chikungunya (n=10), and Rheumatoid factor (n=20) positive human samples. The precision analysis revealed that the assay is robust and reproducible in different sets of conditions. Intra and inter assay variability (%CV) was 3% and 5% respectively.

f.   External Quality Assurance:

These National level laboratories (NIHSAD-Bhopal, BJMC-Ahmadabad, ICMR-NIRTH-Jabalpur) were provided with a panel of 10 coded samples (2 positive and 8 negative) and assay reagents. All three laboratories provided concordant results.

g.   Intended use:

The serological assay is designed for providing presumptive diagnosis of CCHFV in sporadic cases as well as during outbreak investigations (PLoS Negl Trop Dis 6(5): e1653). It can be used at clinical set up, PHCs and hospitals.

h.   Translational Status:

Technology of the Human CCHF IgM ELISA has been transferred to biotech industry for the purpose of commercialization.

I.   Impact:

An indigenously developed Human CCHF IgM ELISA kit was of immense use for screening of antibodies against this virus in India. Using this acute case was detected and antibody kinetics was studied using the clinical specimens of CCHF cases and their follow-up referred from various part of the county.

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4
Human Crimean Congo hemorrhagic fever IgG ELISA

a.   Name of the test / assay: Human CCHF IgG ELISA.

b.   Authors: Dr. PD Yadav, Dr. AM Shete and Dr. DT Mourya

c.   Background:

Crimean Congo Hemorrhagic Fever (CCHF) is a highly infectious zoonotic disease transmitted by *Hyalomma* ticks. Earlier studies have shown CCHF seroprevalence in livestock throughout India, Occupational hazards of CCHF for animal handlers, veterinarians, abattoir workers, and healthcare workers have been documented. CCHFV is maintained by vertical and horizontal transmission cycles involving ixodid ticks and a variety of wild and domestic vertebrates, which do not show signs of illness. Laboratory tests that are used to diagnose CCHF include antigen-capture enzyme-linked immunosorbent assay (ELISA), real time polymerase chain reaction (RT-PCR), virus isolation and detection of virus specific antibody by ELISA (IgG and IgM). There is only one commercial ELISA kit from Vector Best for detection of anti-CCHF IgG antibodies in Humans. Human CCHF IgG screening ELISA test developed by CDC, USA is not available commercially. There is need for a rapid, sensitive & specific ELISA for detection of CCHF IgG in Human.

d.   Principle of the test:

Diluted serum samples, Positive and Negative controls are added to the earmarked ELISA wells coated with inactivated CCHF antigen. After incubation & washing, HRP labelled anti-human IgG (conjugate) is added to each well. Excess conjugate is removed by washing. In the next steps ELISA substrate (TMB) is added to each well. The reaction is terminated by addition of Stop solution (1N H_2SO_4) and absorbance is read at 450 nm using an ELISA plate reader.
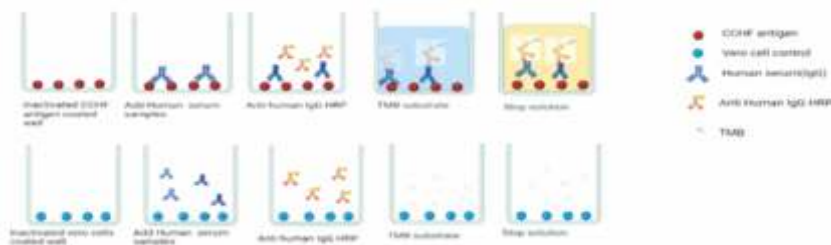
e. Performance characteristics:

One hundred & ninety-three human sera (36 CCHF IgG positive and 157 CCHF IgG negative by the reference kit (Vector Best) were used for validation (sensitivity and specificity) of the Human CCHF IgG ELISA (NIV, Pune).

		CCHF IgG Positive or Negative confirmed by Vector Best (Reference kit)	
		Positive (n=36)	Negative (n=157)
Human CCHF IgG ELISA (NIV)	Positive	35	01
	Negative	01	156

Sensitivity	97.22%
Specificity	99.36%
Positive Predictive Value	97.22%
Negative Predictive Value	99.36%

The Human CCHF IgG ELISA did not show any cross reactivity with Hantaan (n=10), RVF (n=5), Chikungunya (n=10), Dengue (n= 10), RF positive samples (n= 10) indicating that the test is specific for detection of CCHF IgG antibody from human samples. Intra and inter assay variability (%CV) was 2% and 4% respectively.

f. External Quality Assurance:

The three national level laboratories (BJMC-Ahmedabad, GMERS Medical College-Ahmedabad, NIRTH-Jabalpur) were provided with 30 coded samples (5 positive and 25 negative samples) and assay reagents. All three laboratories provided 100% concordant results with the NIV laboratory.

h. Intended use:

Human CCHF IgG ELISA test is intended for qualitative detection of IgG antibodies in the human serum samples (BMC Infect Dis 19, 104 (2019)). It can be used as a tool for sero surveillance to understand the activity of CCHF in any area.

i. Translational Status:

Technology of the Human CCHF IgG ELISA has been transferred to biotech industry for the purpose of commercialization.

j. Impact:

An indigenous developed Human CCHF IgG ELISA kit was of great use for screening of IgG antibodies against this virus in India. The seroprevalence of CCHF among human population was determined using the developed assay.

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5
# Human Kyasanur Forest Disease IgM ELISA

a. **Name of the test/ assay:** Human KFD IgM ELISA

b. **Authors:** Dr. PD Yadav, Dr. AM Shete and Dr. DT Mourya

c. **Background:**

Kyasanur Forest disease virus (KFDV); a member of the genus *Flavivirus* (family *Flaviviridae*), was first recognized in Sagar and Sohrab taluks of Shimoga, Karnataka in 1957. It causes seasonal outbreaks during the months of January to June. The disease; endemic to Karnataka state, has also been reported from neighboring states like Tamil Nadu, Goa, Maharashtra and Kerala during the last decade. All these outbreaks alert about circulation of KFDV outside Karnataka and need for a rapid, sensitive and specific assay for monitoring the disease activity in human. At present, there is no commercial diagnostic ELISA kit available for detection of anti-KFD IgM antibodies in human.

d. **Principle of the test:**

Diluted patient's serum samples, known Positive and Negative controls are added to the earmarked ELISA wells coated with anti-human IgM (μ chain specific). In the next step, KFD antigen is added which binds to captured human IgM, unbound antigen is removed during the washing step. In the next step, biotinylated anti KFD antibodies are added to each well. After incubation and washing this is followed by addition of Streptavidin–HRP. Subsequently after washing, chromogenic substrate (TMB/H_2O_2) is added, the reaction is stopped by 1N H_2SO_4. Optical Density is measured at 450nm. The kit is for in vitro use only for monitoring KFDV specific IgM antibodies in human serum.
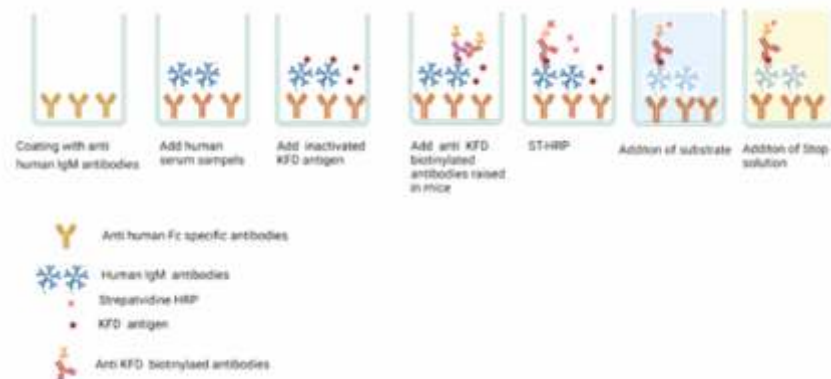
e. **Performance characteristics:**

Assay has been checked for the specificity using panel of IgM antibody positive human serum samples against Japanese encephalitis (n=10), Chandipura (n=10), West Nile (n=10), Dengue (n=50) and Leptospira samples (n=10), RH (rheumatoid) positive and negative samples (n=10). Dengue virus IgM positive human serum samples (20 %) showed cross reactivity with human KFD IgM ELISA, which is common across the flaviviruses.

In the absence of gold standard; for comparison of assay sensitivity, serum samples from 65 follow-up cases of known KFDV real time RT-PCR positive cases were used. Intra and inter assay variability (%CV) was 4% and 5% respectively.

f. **External Quality assurance:**

Three National level laboratories (NIRTH-Jabalpur, NIHSAD-Bhopal, Microbiology department, IISc-Bangalore) were provided with 10 coded samples and assay reagents. All three laboratories showed 100% concordance with the reference results

g. **Intended use:**

KFD IgM capture ELISA kit is intended for qualitative detection of IgM antibodies in the serum of patients presenting clinical signs and symptoms consistent with febrile illness and hemorrhages suspected of KFD (*Journal of virological methods, 186(1-2), 49–54*). The serological assay is designed for outbreak investigations and identifying etiological agent

causing KFD. It can be used at clinical set up, public health centers and hospitals.

h.   Translational Status:

Technology of the Human KFD IgM ELISA has been transferred to biotech industry for the purpose of commercialization.

I.   Impact:

Human KFD IgM ELISA had been instrumental in understanding the antibody kinetics in KFD infected patients. This assay has been helpful in the serodiagnosis of the KFD.

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6
Human Kyasanur Forest Disease IgG ELISA

a.   Name of the test/ assay developed: Human KFD IgG ELISA.

b.   Authors/Inventors: Dr. DT Mourya, Dr. PD Yadav and Dr. AM Shete

c.   Background:

Kyasanur forest disease virus (KFDV), belonging to the family *Flaviviridae*, is considered as one of the high-risk category pathogens. There is no commercial ELISA kit for detection of KFD IgG antibodies in humans. Availability of Human KFD IgG ELISA test would be helpful in serosurveys to understand how widespread the disease is.   In spite of routine vaccination and control measures, KFD cases are reported in Karnataka, Kerala and Maharashtra.

d.   Principle of the test:

In the KFD IgG ELISA assay, the polystyrene wells are coated with KFD antigen (inactivated virus). Diluted serum samples and controls are added to the earmarked wells and incubated to allow specific antibody present in the samples to bind to the antigen. Nonspecific reactants are removed by washing and peroxidase-conjugated anti-human IgG is added. Excess conjugate is removed by washing. In the next step ELISA substrate (TMB/H_2O_2) is added to each well. TMB is catalyzed by the HRP to produce a blue color product. The reaction is terminated by addition of Stop solution (1N H_2SO_4) that changes the blue color to yellow. Absorbance is read at 450 nm using an ELISA plate reader.
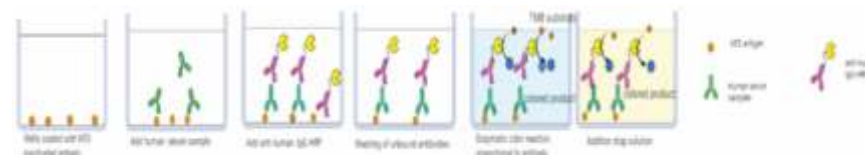
e. Performance characteristics:

Specificity of the assay was evaluated using panel of Flavivrus IgG positive and negative human serum samples (Japanese encephalitis (n=10), West Nile (n=10) and Dengue IgG (n=50) in addition to human sera from RH (rheumatoid) positive (n=5) and Leptospira IgG (n=10) positive and negative samples. None of them showed any cross reactivity except Dengue virus IgG positive human serum samples. 20% of cross reactivity of dengue IgG positive samples was observed which is *common* across the flaviviruses.

Intra and inter assay variability (%CV) was 2% and 3% respectively.

f. External Quality assurance:

External quality assessment was performed at National Institute for Research in Tribal Health, Jabalpur, National Institute of High Security Animal Disease, Bhopal VRDL and King George's Medical University, Lucknow. A panel of coded samples (n= 20) and assay reagents were sent to these laboratories All laboratories provided 100% concordant data with coded samples.

g. Intended use

Human KFD IgG ELISA is intended for qualitative detection of IgG antibodies in the serum of KFD patients *(Indian J Med Res. 2019;150(2):186-193)*. The serological assay is designed to understand the prevalence of KFD. It can be used at clinical set up, public health centers or Sentinel hospitals.

h. Translational Status:

Technology of the Human KFD IgG ELISA has been transferred to biotech industry for the purpose of commercialization.

i. Impact:

The seroprevalence of KFD among human population of different geographical location was determined using the developed assay.

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7
Human Nipah IgM ELISA

a. Name of the test / assay: Human Nipah IgM ELISA

b. Authors: Dr. PD Yadav, Dr. AM Shete and Dr. Sreelekshmy M

c. Background:

Nipah virus (NiV) is an emerging *paramyxovirus* capable of causing lethal infections in a number of mammalian species including humans. NiV is classified as a Biosafety level 4 pathogen due to the high morbidity and mortality associated with the virus andlack of approved vaccine or treatment. Low cost, easy-to-use and sensitive diagnostic assays would be useful to curb the Nipah infection and would help possible interventions.

d. Principle of the test:

The human sera to be tested for Nipah IgM are diluted and added to the earmarked ELISA wells coated with anti-human IgM (μ chain specific). Similarly known positive and negative controls are also added to the respective wells. IgM antibodies in the human serum will bind with anti-human IgM antibodies coated on the ELISA wells. After incubation and washing, inactivated Nipah antigen is added to each well. Unbound antigen is removed during the washing step and anti Nipah IgG raised in mice was added to wells. After further incubation and washing, anti mouse IgG HRP was added. Thereafter, chromogenic substrate (TMB/H_2O_2) is added, and the reaction is stopped by 1N H_2SO_4. The intensity of color / Optical Density is measured at 450nm.
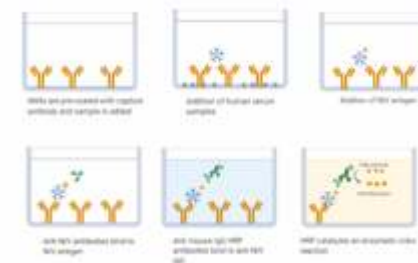
e. Performance characteristics:

Two hundred and ninety-seven human sera (18 Nipah IgM positive and 279 negatives by reference test) were used for validation (sensitivity and specificity) of the Human Nipah IgM ELISA (NIV, Pune)

The Human Nipah IgM ELISA did not show any cross reactivity with Rubella (n=10), Measles (n=10), Influenza (n=15), Mumps (n=5), Herpes (n=5), Crimean Congo hemorrhagic fever (CCHF) (n=5), Kyasanur forest disease (KFD) (n=5) indicating that the test was specific for detection of Human Nipah IgM antibody from human samples. Intra and inter assay variability (%CV) was 2% and 4% respectively.

		Nipah IgM Positive or Negative confirmed by CDC, USA	
		Positive (n=18)	Negative (n=279)
In-house Human Nipah IgM ELISA	Positive	18	01
	Negative	0	278

Sensitivity	100%
Specificity	99.64%
Positive Predictive Value	94.74%
Negative Predictive Value	100%

f. External Quality Assurance:

External quality testing was performed at Institute of Epidemiology Disease Control and Research (IEDCR), Dhaka Bangladesh using a panel of 20 coded samples (3 positive and 17 negative). The results from the laboratory were in 100% concordance with the results of ICMR–NIV, Pune.

g. Intended use:

Anti-NiV IgM capture ELISA is intended for qualitative detection of IgM antibodies in the serum of patients presenting clinical signs and symptoms consistent with febrile illness and suspected of NiV. The serological assay is designed for providing presumptive diagnosis of NiV in sporadic cases / outbreak investigations. It can be used at public health centers or sentinel hospitals.

h. Translational Status:

This test is being used within ICMR-NIV laboratories.

I. Impact:

Understanding of NiV IgM antibody kinetics could be possible due to this indigenous assay. It was first time reported from India that IgM could be detected $\leq$ 2 months after NiV infection. NiV-specific IgM ELISA is an alternative serological approach where PCR is not available.

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8
Human Nipah IgG ELISA

a. Name of the test / assay: Human Nipah IgG ELISA

b. Authors: Dr. PD Yadav and Dr. AM Shete

c. Background:

Nipah is one of the priority diseases that need urgent action. Since its detection in 1998, many outbreaks of Nipah have been reported from Malaysia, Singapore, Bangladesh and India. It is a serious public health threat for the countries in Southeast Asia. During last two decades (2001 – 2021), India has reported five Nipah outbreaks among human population in West Bengal and Kerala. There is no information available on the persistence of the antibodies in Nipah positive cases amongst symptomatic and asymptomatic contacts.

d. Principle of the test:

Positive / Negative Control and Human sera samples to be tested for NiV IgG are diluted and added to the earmarked ELISA wells coated with inactivated NiV antigen. IgG antibodies from human serum (if present) & Positive Control will bind to the inactivated NiV antigen coated on the microplate strips. Unbound material is removed by washing. Formation of NiV antigen-IgG complex is detected by addition of HRP labelled anti-human IgG antibody (probe). Subsequently, after incubation and washing, chromogenic substrate (TMB/H_2O_2) is added, the reaction is stopped by 1N H_2SO_4. The intensity of color / Optical Density is measured at 450nm.

e. Performance characteristics:

Three hundred and ten human sera (31 Nipah IgG positive and 279 negatives by CDC reference test) were used for validation of the Human Nipah IgG ELISA (NIV, Pune).

The Human Nipah IgG ELISA did not show any cross reactivity with Measles (n=5), Mumps (n=5), Rubella (n=5), CCHF (n=5) indicating that the test was specific for detection of Human Nipah IgG antibody from human samples. Intra and inter assay variability (%CV) was 4% and 6% respectively.

		Nipah IgG Positive or Negative confirmed by Reference test (CDC)	
		Positive (n=31)	Negative (n=279)
In-house Human Nipah IgG ELISA	Positive	31	02
	Negative	0	277

Sensitivity	100%
Specificity	99.28%
Positive Predictive Value	93.94%
Negative Predictive Value	100%

f. External Quality assurance:

Inter laboratory comparison of indigenously developed anti-NiV Human IgG was performed at Institute of Epidemiology Disease Control and Research (IEDCR), Dhaka, Bangladesh using a panel of 20 coded samples. The results from the laboratory were in 100% concordance with the results of ICMR –NIV, Pune.

g. Intended use:

Human Nipah IgG ELISA is intended for qualitative detection of IgG antibodies in the convalescent serum of patients presenting clinical signs and symptoms consistent with febrile illness and suspected of NiV. The

serological assay can be used in serosurvey studies. The test can be performed at a laboratory with basic clinical set up, public health centers or sentinel hospitals.

h. Translational Status:

This test is being used within ICMR-NIV laboratories.

i. Impact:

Important information about the antibody kinetics of NiV was investigated using this in house developed assay. Understanding of the immunoglobulin class switch to IgG was studied and IgG antibody persistence was noted for more than one year.

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9
Bat Nipah IgG ELISA

a. Name of the test / assay: Bat Nipah IgG ELISA.

b. Authors: Dr. PD Yadav and Dr. AM Shete

c. Background:

Nipah virus (NiV); belongs to the genus *Henipavirus (Paramyxoviridae)* and causes high fatality in humans. Studies have shown that bats of Pteropus spps. act as the reservoir for NiV. These bats are widespread in South Asia as well as Northern Australia. The presence of NiV has been reported from neighboring countries viz Singapore, Malaysia, and Bangladesh, with a mortality rate ranging from 40 to 70%. In 2018, the NiV outbreak; reported from Kozhikode, Kerala, had a case fatality rate (CFR) of approximately 89%. It was found that *Pteropus spp.* of bats were the probable source of the NiV infection. To ascertain the NiV virus circulation in *Pteropus* and *Rousettus* bats the assay (Bat Nipah IgG ELISA) will be helpful.

d. Principle of the test:

Positive / Negative Control and bat sera samples to be tested for NiV IgG are diluted and added to the earmarked ELISA wells coated with inactivated NiV antigen. Unbound material is removed by washing. The bat IgG bound to the coated antigen is detected using HRP labeled probe (Anti bat IgG HRP). Subsequently, after incubation and washing, chromogenic substrate (TMB/H_2O_2) is added, the reaction is stopped by $1N\ H_2SO_4$. The Optical Density is measured at 450nm.

e. Performance characteristics:

Sensitivity and specificity of indigenous anti-NiV bat IgG ELISA was compared with reference test from CDC. Sensitivity / specificity was found to be 73.04 % and 87.63 % respectively. Intra and inter assay variability (%CV) was 2% and 3% respectively.

		Nipah IgG Positive or Negative confirmed by Reference test (CDC)	
		Positive (n=84)	Negative (n=255)
In-house Human Nipah IgG ELISA	Positive	84	36
	Negative	31	255

Sensitivity	73.0%
Specificity	87.6%
Positive Predictive Value	70.0%
Negative Predictive Value	89.1%

f. External Quality assurance: Not done

g. Intended use:

Bat NiV IgG ELISA is intended for qualitative detection of IgG antibodies in serum of bat collected during the NiV serosurvey studies to understand the possible circulation of NiV in bat population (*BMC Infect Dis 21, 162 (2021)*.

h. Translational Status: This test is being used within ICMR - NIV laboratories.

i. wImpact:

With the help of this technology Bat Nipah IgG antibodies were screened from Bats captured throughout India. Nipah antibodies were not only reported from *Pterpopus* spp but also form *Rousettus* and *pipistrellus* spp.

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10
Human Severe acute respiratory syndrome coronavirus 2 IgG ELISA

a. Name of the test / assay: Human SARS CoV-2 IgG ELISA.

b. Authors: Dr. PD Yadav, Dr. GN Sapkal and Dr. AM Shete

c. Background:

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has recently emerged as the cause of a pandemic and the Coronavirus Disease 2019 (COVID-19). The outbreak of COVID-19 is wreaking havoc worldwide due to inadequate risk assessment. COVID-19 pandemic has entered a dangerous new phase. An indigenous Human SARS CoV-2 IgG ELISA was developed to understand the persistence of anti- SARS CoV-2 IgG antibodies in the human population.

d. Principle of the test:

Positive / Negative control and human samples to be tested for virus specific IgG are added to the ELISA wells coated with inactivated SARS-CoV-2 antigen. Virus specific IgG antibodies; if present in human serum, will bind to the coated SARS-CoV-2 antigen. Unbound material is removed by washing. Formation of SARS-CoV-2 antigen-IgG complex is detected by addition of HRP labelled anti-human IgG antibody (probe). Subsequently, after incubation and washing, chromogenic substrate (TMB/H_2O_2) is added, the reaction is stopped by 1N H_2SO_4. The intensity of color / Optical Density is measured at 450nm

e. Performance characteristics:

A total of 513 blood samples (131 positive and 382 negative for SARS-CoV-2) confirmed by microneutralization test (MNT) were used for validation of the Human SARS-CoV-2 IgG ELISA.

		SARS-CoV-2 IgG Positive or Negative by Microneutralization Test (MNT)	
		Positive (n=131)	Negative (n=382)
In-house Human SARS-CoV-2 IgG ELISA	Positive	121	08
	Negative	10	374

Sensitivity	92.37%
Specificity	97.91%
Positive Predictive Value	93.80%
Negative Predictive Value	97.40%

The Human SARS CoV-2 IgG ELISA did not show cross reactivity with influenza A(H1N1) pdm09 (n=13), influenza A(H3N2) (n=5), human corona virus OC43(n=5), rhinovirus (n=2), RSV, influenza A (n=3) influenza B (n=3), Parainfluenza type 4(n=3), hepatitis B virus (n=5), hepatitis C virus (n=5), dengue IgM and chikungunya IgM samples (n=10 each). Intra & inter assay variability (%CV) was 9 and 6% respectively.
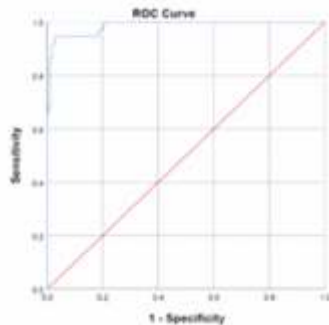
Receiver operating characteristic (ROC) analysis showing sensitivity versus specificity for discrimination of positive and negative serum samples, which were derived for in-house IgG ELISA compared to microneutralization test results (ROC area under the curve: 0.986).
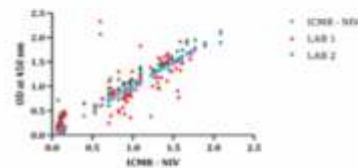
Inter-laboratory correlation analysis: A panel of 150 serum samples tested at two external laboratories and compared with the ICMR-National Institute of Virology reference laboratory indicated a positive correlation between the reference laboratory and the other laboratories

f.   External Quality Assurance:

External validation of indigenously developed anti-SARS human IgG ELISA was performed at two centers (KEM Hospital, Mumbai and ICMR –NIV, Mumbai). A panel of 150 (75 positive and 75 negative) coded human serum samples (including the panel to test cross-reactivity) were provided along with four ready-to-use SARS-COV-2 IgG ELISA kits. The concordance obtained was 99.33% and 100 % respectively at both the centers when compared with the results of ICMR-NIV, Pune.

g.   Intended use:

Human SARS-CoV-2 IgG ELISA is intended for qualitative detection of IgG antibodies in serum of patients presenting clinical signs and symptoms consistent with febrile illness and suspected of COVID-19 (*Indian J Med Res. 2020 May; 151(5):444-449*). The assay can be used for serosurvey. It can be used at any level of clinical setup, public health centers and hospitals

h.   Translational Status:

Technology has been transferred to seven commercial companies.

I.   Impact:

This technology was developed quickly and further transferred for commercialization. COVID Kavach Elisa has been used for nationwide serosurvey SARS CoV-2 in Indian population.

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11
Hamster SARS CoV-2 IgG ELISA

a. **Name of the test / assay:** Hamster SARS CoV-2 IgG ELISA.

b. **Authors:** Dr. PD Yadav, Dr. AM Shete and Dr. Sreelekshmy M

c. **Background:**

Multiple animal models have been used to evaluate the efficacy of SARS-CoV-2 vaccine candidates. Syrian hamster (*Mesocricetus auratus*) is one the model which has been used in diverse research studies on SARS-CoV-2 and seems to be the appropriate model as it mimics the human disease in comparison to other animals. Since SARS-CoV-2 vaccination experiments are conducted in hamster model, hamster SARS CoV-2 IgG ELISA will be useful in vaccination studies.

d. **Principle of the test:**

At the first stage of the assay, positive / negative control and hamster sera to be tested for virus specific IgG are added to the ELISA wells coated with inactivated SARS-CoV-2 antigen. Virus specific IgG antibodies; if present in hamster sera, will bind to the coated SARS-CoV-2 antigen. Unbound material is removed by washing. Virus specific IgG bound to the coated antigen is detected using anti hamster IgG HRP system. Subsequently, after incubation and washing, chromogenic substrate (TMB/H_2O_2) is added, and the reaction is stopped by 1N H_2SO_4. The intensity of color / Optical Density is measured at 450nm.

Anti SARS-CoV-2 Hamster qualitative IgG ELISA

e. **Performance characteristics:**

PRNT was used to check the performance characteristics of the developed assay and it was found to be comparable.

		SARS-CoV-2 IgG Positive or Negative by (PRNT).	
		Positive (n=79)	Negative (n=31)
In-house Human SARS-CoV-2 IgG ELISA	Positive	66	0
	Negative	13	18

Sensitivity	83.5%
Specificity	79.5%
Positive Predictive Value	89.1%
Negative Predictive Value	70.4%

Intra and inter assay variability (%CV) was 6% and 5% respectively.

f. **External Quality Assurance:** Not done

g. **Intended use:**

To understand antibody level in experimentally infected hamsters with SARS CoV-2. (STAR Protoc. 2021 May 10:100573).

h. **Translational Status:** Used in ICMR-NIV laboratory.

I. **Impact:**

This assay helped in understanding the effectiveness of various vaccine candidates against SARS CoV-2 in hamster model. Successful evaluation of BBV152 as well as ZyCoVD vaccine candidates was studied with this technology

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12
Rotavirus Antigen Capture ELISA

a.   Name of the test / assay developed: Rotavirus Antigen Capture ELISA.

b.   Authors / Inventors: Dr. SD Kelkar, Dr. SD Chitambar, Dr. SS Ranshing, Dr V Gopalkrishna, Dr. MS Joshi and Dr. PS Sathe.

c.   Background:

Rotavirus is a non-enveloped, segmented, double-stranded RNA virus (genus 'Rotavirus', family Reoviridae). The nine species of the genus referred to as A-D and F-J are identified on the basis of epitopes of the major structural protein VP6 of rotavirus capsid. VP6 is highly antigenic and is used for identification of rotavirus A (RVA) infections.

RVAs account for >90% of rotavirus gastroenteritis cases in humans and is the leading cause of mortality in infants and young children worldwide. The virus is transmitted by faecal-oral route and possibly by the respiratory route. Clinically, rotavirus gastroenteritis is characterized by profuse diarrhea, mild fever and vomiting leading to mild to severe dehydration associated with shedding of 10^9-10^{10} rotavirus particles/gm of faeces. The clinical manifestations of rotavirus induced diarrhea alone are not sufficiently distinctive to differentiate it from diarrhea caused by other viral or bacterial pathogens.

Rapid detection of rotavirus infection is desirable to avoid unnecessary usage of antibiotics. Enzyme immunoassay is the method of choice because it has overcome the limitations of earlier methods (TEM / virus isolation) like requirement of specialized facility, technical expertise and long duration for completion of the test.

Rotavirus antigen capture ELISA is a rapid, sensitive, specific and cost-effective method for detection of RVA antigen(s) from faecal samples of diarrhea patients during acute phase of the disease.

d.   Principle of the test:

Faecal samples from patients with diarrhea, known RVA positive and negative controls are added to the respective earmarked wells coated with rabbit anti-SA11 polyclonal IgG and post-coated to block non-specific binding of proteins. RVA antigen; if present, in the faecal sample binds to coated rabbit anti-SA11 IgG and
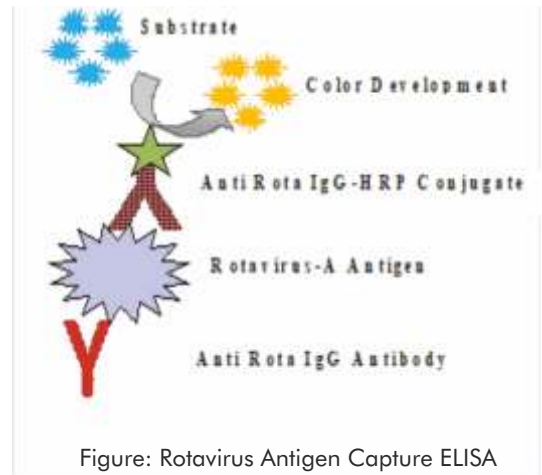
Figure: Rotavirus Antigen Capture ELISA

unbound antigen is removed by washing the wells. In the next step, RVA species specific polyclonal antibody conjugated to horseradish peroxidase is added. After incubation, the wells are washed to remove unbound conjugate before addition of chromogenic substrate (TMB/H_2O_2) to each well. The reaction is stopped by addition of 1N H_2SO_4. The optical density (OD) is measured at 450 nm. Considering the cut off value, results are interpreted as positive or negative.

e.   Performance characteristics:

The performance of the Rotavirus antigen capture ELISA was compared with the commercially available Premier Rotaclone (Meridian Bioscience) which has been widely used in National Rotavirus surveillance studies (Vaccine (2020); 38(51): 8154-60) and is considered as gold standard. The in house "Rotavirus antigen capture ELISA" was evaluated for performance using 32 RVA positive & 50 RVA negative faecal samples.

		Stool samples confirmed as Positive or Negative by Premier Rotaclone	
		Positive (n=32)	Negative (n=50)
Rotavirus antigen capture ELISA	Positive	32	1
	Negative	0	49

Sensitivity	100%
Specificity	98.00%
Positive Predictive Value	96.97%
Negative Predictive Value	100%

Rotavirus antigen capture ELISA assay showed no cross reactivity with RT-PCR positive faecal samples eg norovirus (n=8), enteric adenovirus (n=9), astrovirus (n=11), aichivirus (n=3), enterovirus (n=9), human bocavirus (n=5) and salivirus (n=4).

The lowest detection limit of the NIV assay was determined by spiking rotavirus negative stool sample with purified rotavirus strain SA-11 and is estimated to be ~10 ng viral protein.

f. Inter laboratory comparison / External validation:

Three National level laboratories (ICMR-NIE Chennai, ERC Mumbai and RMRC Belgaum) were provided with coded samples (18 RVA positive & 18 RVA negative stool samples) and assay reagents. All three laboratories showed 100% concordance with the reference results.

g. Intended use:

The Rotavirus antigen capture ELISA is intended for the qualitative detection of RVA from faecal samples collected during acute phase of the diarrheal disease. The test can be used to estimate impact of RVA vaccination on the disease burden.

h. Translational Status:

Technology of the Rotavirus antigen capture ELISA has been transferred to biotech industry for the purpose of commercialization.

I. Impact:

Rotavirus Antigen Capture ELISA test developed at NIV has been used to test more than 7000 stool samples collected from sporadic cases of diarrhea patients. The test proved to be useful in management of the patients and their close contacts. The test has been used in investigation of 12 diarrheal outbreaks in Maharashtra and nearby states. In-house use of this test could facilitate accelerated Rotavirus research (Identification and characterization of unusual Rotavirus strains circulating in human and animal species, Development of Real-time RT-PCR for Rotavirus and intervention strategies against Rotavirus diarrhea).

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13
Human HAV IgM ELISA

a. **Name of the test/ assay:** Human HAV IgM ELISA

b. **Authors / Inventors :** Dr. SD Chitambar, Dr. MS Joshi, Ms SM Grewal, Dr VA Arankalle and Dr. KS Lole

c. **Background:**

Hepatitis A virus (HAV) is a 27-32 nm, non-enveloped, single stranded RNA virus (genus 'Hepatovirus', family 'Picornaviridae'). Human HAV strains are divided into four genotypes, however, appear to be of only one serotype. HAV is transmitted by fecal-oral route. Following ingestion, the virus traverses through intestines into the blood stream and is transported to the liver where it resides and replicates. The virus is mainly excreted through the feces. Symptoms of the disease usually include nausea, vomiting, diarrhea, jaundice, fever and abdominal pain.

Hepatitis A occurs sporadically and in epidemics worldwide. Geographical distribution indicates areas characterized as having high, intermediate or low levels of HAV infection. The disease is common in low and middle income countries with poor sanitary/hygienic conditions and low in high income countries with good sanitary/hygienic conditions. In India, the disease is endemic. Most of the population is infected asymptomatically in early childhood with lifelong immunity. However, several outbreaks of hepatitis A in various parts of India have been recorded in the past decade with anti-HAV positivity ranging from 26% to 85%.

Cases of hepatitis A are not clinically distinguishable from other types of acute viral hepatitis. In majority of patients, anti-HAV IgM serum becomes detectable 5-10 days before onset of symptoms and declines to undetectable levels in less than 6 months.

'Human HAV IgM capture ELISA' is a rapid, sensitive and specific method to detect HAV specific IgM in serum samples of viral hepatitis patients during acute phase of the disease.

d. **Principle of the test**

Serum samples from patients with acute hepatitis, known as anti-HAV IgM positive and negative control samples are added to the identified wells coated with rabbit IgG against human IgM and post coated to block non-specific binding of proteins. IgM present in the serum samples binds to the coated rabbit anti-human IgM. After incubation, unbound IgM and other proteins are removed by washing the wells. Lysate from HAV infected Buffalo Green Monkey Kidney cell cultures is added to the wells. HAV from the lysate binds to anti-HAV IgM if present in the serums sample. Unbound HAV is removed by washing the wells. Human anti-HAV IgG conjugated to HRP is added to the wells. After incubation, wells are washed to remove unbound conjugate before addition of chromogenic substrate (o-phenylenediamine / urea peroxide) to each well. The reaction is stopped by addition of 4N H_2SO_4. The optical density is measured at 492 nm in the ELISA reader. Results are interpreted as positive or negative on the basis of cut off value.

Figure: Human HAV IgM capture ELISA

e. **Performance characteristics**

The performance of 'Human HAV IgM ELISA' was compared with that of the commercially available HAVAB-M EIA test, a microparticle enzyme immunoassay of Abbott Laboratories, using sera from healthy individuals (6), hepatitis A patients (18), hepatitis B patients (9), hepatitis E patients (5), HBsAg carriers (2) with super imposed infection of non A-non B hepatitis,

Rheumatoid factor positive patients and commercially available human immunoglobulins (with anti-HAV IgG titer of 1:800). Only sera from hepatitis A patients showed reactivity in both tests, thus indicating 100% sensitivity and specificity of the developed test.

f.   External validation

The performance of 'Human HAV IgM ELISA' was validated at the Department of Gastroenterology, AIIMS, New Delhi using coded serum samples. The results showed 100% concordance with the reference record.

g.   Intended use

'Human HAV IgM ELISA' has been utilized for diagnosis of hepatitis A in the serum samples received from different hospitals/ clinics and investigations of outbreaks of hepatitis. The data has been documented in National and International journals. Work has been recognized by Department of Biotechnology (DBT)/ Biotech Consortium India Limited (BCIL), New Delhi.

h.   Translational status:

Technology of 'Human HAV IgM ELISA' has been transferred to biotech industry for the purpose of commercialization.

I.   Impact:

Human HAV IgM capture ELISA has been useful in serological diagnosis of hepatitis A in referred sporadic cases and >100 outbreaks of viral hepatitis. Use of the assay within laboratory expedited research (Genomic characterization of Indian HAV strains, Evaluation of clinical specimens collected by non-invasive methods for detection of HAV specific antibodies and ultrafiltration based membranes for removal of HAV) on hepatitis A.

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14
HEV IgM Rapid Test

a.   Name of the test/assay: HEV IgM Rapid Test

b.   Authors/Inventors: Dr. TM Deshmukh, Ms. MT Dudhmal and Dr. KS Lole

c.   Background:

Hepatitis E (HE) contributes significantly to infectious disease burden in India. Hepatitis E virus (HEV) is mainly responsible for water-borne epidemics of varying magnitudes in rural and urban India. More than 50% of reported sporadic acute viral hepatitis cases are due to HEV. HEV causes significant mortality in pregnant women (10-30%). HEV is the major cause of acute viral hepatitis in laboratory confirmed cases and outbreaks amongst the other known viral causes [hepatitis A (HAV), B (HBV) and C (HCV) viruses] and highlights importance of surveillance of viral hepatitis with strengthening of laboratory component. Management of acute viral hepatitis is a function of its virus specific mode of transmission (enteric vs. parenteral) making rapid diagnosis mandatory for effective management and prevention of this water-borne disease. Availability of a simple rapid strip test immensely eases the task of HE diagnosis and its management particularly where laboratory facilities are not readily accessible. Presence of anti-HEV IgM antibodies in human serum or plasma indicates ongoing or recent infection. Anti-HEV IgM antibodies are detectable from approximately day 2 to 6 months post-infection/onset of symptoms. Detection of HEV specific antibodies is done using laboratory based assays or Point-of-Care (PoC) assays. However, laboratory based assays require trained personnel and appropriate equipment, facility and cannot be performed in field or POC settings.

HEV IgM Rapid Test is a lateral flow assay (immunochromatographic)

developed for use in field/PoC settings. The test utilizes mouse anti-human IgM antibodies to capture IgM antibodies in the test samples. The captured HEV specific IgM antibodies are detected by complex consisting of recombinant HEV protein-colloidal gold labeled anti-HEV monoclonal antibody.

d.   Principle of the test:

HEV IgM Rapid Test is an IgM capture solid-phase lateral-flow immunochromatographic assay. IgM antibodies present in test sample will be captured by anti-human IgM antibodies immobilized at Test Line (TL). The presence of HEV specific IgM antibodies can be differentially detected by colloidal gold labeled anti-HEV monoclonal antibody complexed with recombinant HEV protein (neutralizing epitope/s region of genotype 1 HEV ORF2 based) immobilized within the device, and can be visualized as pink/purple lines after completion of the assay. Mouse anti-HEV antibodies immobilized at Control Line (CL) will capture colloidal gold labeled anti-HEV monoclonal antibody complexed with recombinant HEV protein indicating proper functioning of assay reagents and visualized as pink/purple CL.
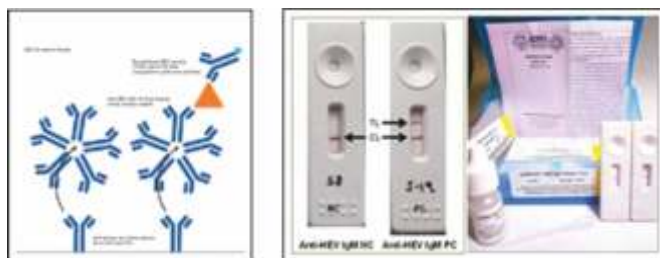
e.   Performance characteristics:

In house assay ↓	Reference Assay →	*Wantai# (IgM Capture)*	
		Positive	Negative
ICMR-NIV HEV IgM Rapid Test	Positive	19	35
	Negative	24	372
Total number of samples tested			

In house assay ↓	Reference Assay →	*Wantai# (IgM Capture)*
ICMR-NIV HEV IgM Rapid Test	Sensitivity (95% CT)	88.9% (84.1 92.5)
	Specificity (95% CT)	98.7% (96.9, 99.4)
	Positive Predictive Value (PPV)	97.5%
	Negative Predictive Value (NPV)	93.9%
	Concordance, Kappa, P value	95.1%, 0.89, 0.0000

 # Wantai (#WE-7196) Beijing Wantai Biological Pharmacy Enterprise Co. Ltd. China. Wantai ELISA Cut-off = mean 3X NC + 0.26. ICMR-NIV HEV IgM Rapid Test requires 10µl of serum or plasma for detection of specific IgM antibodies and assay turnaround time is 10 minutes. Wantai ELISA requires 10µl of serum or plasma for detection of specific IgM antibodies and assay turnaround time is ~150 minutes.

f.   Validation:

Validation of HEV IgM Rapid was done in 2 external laboratories (ICMR-NARI, Pune and ARI, Pune) and 3 ICMR-NIV internal laboratories (Encephalitis Group, Dengue/Chikungunya Group, Polio Virus Group). All these laboratories were given 20 coded serum samples and ICMR-NIV HEV IgM Rapid Tests. An agreement of 95% and 100% were noted between the results obtained at 2 external laboratories, respectively. Agreement of 90%, 95% and 85% was noted in 3 of the aforementioned internal laboratories, respectively.

g.   Intended use:

HEV IgM Rapid Test is intended for the rapid qualitative detection of IgM antibodies to HEV in human serum or plasma.

h.   Translational status:

The technology for HEV IgM Rapid Test has recently been developed under DHR funded project and is being used at ICMR-NIV laboratory. A patent application titled, "A method for developing a rapid immunochromatographic assay for identifying hepatitis E infection" has been filed (No. 202011035352) (Inventors: Dr. T. M. Deshmukh, Ms. M. T. Dudhmal, Dr. K. S. Lole; Applicant: Indian Council of Medical Research).

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15
Human HEV IgM ELISA

a.   Name of the test/assay: Human HEV IgM ELISA

b.   Authors/Inventors: Dr. VA Arankalle, Dr. KS Lole and Dr. TM Deshmukh

c.   Background:

Hepatitis E (HE); formerly referred as enterically transmitted non-A non-B (ET-NANB) hepatitis, is predominantly transmitted feco-orally with highest transmission rates associated with consumption of water contaminated with feces. Hepatitis E virus (HEV) is the causative agent of HE. It occurs in sporadic and epidemic forms. HE is highly endemic in several developing countries of Central and South Asia, North and West Africa, Mexico where inadequate sanitation and supply of unsafe drinking water is very common. Several water-borne epidemics of varying magnitudes have been documented in developing countries. The disease is emerging in developed countries including United States, Japan and Europe. In India, more than 50% of reported sporadic acute viral hepatitis cases have been attributed to HEV. Though mortality rate in adult population is 1-3%, it is significantly higher in pregnant women especially in their third trimester (10-30%). Highest attack rate is noted in young adults aged 15-40 years. Disease is usually self-limiting and spectrum ranges from asymptomatic to acute hepatitis which may occasionally lead to acute liver failure. HEV causes clinically indistinguishable acute hepatitis from other infectious/non-infectious causes highlighting importance of differential diagnosis. Chronic HE also has been reported in immune-compromised/immune-suppressed patients.

Definitive diagnosis of HEV infection is done by detection of virus specific genomic RNA in blood/feces and antigens or antibodies in blood. Identification of infectious virus is not feasible as HEV shows limited growth

in tissue culture. HEV viremia and its excretion in feces are restricted to early acute phase of infection for a short period and usually precede appearance of specific antibodies or onset of symptoms. Thus, detection of anti-HEV IgM antibodies in human serum or plasma samples is the most feasible method used for diagnosis of HE and indicates ongoing or recent infection. Anti-HEV IgM antibodies are detectable during the acute phase of infection from approximately day 2 to 6 months post-infection/onset of symptoms. HEV specific IgM and IgG antibodies are detectable in sera obtained during the acute phase of infection. A low anti-HEV IgG antibody titer together with the absence of anti-HEV IgM antibodies indicates prior infection.

Human HEV IgM ELISA utilizes open reading frame 2 (ORF2) protein of HEV. Presence of IgM antibodies in the specimen is detected using monoclonal anti-human IgM antibodies developed in goat and labeled with HRP.

d. Principle of the test:

Diluted human serum or plasma, known Positive and Negative Control are added to the earmarked wells coated with HEV recombinant antigen (rAg) (genotype 1 HEV ORF2 based) and incubated. HEV specific antibodies, if present, will bind to the antigens immobilized on the solid phase. After incubation, the wells are thoroughly washed and HRP labeled monoclonal anti-human IgM antibodies developed in goat is added to each well. This labeled antibody binds to the antigen-antibody complexes previously formed and excess unbound labeled antibodies are removed by washing. A colorless substrate solution TMB/H_2O_2 is then added to each

well. The presence of HEV specific antibodies is indicated by the blue color after incubation, which changes to yellow when the reaction is terminated by 1N H_2SO_4. The Optical Density (OD) is measured at 450nm using a spectrophotometer.

e. Performance characteristics:

In house assay ↓	Reference Assay →	*Wantai#* *(IgM Capture)*		*MP Dianostics$* *(Indirect)*	
		Positive	Negative	Positive	Negative
Human HEV IgM ELISA	Positive	19	35	200	4
	Negative	19	374	58	335
Total number of samples tested					

In house assay ↓	Reference Assay →	*Wantai#* *(IgM Capture)*	*MP Dianostics$* *(Indirect)*
Human HEV IgM ELISA	Sensitivity (95% CI)	91.4% (86.9, 94.4)	77.5% (72.0, 82.2)
	Specificity (95% CI)	99.2% (97.7, 99.7)	98.8% (97.0, 99.)
	Concordance, Kappa, P value	96.3%, 0.92, 0.0000	89.6%, 0.78, 0.0000

 # Wantai (#WE-7196) Beijing Wantai Biological Pharmacy Enterprise Co. Ltd. China.

$ HEV IgM ELISA 3.0 (#23160-096) MP Diagnostics formerly Genelabs, MP Biomedicals Asia Pacific Pte. Ltd. Singapore.

Human HEV IgM and MP Diagnostics ELISA Cut-offs = mean 3X NC + 0.4; Wantai ELISA Cut-off = mean 3X NC + 0.26. NIV Human HEV IgM ELISA requires 1µl of serum/plasma while Wantai and MP Diagnostics ELISAs require 10µl of serum/plasma for detection of specific IgM antibodies.

f.   Validation:

Four external laboratories (ICMR-NIRTH, Jabalpur; ICMR-NICED, Kolkata; CMC, Vellore and Pt. JNMC, Raipur) and 3 internal NIV laboratories (BSL-4, Dengue and Encephalitis Groups) were provided with 20 coded serum samples and in house assay reagents. Results of all laboratories showed 100% concordance with the in house assay results. Additionally, NIV Human HEV IgM ELISA was 76% and 97% sensitive and specific, respectively in detecting anti-HEV IgM antibodies in 84 serum samples collected at CMC Vellore laboratory and previously tested in ELISA from MP Biomedicals Asia Pacific Pte. Ltd., Singapore formerly Genelabs). Human HEV IgM ELISA showed 100% agreement in detecting HEV specific IgM antibodies in 13 serum samples collected at ICMR-NICED Kolkata and previously tested in ELISA from Asia-lion Biotechnology Co. Ltd., China.

g.   Intended use:

Human HEV IgM ELISA; an indirect assay, is intended for in *vitro* qualitative detection of IgM antibodies to HEV in human serum or plasma. Human HEV IgM ELISA can be modified for in vitro qualitative detection of IgG antibodies to HEV in human, monkey and swine serum or plasma [Arankalle VA et al (2007) J Viral Hepatitis 14: 435-45].

h.   Translational status:

Technology for Human HEV IgM ELISA was non-exclusively transferred to a biotech industry which subsequently received manufacturing license and approval for commercialization. Human HEV IgM ELISA is being used in ICMR-NIV laboratory.

I.   Impact:

Human HEV IgM ELISA has been the core laboratory component in viral hepatitis outbreak investigations, hepatitis sero-prevalence studies and providing diagnosis for referred cases at ICMR-NIV. Since 2003, the assay has been used to test ~4306 serum samples collected during ~65 HEV outbreaks from different states like Maharashtra, Odisha, Gujarat, Andhra Pradesh, Kerala and Himachal Pradesh. Diagnosis for ~5840 referred cases of acute viral hepatitis has been provided using Human HEV IgM ELISA. Twenty-three research articles published from ICMR-NIV involved use of Human HEV IgM ELISA. The publication based on development of this assay has received more than 50 citations till date [Arankalle VA et al (2007) J Viral Hepatitis 14: 435-45].

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16
Human HEV IgG ELISA

a. **Name of the test/assay:** Human HEV IgG ELISA

b. **Authors/Inventors:** Dr. VA Arankalle, Dr. KS Lole and Dr. TM Deshmukh

c. **Background:**

Enterically transmitted hepatitis E (HE) has emerged as an important public health concern of global significance. First epidemiological evidence of HE in the world was reported from New Delhi, India in 1957 which was described as waterborne infection due to sewage contamination of the Yamuna River. Estimated 20 million HEV infections occur worldwide which include 3.3 million symptomatic cases every year. Since its discovery in 1980, HEV has been responsible for several epidemics of varying magnitudes involving contamination of water supplies with human feces in developing countries. HEV is endemic in India and the major cause of acute hepatitis and acute liver failure. Asymptomatic infections are more common than symptomatic infections. HEV causes clinically indistinguishable acute hepatitis from those caused by other infectious/non-infectious causes highlighting importance of differential diagnosis. Serological detection of anti-HEV IgM antibodies in human serum or plasma indicates ongoing or recent infection and is routinely used for diagnosis of HE. Anti-HEV IgM antibodies are detectable as early as day 2 to nearly 6 months post-infection/onset of symptoms. HEV specific IgM and IgG antibodies are detectable in sera obtained during the acute phase of infection. A low anti-HEV IgG antibody titer together with the absence of anti-HEV IgM antibodies indicates prior infection. Anti-HEV IgG antibodies are detectable for decades post infection. Detection of specific IgG antibodies has been considered as diagnostic marker in HE non-endemic countries.

Human HEV IgG ELISA utilizes open reading frame 2 (ORF2) proteins of HEV. Presence of IgG antibodies in the specimen is detected using horseradish peroxidase enzyme (HRP) labeled monoclonal anti-human IgG antibodies developed in goat.

d. **Principle of the test:**

Diluted human serum or plasma, known Positive and Negative Control are added to the earmarked wells coated with HEV recombinant antigen (rAg) (genotype 1 HEV ORF2 based) and incubated. HEV specific antibodies, if present, will bind to the antigens immobilized on the solid phase. After incubation, the wells are thoroughly washed to remove unbound materials and goat monoclonal anti-human IgG antibodies labeled with horseradish peroxidase is added to the wells. This labeled antibody will bind to the antigen-antibody complex previously formed and excess unbound labeled antibodies would be removed by washing. A colorless substrate solution 3, 3', 5, 5'-TMB/H_2O_2 is then added to each well. The presence of specific antibodies is indicated by a blue color after incubation, which changes to yellow when the color reaction is terminated by the addition of acid. The intensity of the resulting yellow product is measured at 450nm using a spectrophotometer.
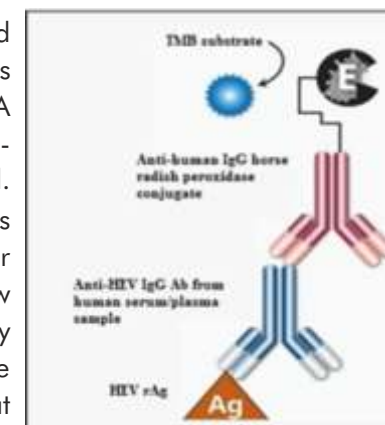
e. **Performance characteristics:**

In house assay ↓	Reference Assay ⟶	Genelabs (#21150-096T, Singapore) (Indirect)	
		Positive	Negative
Human HEV IgG ELISA	Positive	203	28
	Negative	3	182
Total number of samples tested		416	

In house assay ↓	Reference Assay ⟶	*Genelabs (#21150-096T,*
Human HEV IgG ELISA	Sensitivity (95% CT)	98.5% (95.8, 99.5)
	Specificity (95% CI)	86.7% (81.4, 90.6)
	Concordance, Kappa, P Value	92.6%, 0.85, 0.0000

Human HEV IgG ELISA Cut-off = mean 3X NC + 0.4; Human HEV IgG ELISA requires 1µl of serum/plasma for detection of specific IgG antibodies.

f. Validation: Not done.

g. Intended use:

Human HEV IgG ELISA is intended for *in vitro* qualitative detection of HEV IgG antibodies in human serum or plasma [Arankalle VA *et al* (2007) J Viral Hepatitis 14: 435-45].

h. Translational status:

Human HEV IgG ELISA is being used in ICMR-NIV laboratory.

I. Impact:

Human HEV IgG ELISA or its modified version has been used in hepatitis E sero-prevalence studies, monitoring experimental infections in laboratory animals (monkeys, pigs and rabbits), assessment of humoral immune responses in animals induced by experimental anti-HEV vaccine candidates (mice and monkeys) and detection of anti-HEV antibodies in different animals (dog, cat, emu, cattle, sheep, goat, rat). Seventeen research articles published from ICMR-NIV involved use of Human HEV IgG ELISA.

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17
Swine HEV IgG ELISA

a. Name of the test/assay: Swine HEV IgG ELISA

b. Authors/Inventors: Dr. VA Arankalle, Dr. KS Lole, Dr. TM Deshmukh and Dr. LP Chobe

c. Background:

Enterically transmitted hepatitis E (HE) is emerging as an important public health concern of global significance. Hepatitis E virus (HEV), the causative agent of HE shows worldwide distribution and follows distinct epidemiological patterns in developing and developed countries. Large epidemics have been reported from several developing countries. Earlier, sporadic acute HE cases occurring in developed countries were often travel associated. However, recently reports of several autochthonous HE cases are on rise from developed regions of the world. HEV outbreaks have not been reported from developed countries (USA, Japan and Europe). HEV shows highest clinical attack rate in young adults aged 15-40 years in developing countries while in developed countries, elders aged ⩾ 65 years are common targets. HEV infections in immune-compromised/suppressed patients are common in this region. HEV exhibits a broad host range. Antibodies to HEV or HEV like agents and/or HEV genome (RNA) have been detected in several wild and domestic animals (wild monkeys, wild boars, mongoose, bats, rats, cattle, chicken, rabbits, dogs, cats, pigs, horses, camel, trout). Clinical disease has not been reported in animals. HEV transmission patterns are distinct in developing (water-borne) and developed (zoonotic) countries. This expanding virus family has significant implications in achieving One Health.

HEV is a non/quasi-enveloped virus having a linear, single-stranded

positive sense RNA genome of about 7.2 kb. The genome has short untranslated regions at 5' and 3' ends, a 5' methylguanine cap, a 3' poly A tail and three partially overlapping open reading frames (ORF1-3). ORF1 codes for non-structural polyprotein, ORF2 codes for capsid protein (660 a.a) and ORF3 codes for smallest multifunctional protein. HEV is classified as a member of *Hepeviridae* family containing two genera, *Orthohepevirus* and *Piscihepevirus*. *Orthohepevirus* contains four species (A-D). Previously recognized four genotypes (HEV1-4) belong to *Orthohepevirus* A which has additional three genotypes (HEV5-7). Genotypes HEV1-4 and 7 infect humans. Only one serotype of HEV exists. An ORF4 was identified from HEV1 genotype solely. HEV1 and 2 are restricted to humans. HEV3 and 4 are found in several animal species along with humans. In India, HEV1 and 4 circulate in human and swine population, respectively. ORF2p is the most conserved protein between HEV1-4 genotypes. ORF2 has been the major target for vaccine and diagnostics development.

Swine HEV IgG ELISA utilizes ORF2 protein of HEV genotype 4. Presence of IgG antibodies in the specimen is detected by monoclonal anti-swine IgG antibodies developed in goat and labeled with horseradish peroxidase enzyme (HRP).

d.   Principle of the test:

Diluted swine serum or plasma, known Positive and Negative Control are added to the earmarked wells coated with HEV recombinant antigen (rAg) (genotype 4 HEV ORF2 based) and incubated. HEV specific antibodies, if present, will bind to the antigens immobilized on the solid phase. After incubation, the wells are thoroughly washed to remove unbound materials and HRP labeled anti-swine IgG monoclonal antibodies developed in goat are added to the wells. This labeled antibody will bind to the antigen-antibody complexes previously formed and excess unbound labeled antibodies are removed by washing. A colorless substrate solution containing o-phenylenediamine dihydrochloride (OPD) and urea peroxide is then added to each well. Presence of specific
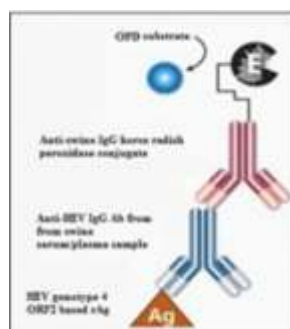
antibodies is indicated by the presence of yellow-orange color after incubation. The reaction is terminated by addition of acid. The intensity of the resulting colored product is measured at 492nm using a spectrophotometer.

e.   Performance characteristics:

In house assay ↓	Reference Assay (*in house* or Commer-cial) →	Modified NIV Human HEV IgG ELISA		*Genelabs (#21160-096T, Singapore)*		*Genelabs (#21150-096T, Singapore)*	
		Positive	Negative	Positive	Negative	Positive	Negative
Modified or Swine HEV IgG ELISA	Positive	48	0	184	35	204	27
	Negative	0	6	0	219	2	183
Total number of samples tested		Anti-HEV IgG antibodies detected in 54 swine serum samples		Anti-HEV IgM antibodies detected in 438 human serum samples		Anti-HEV IgG antibodies detected in 416 human serum samples	

In house assay	Reference Assays (*in house or commercial*)	Modified NIV Human HEV IgG ELISA (n=54)	*Genelabs (#21160-096T, Singapore)*	*Genelabs (#21150-096T, Singapore)*
Modified or Swine HEV IgG ELISA	Sensitivity (95% CI)	-	100.0% (98.0, 100.0)	100.0% (96.5, 99.7)
	Specificity (95% CI)	-	86.2% (81.4, 89.9)	87.1% (81.9, 91.0)
	Concordance, Kappa, P Value	100.0%, 1.00, 0.0000	92.0%, 0.84 0.0000	93.0%, 0.86 0.0000

Modified NIV Swine HEV IgG and Modified NIV Human HEV IgG ELISA Cut-off = Thrice of mean 3X NC. All *in house* assays require 1μl of serum/plasma for detection of specific IgM or IgG antibodies.

f.   Validation: Not done.

g.   Intended use:

Swine HEV IgG ELISA is intended for *in vitro* qualitative detection of IgG antibodies to Hepatitis E Virus (HEV) in swine serum or plasma. Swine HEV

IgG ELISA can be modified for *in vitro* qualitative detection of IgM and/or IgG antibodies to HEV in human and monkey serum or plasma [Arankalle VA *et al* (2007) J Viral Hepatitis 14: 435-45].

h.   Translational status:

Swine HEV IgG ELISA is being used in ICMR-NIV laboratory.

i.   Impact:

ICMR-NIV was first to report circulation of distinct HEV genotypes in human and swine population in India (HEV1 and HEV4, respectively) [Arankalle VA *et al.,* 2002]. Swine HEV IgG ELISA or its modified version has been used in detecting HEV infections in swine population and monitoring experimental infections in laboratory animals (monkeys, pigs and rabbits). Six research articles published from ICMR-NIV involved use of Swine HEV IgG ELISA.

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18
Human Measles IgM ELISA

a.   Name of the test / assay: Human Measles IgM ELISA.

b.   Authors: Dr. GN Sapkal, Dr. SR Vaidya and Dr. DT Mourya
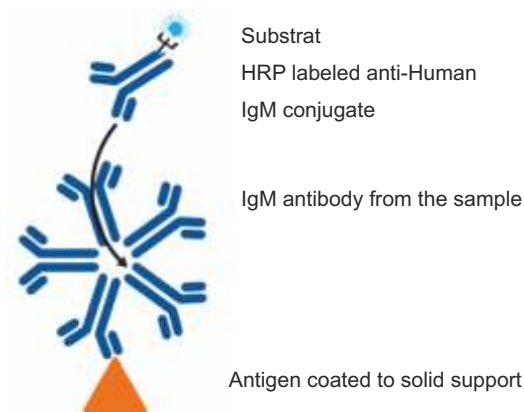
c.   Background:

Measles; a highly contagious viral disease, is a leading cause of morbidity and mortality with around 2.6 million deaths worldwide every year. Though the vaccination programs have achieved some success in control of the virus spread, epidemic outbreaks are still reported. India stands 4th in the top 10 countries with global measles outbreaks as of October 2021 as per WHO report. Disease is caused by Measles virus, a member of the *Morbillivirus* genus of the family *Paramyxoviridae*.

The virus is highly infectious & is primarily transmitted by large droplet spread or direct contact with nasal or throat secretions from an infected person. The incubation period is usually 11–12 days from exposure until the symptoms appear. The virus is infectious 4 days before & after the rash appears.

In India, death in children is due to devastating complications of the measles virus which include pneumonia, diarrhea and Subacute sclerosing panencephalitis (SSPE). Measles confirmatory tests include RT-PCR (throat or nasopharyngeal swab) and/or presence of measles virus specific IgM (serum). Measles IgM ELISA has been standardized for qualitative determination of virus specific IgM in the human serum.

d.   Principle of the test:

Indirect IgM ELISA

Substrat

HRP labeled anti-Human

IgM conjugate

IgM antibody from the sample

Antigen coated to solid support

The serum samples to be tested for Measles virus specific IgM are pre-treated with IgG / RF absorbent. The pre-treated samples are diluted and added to earmarked wells coated with Measles antigens (inactivated virus) and control antigen (uninfected cell slurry). Similarly, Positive and Negative Controls are also added to the respective earmarked wells. After incubation and washing, a prediluted HRP labelled anti-Human IgM conjugate is added to each well. The conjugate binds to the immobilized Measles virus-specific IgM antibodies. After incubation & washing, chromogenic substrate (TMB/H_2O_2) is added to each well. The reaction is terminated by addition of 1N H_2SO_4. The intensity of color / optical density is measured at 450 nm.

e. Performance characteristics:

Two hundred and seven sera samples (103 Measles IgM positive and 104 Measles IgM Negative) were compared with Siemens Measles IgM ELISA to calculate sensitivity & specificity of the assay.

		Measles IgM positive / Negative by Siemens Measles IgM ELISA (Reference test)	
		Positive (n=103)	Negative (n=104)
Human Measles IgM ELISA (NIV)	Positive	103	4
	Negative	0	100

Sensitivity	100% %	95% CI : 95.07% to 100%
Specificity	96.15 %	95% CI : 89.68% to 99.24%
Positive Predictive Value	96.26 %	95% CI : 88.91% to 98.66%
Negative Predictive Value	100%	

f. External Quality Assurance:

Three national level laboratories (HIMS- Hassan, KIPM- Chennai, OLS- Kolkata) were provided with a panel of coded samples (n=30) and assay reagents. Test results of all three laboratories showed 100% concordance with reference results.

g. Intended use:

Human Measles IgM ELISA is intended for qualitative detection of IgM antibodies in serum of patients presenting clinical signs & symptoms of measles virus infection. The serological assay is designed for providing presumptive diagnosis of recent infection with measles virus.

h. Translational Status:

Technology of Human Measles IgM ELISA has been transferred to biotech industry for the purpose of commercialization.

i. Impact:

This kit will serve for quick identification measles cases in the community and would help in disease control and containment efforts.

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19
Mosquito JE Antigen Capture ELISA

a. **Name of the test / assay:** Mosquito JE Antigen Capture ELISA.

b. **Authors:** Dr. GN Sapkal, Dr. MM Gore, Dr. DT Mourya and Dr. PS Sathe

c. **Background:**

Japanese encephalitis (JE) is a serious public health problem in India. JE epidemics have been reported from many parts of the country. Furthermore, JE cases are being reported from newer areas. Identification of the etiological agent from patients / vectors or antibody response is some of the parameters for confirming JE virus circulation in the area. JE virus is maintained in nature by a complex cycle that involves pigs as an amplifying host, ardeid birds as a reservoir and mosquitoes as vectors. In India, JE virus (JEV) has been isolated from 16 species of mosquitoes - *Culex vishnui* subgroup of mosquito consisting of *Cx. tritaeniorhynchus Giles, Cx. vishnui Theobald* and *Cx. pseudovishnui Colless* and have been implicated as major vectors.
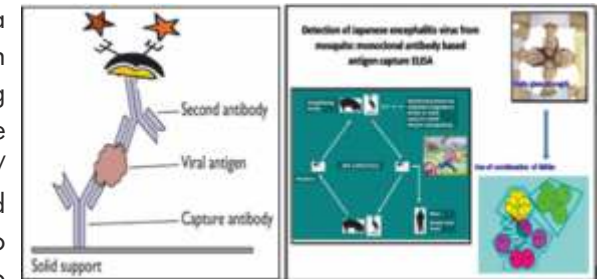
For implementing rapid public health measures, it is essential to monitor JEV in mosquito vector population. For virus isolation, inoculating a susceptible cell line or suckling mice has been employed. More recently mosquito inoculation techniques and PCR have been reported for detection and propagation of *flaviviruses*. Many of these methods are either time consuming or expensive. Therefore, antigen capture ELISA has been standardized using a panel of indigenously developed JEV specific monoclonal antibodies.

No antigen capture ELISA kit is commercially available for detection of JEV. The inhouse "Mosquito JE Antigen Capture ELISA" would be useful for surveillance of JEV in mosquito to identify regions of high risk. Moreover, the test has the added advantage of high sensitivity and specificity due to the use of monoclonal antibodies. The test can be performed in resource limited setting.

d. **Principle of the test:**

On the day of the test, a mosquito suspension prepared by grinding 50 mosquito as one pool. Positive / Negative Controls and mosquito suspension to be tested are added to the earmarked ELISA wells coated with anti-JEV monoclonal antibodies. JE viral antigen from mosquito suspension as well as antigen from PC is captured by the immobilized JE monoclonals. At the end of incubation; the wells are washed and biotin labelled flavivirus cross reactive monoclonal antibody is added to each well. In the next step, Avidin-HRP is added to each well followed by ELISA substrate (TMB/H_2O_2). The reaction is terminated by addition of Stop solution ($1N\ H_2SO_4$). Absorbance is read at 450 nm using an ELISA plate reader.

e. **Performance characteristics:**

Sixty mosquito pools (17 JEV positive and 43 JEV Negative) by the gold standard test (PCR / real time PCR), were used for validation of the inhouse mosquito JE antigen capture ELISA. The results are as shown below-

		Mosquito pools confirmed as JEV Positive or Negative by PCR / real time PCR	
		Positive (n=17)	Negative (n=43)
Mosquito JE Antigen Capture ELISA (NIV)	Positive	16	1
	Negative	1	42

Sensitivity	94.12 %	95% CI : 71.31% to 99.85%
Specificity	97.67 %	95% CI : 87.71% to 99.94%
Positive Predictive Value	96.12 %	95% CI : 69.68% to 99.11%
Negative Predictive Value	97.67 %	95% CI : 86.24% to 99.65%

f.   External Quality Assurance:

Three National level laboratories (NIRTH-Jabalpur, CRME-Madurai, RMRC- Dibrugarh) were provided with coded 13 mosquito pools (Each pool included 50 mosquito per pool) and assay reagents. All three laboratories showed 100% concordance with the reference results.

g.   Intended use:

Japanese encephalitis Antigen Capture ELISA is intended for qualitative detection of JE Antigen in mosquitoes. The serological assay is designed for identification of high-risk geographic areas of JE.

h.   Translational Status:

Technology of the inhouse JE Antigen Capture ELISA has been transferred to biotech industry for the purpose of commercialization.

i.   Impact:

Mosquito JE Antigen Capture ELISA would be useful for implementing rapid public health measures, it is essential to monitor JEV in mosquito vector population. Surveillance of JEV in mosquito helps to identify regions of high risk. Moreover, the test has the added advantage of high sensitivity and specificity due to the use of monoclonal antibodies. The test can be performed in resource limited setting. Additionally presently such kits are not available commercially.

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20
Human Chandipura IgM ELISA

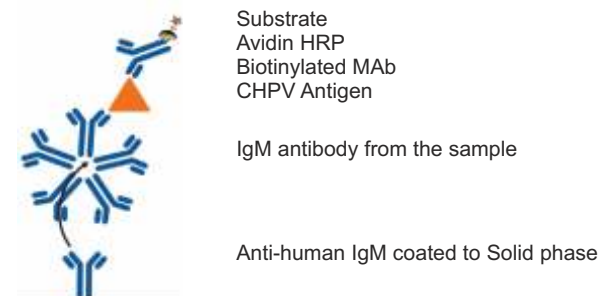**a.   Name of the test / assay:** Human Chandipura (CHP) IgM Capture ELISA.

b.   Authors: Dr. GN Sapkal and Dr. VP Bondre

c.   Background:

Chandipura virus (CHPV) has been implicated as the etiological agent of larger scale outbreaks with high case fatality rate in children in various districts of Andhra Pradesh, Gujarat and Maharashtra. In the absence of vaccine or antivirals, patient management is the only supportive measure for treating viral encephalitis. Monitoring virus specific IgM in patient's serum / cerebrospinal fluid (CSF) helps in diagnosing acute encephalitis due to CHPV during early stages of the disease.

Chandipura IgM Capture ELISA has been standardized for detection of virus specific IgM in serum and CSF of encephalitic patients. The indigenous ELISA uses inactivated CHPV antigen. The high sensitivity and specificity of the inhouse test is due to Biotin labelled monoclonal antibody and Avidin -HRP.

d.   Principle of the test: IgM Capture ELISA

Substrate
Avidin HRP
Biotinylated MAb
CHPV Antigen

IgM antibody from the sample

Anti-human IgM coated to Solid phase

Positive Control (PC), Negative Control (NC) and the patient's sera / CSF after dilution are added to the earmarked ELISA wells. IgM antibodies in the patient's serum / CSF (if present) and IgM antibodies from Positive Control are captured by anti-human IgM (*μ* chain specific) coated on to the solid surface (wells). In the next step, CHPV antigen is added which binds to captured human IgM, unbound antigen is removed during the washing step. In the subsequent step, biotinylated anti-CHPV monoclonal antibodies are added followed by Avidin–HRP. Subsequently, chromogenic substrate (TMB/H_2O_2) is added, the reaction is stopped by 1N H_2SO_4. The intensity of colour / Optical Density is measured at 450nm.

The kit is for *in vitro* use only, for monitoring CHPV specific IgM antibodies in human serum and/or CSF.

e.   Performance characteristics:

Two hundred & sixty-one samples (101 CHP IgM positive and 160 CHP IgM Negative) confirmed by PCR and/or PRN assay were used for validation of the Human Chandipura IgM Capture ELISA. The panel had 223 serum & 38 CSF samples.

		CHP IgM Positive or Negative confirmed by PCR and PRN assay	
		Positive (n=101)	Negative (n=160)
Human CHP IgM Capture ELISA (NIV)	Positive	93	07
	Negative	08	153

Sensitivity	92.07 %	95% CI : 85.12% to 96.54%
Specificity	95.63 %	95% CI : 91.08% to 98.19%
Positive Predictive Value	93.00 %	95% CI : 86.23% to 97.16%
Negative Predictive Value	95.03 %	95% CI : 90.32% to 97.80%

f.   External Quality Assurance:

Three National level laboratories (KIPM - Chennai, CMC- Vellore and PGIMER- Chandigarh) were provided with coded samples (n=30) and assay reagents. All three laboratories showed 100% concordance with reference results.

g.   Intended use:

CHP IgM Capture ELISA is intended for qualitative determination of IgM antibodies in serum/CSF of patients presenting clinical signs and symptoms consistent with CHP encephalitis. The serological assay is designed for providing laboratory diagnosis of CHP at sentinel hospitals and primary health centers.

h.   Translational Status:

Technology of the inhouse CHP IgM Capture ELISA has been transferred to biotech industry for the purpose of commercialization.

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21
Human Dengue IgM ELISA

a. **Name of the test / assay:** Human Dengue (DEN) IgM Capture ELISA.

b. **Authors:** Dr. PS Sathe, Dr. PS Shah, Dr. D Parashar, Dr. KP Patil and Team.

c. **Background:**

Dengue is an acute febrile illness caused by positive-sense ssRNA virus (family *Flaviviridae*, genus *Flavivirus*); has 4 distinct serotypes (DV-1, -2, -3, -4), and is primarily transmitted by *Aedes aegypti* mosquito. It may cause a subclinical infection or severe flu-like illness and could be fatal - Dengue Haemorrhagic Fever (DHF) or Dengue Shock Syndrome (DSS). Dengue poses a severe public health threat throughout the tropical and subtropical regions with approximately 30,000 deaths annually.

Dengue virus specific IgM is detectable $\geqslant$ 5 days after onset of symptoms until $\approx$ 6 months. Although detection of dengue, IgM is one of the important diagnostic markers, the test results must be interpreted in conjunction with history of exposure and/or clinical presentation. The clinical symptoms of dengue infection by any of the 4 DV serotypes include fever, headache, muscle and/or joint pain. There is no specific treatment or vaccine available to treat or prevent dengue infection. Therefore, precise diagnosis of dengue at an early stage is very essential for disease control and its effective management.

Dengue IgM Capture ELISA; developed by ICMR-NIV, is intended for qualitative determination of dengue virus specific IgM antibodies in serum of patients presenting clinical signs and symptoms consistent with Dengue.

d. **Principle of the test:**

Positive Control (PC), Negative Control (NC) and the patient's sera after dilution are added to the earmarked ELISA wells. IgM antibodies in the patient's serum (if present) and IgM from Positive Control are captured by anti-human IgM (μ chain specific) coated on to the solid surface (wells). In the next step, dengue antigen (inactivated dengue virus) is added that binds to captured dengue virus specific IgM. Unbound antigen is removed during washing step. In the subsequent step, biotinylated anti-DEN monoclonal antibodies are added. After incubation & washing, the step is followed by addition of Avidin-HRP. Subsequently, chromogenic substrate (TMB/H_2O_2) is added, the reaction is stopped by 1N H_2SO_4. Intensity of color / optical density (OD) is measured at 450 nm.
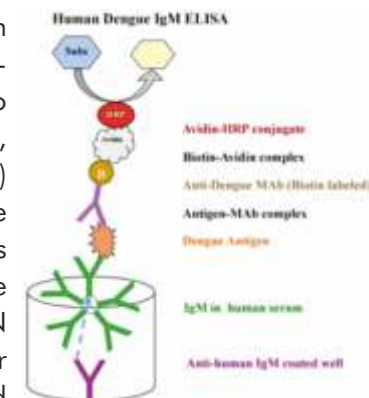
Interpretation of Results:

- IgM Negative: If sample OD $\leqslant 2 \times$ OD of Negtive Control (NC).
- IgM Positive: If sample OD $\geqslant 3 \times$ OD of NC.
- Equivocal: If sample OD $\geqslant 2 \times$ OD of NC but $\leqslant 3 \times$ OD of NC

Note: Interpretation of test results with reference to Clinical history is important because DV specific IgM may persist in persons who have received blood transfusions or blood products whereas immunosuppressed patients may show negative results.

e. **Performance characteristics:**

Three hundred and seventy-seven characterized sera (204 Dengue IgM positive & 173 Dengue IgM negative) were used for validation of Human Dengue IgM ELISA.

		Reference sera characterized as IgM Positive or Negative	
		Positive (n=204)	Negative (n=173)
Human Dengue IgM ELISA (NIV)	IgM Positive	201	2
	IgM Negative	03	171

Sensitivity	98.53%
Specificity	98.84%
Positive Predictive Value	99.01%
Negative Predictive Value	98.28%

The Human Dengue IgM ELISA did not show any cross reactivity with sera from chikungunya, leptospirosis and Rheumatoid factor positive patients indicating that the test was specific for detection of Human dengue IgM antibody Furthermore, the test had high precision. The set of reagents are stable for six months when stored at 2-8°C.

f.   External Validation:

The performance of the test was evaluated by Christian Medical College (CMC), Vellore.

g.   Intended use:

Human Dengue IgM ELISA is used as a diagnostic tool to confirm suspected / probable dengue at late post onset days ($\geqslant$ 5 POD) of infection. The test can be performed at public health centres, sentinel hospitals or clinical set up.

h.   Translational Status:

The technology has been standardized for up-scaling. Ready to use reagents in kit form are being supplied to sentinel surveillance hospitals and apex referral laboratories under the national program (NVBDCP) since 2006.

i.   Impact:

From the year 2006 to 2021, around 61000 sets of Human Dengue IgM ELISA reagents in kit form were supplied to various sentinel surveillance hospitals/ centers and VRDLs, which is a significant contribution to the public health system of the country.

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22
Human Chikungunya IgM ELISA

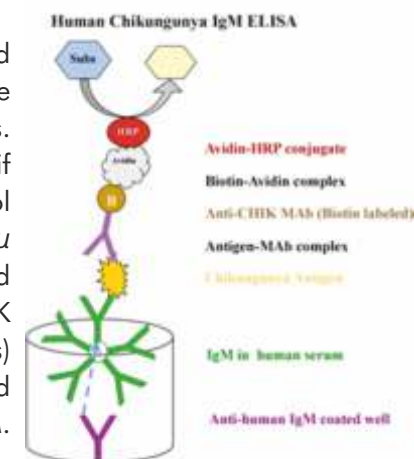**a.   Name of the test / assay:** Human Chikungunya (CHIK) IgM Capture ELISA.

b.   Authors: Dr. PS Sathe, Dr. PS Shah, Dr. D Parashar, Dr. KP Patil and Team.

c.   Background:

Chikungunya is a viral disease transmitted to humans by the bite of infected *Aedes aegypti* mosquito. The etiological agent causing the crippling disease is a member of the genus *Alphavirus*, family *Togaviridae*. Since the first report of chikungunya infection in Tanzania (1953), the virus has been associated with many epidemics in tropical regions of Africa, South Asia etc. CHIK IgM capture ELISA developed by ICMR-NIV is intended for qualitative determination of chikungunya virus specific IgM antibodies in serum of patients presenting clinical signs and symptoms consistent with chikungunya.

d.   Principle of the test:

Positive Control, Negative Control and the patient's sera after dilution are added to the earmarked ELISA wells. IgM antibodies in the patient's serum (if present) and IgM from Positive Control are captured by anti-human IgM (μ chain specific) coated on to the solid surface (wells). In the next step, CHIK antigen (inactivated chikungunya virus) is added which binds to captured human chikungunya specific IgM.

Human Chikungunya IgM ELISA

Avidin-HRP conjugate
Biotin-Avidin complex
Anti-CHIK MAb (Biotin labeled)
Antigen-MAb complex
Chikungunya Antigen
IgM in human serum
Anti-human IgM coated well

Unbound antigen is removed during the washing step. In the subsequent step biotinylated anti-CHIK monoclonal antibodies are added. After incubation & washing, the step is followed by addition of Avidin-HRP. Subsequently, chromogenic substrate (TMB/H_2O_2) is added, the reaction is stopped by 1N H_2SO_4. The intensity of color/ optical density (OD) is measured at 450nm.

Interpretation of Results:

- IgM Negative: If sample OD $\leqslant$ 2×OD of Negtive Control (NC).

- IgM Positive: If sample OD $\geqslant$ 3×OD of NC.

- Equivocal: If sample OD $\geqslant$ 2×OD of NC but $\leqslant$ 3×OD of NC

e.   Performance characteristics:

The inhouse Human CHIK IgM ELISA was evaluated for performance by CDC, CO, USA.

		CHIK IgM Positive & Negative by CDC, Fort Collins, USA	
		Positive (n=19)	Negative (n=36)
Human CHIK IgM ELISA (NIV)	IgM Positive	19	01
	IgM Negative	00	35

Sensitivity	100%
Specificity	97.22%
Positive Predictive Value	95.00%
Negative Predictive Value	100%

Sera samples from dengue, leptospirosis and Rheumatoid factor positive patients were negative when tested with Human CHIK IgM ELISA indicating no cross reactivity of the inhouse test. Furthermore, the test had high precision. The set of reagents of Human Chikungunya IgM ELISA are stable for six months when stored at 2-8˚C.

f.   External validation:

The Human CHIK IgM ELISA has been evaluated for performance by Center for Disease Control (CDC), Fort Collins, CO, USA.

g.   Intended use:

CHIK IgM Capture ELISA is used as a diagnostic tool to confirm suspected / probable chikungunya at late post onset day ($\geqslant$ 5 POD) of infection. The test can be used at the level of clinical set up, public health centres and sentinel hospitals.

h.   Translational Status:

The technology has been standardized for up-scaling. Ready to use reagents in kit form are being supplied to sentinel surveillance hospitals and apex referral laboratories under national program (NVBDCP) since 2006.

i.   Impact:

From the year 2006 to 2021, around 20000 sets of Human Chikungunya IgM ELISA reagents in kit form were supplied to various sentinel surveillance hospitals/ centers and VRDLs, which is a significant contribution to the public health system of the country.
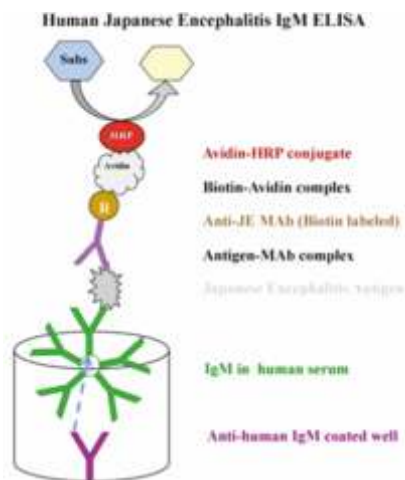
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23
Human Japanese Encephalitis (JE) IgM ELISA

a. **Name of the test / assay:** Human JE IgM Capture ELISA.

b. **Authors:** Dr. PS Sathe, Dr. PS Shah, Dr. D Parashar, Dr. KP Patil and Team.

c. **Background:**

Japanese Encephalitis (JE) is a mosquito borne viral disease and is a major cause of seasonal viral encephalitis. The disease is prevalent in large areas of Asia. The virus is transmitted by *Culex* species of mosquito. The JE virus belongs to family *Flaviviridae*, genus *Flavivirus*. JE IgM Capture ELISA is intended for qualitative determination of virus specific IgM antibodies in serum / CSF of patients presenting clinical signs and symptoms consistent with Japanese Encephalitis. The serological assay is designed for providing presumptive diagnosis of JE.

d. **Principle of the test:**

Positive Control, Negative Control and the patient's sera after dilution are added to the earmarked ELISA wells. IgM antibodies in the patient's serum / CSF (if present) and IgM from Positive Control are captured by anti-human IgM (μ chain specific) coated on to the solid surface (wells). In the next step, JE antigen (inactivated JE virus) is added which binds to captured JE virus specific IgM. Unbound antigen is removed during washing step. In the subsequent step biotinylated anti-JE monoclonal antibodies are added.

Human Japanese Encephalitis IgM ELISA

Avidin-HRP conjugate
Biotin-Avidin complex
Anti-JE MAb (Biotin labeled)
Antigen-MAb complex
Japanese Encephalitis antigen
IgM in human serum
Anti-human IgM coated well

After incubation & washing, the step is followed by addition of Avidin-HRP. Subsequently, chromogenic substrate (TMB/H_2O_2) is added, the reaction is stopped by 1N H_2SO_4. The intensity of color / optical density (OD) is measured at 450 nm.

Interpretation of Results:

- IgM Negative: If sample OD $\leqslant 3 \times$ OD of Negtive Control (NC).

- IgM Positive: If sample OD $\geqslant 5 \times$ OD of NC.

- Equivocal: If sample OD $\geqslant 3 \times$ OD of NC but $\leqslant 5 \times$ OD of NC

e. **Performance characteristics:**

The performance was evaluated by CDC, Fort Collins, CO, USA using validated panel of human serum (n = 248) and CSF (n = 190). The CDC validation results of the inhouse Human JE IgM ELISA kit showed that it was as good as or better than the commercial kits.

Parameter	Serum	CSF
Sensitivity	71	75
Specificity	77	96

Sera samples from chikungunya, leptospirosis and Rheumatoid factor positive patients were negative when tested with Human JE IgM ELISA indicating no cross reactivity of the inhouse test. Furthermore, the test had high precision. The set of reagents of Human JE IgM ELISA are stable for six months when stored at 2-8°C.

f. **External validation:**

Human JE IgM ELISA was evaluated by Center for Disease Control (CDC), Fort Collins, CO, USA [Report of Fourth Biregional Meeting organized by WHO on the Control of Japanese Encephalitis (JE), Bangkok, Thailand, 7-8 June 2009].

g. **Intended use:**

JE IgM Capture ELISA is used as a diagnostic tool to confirm suspected / probable JE at late post onset day ($\geqslant$ 5 POD) of infection. The test can be performed at public health centre, sentinel Hospitals or a clinical set up.

h.   Translational Status:

The technology has been standardized for up-scaling. Ready to use reagents in kit form are being supplied to sentinel surveillance hospitals and apex referral laboratories under national program (NVBDCP) since 2006.

i.   Impact:

From the year 2006 to 2021, around 6000 sets of Human JE IgM ELISA reagents in kit form were supplied to various sentinel surveillance hospitals/ centers and VRDLs, which is a significant contribution to the public health system of the country.

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Molecular Assays

24
Real Time RT-PCR for the diagnosis of Crimean Congo Hemorrhagic Fever

a.   Name of the test/ assay: Real Time RT-PCR for the diagnosis of Crimean Congo Hemorrhagic Fever

b.   Authors/Inventors: Dr. PD Yadav and Dr. DT Mourya

c.   Background:

Crimean-Congo hemorrhagic fever (CCHF) is a severe acute febrile illness caused by the CCHF virus (CCHFV) (*Nairoviridae*, genus *Orthonairovirus*), with an overall case fatality rate of 5–50%. Person-to-person transmission of CCHFV occurs through direct exposure to blood or other secretions and instances of nosocomial transmission are well-documented. CCHFV has been reported in over 30 countries covering Africa, South-Eastern Europe, the Middle East and Western Asia. In India, CCHF was first confirmed in a nosocomial outbreak in 2011 that occurred in Gujarat. After this outbreak, many episodes of sporadic cases and outbreaks were recorded in Gujarat and Rajasthan. Outbreaks of this disease are usually attributable to handling infected animals or patients. The CCHFV genome comprises of three RNA segments: the small (S), medium (M), and large (L) segments. The S segment encodes the nucleocapsid protein (N; nucleoprotein), the M segment encodes the viral glycoprotein, and the L segment encodes an RNA-dependent RNA polymerase (RdRp; the L protein).

d.   Principle of the test:

Qualitative reverse transcription PCR (RT-qPCR) is used when the starting material is RNA. In this method, RNA is first transcribed
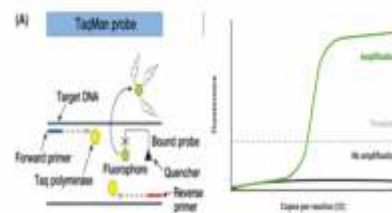
into complementary DNA (cDNA) by reverse transcriptase from total RNA. One-step assays combine reverse transcription and PCR in a single tube containing reverse transcriptase and DNA polymerase. One-step RT-qPCR only utilizes sequence-specific primers for N gene.

e.   Performance characteristics:

The assay is checked for the specificity with known positive samples of chikungunya, ebola, hantaan, Japanese encephalitis, and phlebo virus. The assay is checked for sensitivity detection using in-vitro transcribed RNA.

The real-time RT-PCR could detect CCHFV, 2,779 geq/ml. Intra and inter assay variability (%CV) was 2% and 5% respectively.

f.   External quality assurance:

External validation was performed at National Institute for Research in Tribal Health (NIRTH), Jabalpur with a panel of coded samples and the results were found to be 100% concordant.

g.   Intended use:

The kit is intended for rapid qualitative detection of CCHFV in the serum of patients presenting clinical signs of high fever, petechiae and hemorrhagic signs.

h.   Translational Status:

This test is being used within the ICMR-NIV laboratory.

i.   Impact:

The precise diagnosis of CCHF is necessary in order to reduce the mortality and further transmission of CCHF. Accurate diagnostics helped to save many lives during the nosocomial outbreaks of CCHF from endemic part of the country i.e Gujarat and Rajasthan since the year 2011.

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25
Real Time RT-PCR for the diagnosis of Kyasanur Forest Disease

a. **Name of the test/ assay:** Real Time RT-PCR for the diagnosis of Kyasanur Forest Disease

b. **Authors/Inventors:** Dr. PD Yadav and Dr. DT Mourya

c. **Background:**

Kyasanur forest disease virus (KFDV) is a member of the genus *Flavivirus* and family *Flaviviridae*. It was first recognized in 1956 in Shimoga, Karnataka. It causes seasonal outbreaks during the months of January to June. The symptoms of the human disease include a high fever with frontal headaches, followed by hemorrhagic symptoms. KFDV is a high risk group of pathogen (BSL-4) for other countries and a risk group-3 pathogen for India. The natural cycle of KFDV involves two species of monkeys, the black-faced langur (<u>Presbytis entellus</u>) and the red-faced bonnet monkey (*Macaca radiata*), and various tick species belonging to the genus *Haemaphysalis* that inhabit the forest. In the natural viral cycle, humans are considered a dead-end host. The disease has an acute onset with chills followed by frontal headache, body aches and high temperature. The symptoms last for 5–12 days or longer. Occasionally, coughing, abdominal pain, diarrhea and vomiting occur in a few cases. Bleeding from the nose, gums and gastro-intestinal track occurs in severe cases. Neurological abnormalities have also been reported in a few cases. The genome of KFDV consists of 10,774 nucleotides of single-stranded, positive-sense RNA encoding a single polyprotein that is cleaved post-translationally into three structural (C, prM/M and E) and seven non-structural (NS1, NS2a, NS2b, NS3, NS4a, NS4b and NS5) proteins. We have developed an indigenous Real Time RT-PCR assay to detect KFD viral RNA in samples from human, monkey and ticks.

d. **Principle of the test:**

Qualitative reverse transcription PCR (RT-qPCR) is used when the starting material is RNA. In this method, RNA is first transcribed into complementary DNA (cDNA) by reverse transcriptase from total RNA. One-step assays combine reverse transcription and PCR in a single tube containing reverse transcriptase and DNA polymerase. The one-step RT-qPCR only utilizes sequence-specific primers for the NS5 region.

e. **Performance characteristics:**

The assay is specific for detection of KFDV RNA from the patient sera. It did not cross react with Japanese encephalitis virus, West Nile virus, dengue virus and *Alkhumara* virus (AHFV) from *Flavivirus family* and Ingwavuma, CCHF virus of the *Bunyaviridae family*.
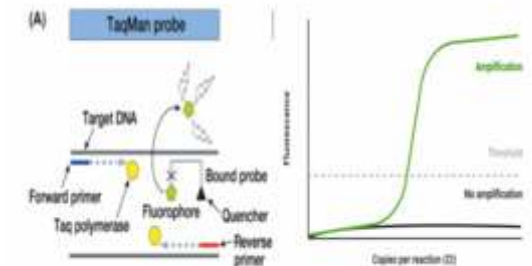
KFDV isolates were used for the one-step real time RT-PCR to test the sensitivity of the assay. The assay worked with RNA from different isolates of KFDV and the cut-off Ct for this assay was found to be 37.

The real-time RT-PCR could detect KFDV with less than 2.67 pfu/ml. As few as 38 RNA copies could be detected with the one-step real time RT-PCR.

Intra and inter assay variability (%CV) was 3% and 4% respectively.

f. **External quality assurance:**

External validation was performed at National Institute for Research in Tribal Health (NIRTH), Jabalpur with coded samples (2 positive and 8 negative) and the results were found to be 100% concordant,

g. **Intended use:**

This kit is intended for rapid qualitative detection of KFDV in the serum of patients presenting clinical signs such as chills, fever, headache and

severe muscle pain with vomiting.

h. Translational Status:

This test is being used within the VRDLs and ICMR-NIV laboratories.

Publication: Mourya, D. T., Yadav, P. D., Mehla, R., Barde, P. V., Yergolkar, P. N., Kumar, S. R., Thakare, J. P., & Mishra, A. C. (2012). Diagnosis of Kyasanur forest disease by nested RT-PCR, real-time RT-PCR and IgM capture ELISA. Journal of virological methods, 186(1-2), 49–54. https://doi.org/10.1016/j.jviromet.2012.07.019https://doi.org/10.1016/j.jviromet.2012.07.019

i. Impact:

Sensitive Taqman based qRT-PCR helped in rapid and accurate diagnosis of suspected Kyasanur Forest Disease (KFD) cases in India. This in turn helped to understand spread of KFDV in different parts of the country.

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26
Nested RT-PCR for the diagnosis of Kyasanur Forest Disease

a. Name of the test/ assay: Nested RT-PCR for the diagnosis of Kyasanur forest disease

b. Authors/Inventors: Dr. PD Yadav and Dr. DT Mourya

c. Background:

KFD is a public health concern in Karnataka and the other neighboring states in the Western ghats of India. Though localized, the disease is a public health problem as a result of the endemicity and occasional epidemic outbreaks with hemorrhagic manifestations and approximately 10% case fatality rate.. Reliable and rapid diagnostic tests are essential to confirm the suspected cases of KFD. We have developed KFD RT-PCR for human, monkey and tick samples. Availability of the kit for diagnosis of KFD would ease the task of investigating acute KFD outbreaks and would also help in the timely diagnosis of sporadic cases.

d. Principle of the test:

Nested PCR uses two sequential sets of primers designed for the NS5 region. The non-structural (NS5) gene, which is highly conserved among the members of flaviviruses, was selected for primer design for the nested PCR. The first primer set binds to sequences outside the target DNA, as expected in standard PCR, but it may also bind to other areas of the template. The second primer set binds to sequences in the target DNA that are within the portion amplified by the first set (that is, the primers are nested). Thus, the second set of primers will bind and amplify target DNA within the products of the first reaction.
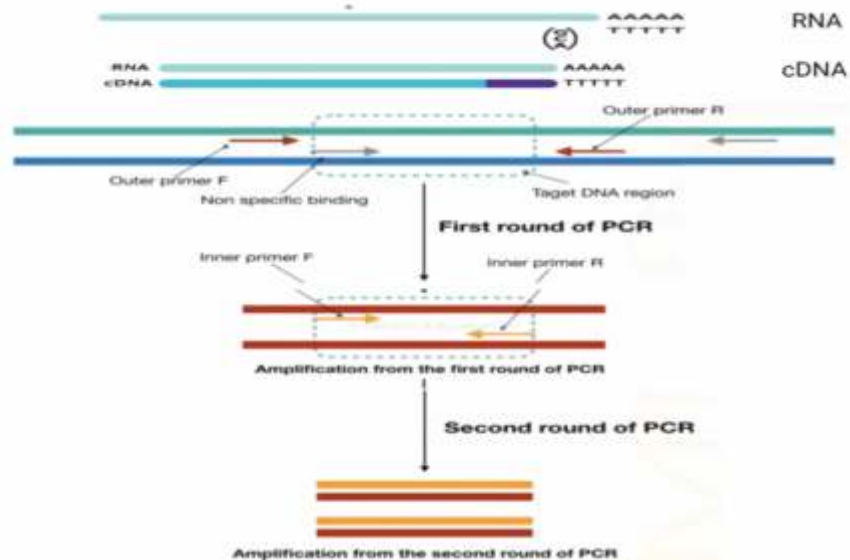
e.   Performance characteristics:

The assay is specific for detection of KFDV RNA from the patient sera. It did not cross react with Japanese encephalitis virus, West Nile virus, dengue and Alkhumara virus from Flavivirus group and Ingwavuma, CCHFV of Bunyaviridae family.

The product specificity was confirmed further by DNA sequencing. The nRT-PCR was highly specific and could distinguish KFDV from the other flaviviruses.

Sensitivity of the assay was checked by serially diluted RNA extracted from KFDV isolates. The sensitivity of diagnostic RT-PCR was 2.67pfu/ml, as determined using log dilutions of the quantified virus stock used for standardization. The test is standardized using KFD viral RNA extracted from the KFDV isolate.

f.   External quality assurance:

Internal validation of the test is complete and 100% concordance was observed with the coded panel of 20 positive and 40 negative samples.

Intra and inter assay variability (%CV) was 2% and 3% respectively.

g.   Intended use:

This kit is intended for qualitative detection of KFDV in the serum of patients presenting clinical signs and symptoms consistent with febrile illness and hemorrhages suspected of KFD. The PCR based assay is designed for providing the diagnosis of KFDV in sporadic cases, outbreak investigations and identifying the causative etiological agent.

h.   Translational Status:

This test is being used in the ICMR-NIV laboratory setting.

Publication: Mourya, D. T., Yadav, P. D., Mehla, R., Barde, P. V., Yergolkar, P. N., Kumar, S. R., Thakare, J. P., & Mishra, A. C. (2012). Diagnosis of Kyasanur forest disease by nested RT-PCR, real-time RT-PCR and IgM capture ELISA. Journal of virological methods, 186(1-2), 49–54. https://doi.org/10.1016/j.jviromet.2012.07.019https://doi.org/10.1016/j.jviromet.2012.07.019

i.   Impact:

This technology has helped in diagnosis of suspected KFD cases with further applications such as sequencing for identification of stains circulating in India.

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27
Single step RT-PCR for the diagnosis of Kyasanur Forest Disease

a. **Name of the test/ assay:** Single step RT-PCR for the diagnosis of Kyasanur Forest Disease

b. **Authors/Inventors:** Dr. PD Yadav and Dr. G Chaubal

c. **Background:**

The recent years have witnessed the emergence of the Kyasanur forest disease (KFD) zoonotic disease as a causative agent for human suffering and monkey deaths in Maharashtra, Kerala, Goa and Tamil Nadu. Currently the methods of laboratory diagnosis of KFDV include real time RT-PCR assay, nested RT-PCR assay, anti-KFD IgM and anti-KFD IgG ELISA. None of the diagnostic assays are currently available commercially. In the Indian setting many laboratories cannot afford to have trained manpower for real-time PCR machine and reagents while RT-PCR is traditionally performed in many sectors. In the current study, we describe an indigenously developed single step RT-PCR assay for the detection of KFDV RNA. The assay is efficient, fast, sensitive and specific to KFD and can be used for any kind of clinical sample.

d. **Principle of the test:**

The NS5 region was used as this region is highly conserved among flaviviruses. In a single tube or single reaction, reverse transcription and amplification are performed (therefore it is named as one-step RT-PCR). The assay having good accuracy, specificity, ease of handling and a simple setup, is useful for
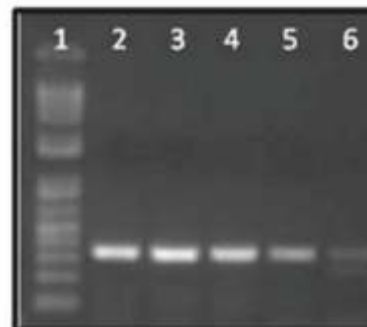
repeat quantification and high throughput screening.

e. **Performance characteristics:**

The assay could specifically detect KFD viral RNA and showed no non-specific PCR amplification with other representative flaviviruses namely, zika, dengue, chikungunya, CCHF, yellow fever, Japanese encephalitis and West Nile viral RNAs.

The present assay (detects upto 100 copies of viral RNA) is more sensitive compared to nested RT-PCR assay (detects upto 104 copies of viral RNA) and nearly as sensitive as real-time RT-PCR assay (detects upto 10 copies of viral RNA)

Intra and inter assay variability (%CV) was 2% and 5% respectively.

f. **External quality assurance:** Not done

g. **Intended use:**

In the Indian setting many laboratories cannot afford to have trained manpower for real-time PCR machine and reagents while RT-PCR is traditionally performed in many sectors.

h. **Translational Status:** This test is being used in the ICMR-NIV laboratory.

Publication: Chaubal G, Sarkale P, Kore P, Yadav P. Development of single step RT-PCR for detection of Kyasanur forest disease virus from clinical samples. Heliyon. 2018;4(2):e00549. Published 2018 Mar 1. doi:10.1016/j.heliyon.2018.e00549

i. **Impact:**

A single step RT-PCR is relatively easy to perform and more cost effective than real time RT-PCR in smaller setups.

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28
Kyasanur Forest Disease (KFD) Point of Care test

a.   Name of the test/ assay: KFD Point of Care test

b.   Authors/Inventors: Dr. PD Yadav and Dr. AM Shete

c.   Background:

The problem of rapid diagnosis of KFD persisted as the sporadic cases and the outbreaks mainly occur in remote, forest areas because of the zoonotic nature of the disease. This study aimed to evaluate the diagnostic performance of Truenat KFD Point-of-care test (PoCT) for detection of KFD and the test comparison for the diagnostic accuracy with conventional real time RT-PCR.

d.   Principle of the test:

The test works on the principle of DNA amplification, hence provides the ability to diagnose the disease early in infection because of its excellent sensitivity and specificity. The Truelab Real

Time Quantitative micro PCR System is a compact, battery-operated system with single testing capability and provides result within 1 hour after collection of samples. Hence, it enables same day reporting and initiation of evidence-based treatment for the patient. It also has real-time data transfer capability (through SMS/E-mail/data push) for immediate reporting of results in emergency cases.

e.   Performance characteristics:

The sensitivity of the Truenat™ KFDV PoCT was determined using RNA

extracted from the serial dilutions of KFD virus stock ($10^{7.79}$ TCID50/ml). The results were found to be comparable with gold standard TaqMan Real Time assay and commercially available Altona RealStar® AHFV / KFDV RT-PCR Kit 1.0. KFDV, Flaviviridae Zika virus (n=2) and dengue virus (n=5), and also samples positive for viruses belonging to Alphaviridae - chikungunya virus (n=3), Togaviridae – rubella virus (n=5), Orthomyxoviridae – influenza virus (n=5) and Paramyxoviridae – measles virus (n=5) indicated no cross-reactive results when tested by the Truenat™ KFDV PoCT.

Intra and inter assay variability (%CV) was 2 and 3% respectively.

f.   External quality assurance:

External validation of the test is complete and results indicated 100% concordance with the coded panel of 40 positive and 60 negative samples at ICMR-NIV, Bangalore unit.

Intra and inter assay variability (%CV) was 2.1% and 3.2% respectively.

g.   Intended use:

The PoC test would be useful in rapid diagnosis of KFD in remote and/or field settings, quick patient management and for controlling the further spread of the virus.

h.   Translational Status:

This test is being used within the VRDLs and ICMR-NIV laboratories.

Publication: Majumdar T, Shete A, Yadav PD, Patil S, Mali D, Waghmare A, Gawande P. Point of care real-time polymerase chain reaction-based diagnostic for Kyasanur forest disease. International Journal of Infectious Diseases. 2021 Jul 1; 108:226-30.

i.   Impact:

Due to the remote forest area locations of sporadic cases and outbreaks of Kyasanur forest disease (KFD), rapid diagnosis poses a significant challenge. This technology is simple, rapid and user-friendly point-of-care test for detection of KFD and at par to diagnostic accuracy with conventional real-time reverse transcription-polymerase chain reaction (RT-PCR) testing. Truenat KFD can be deployed in remote outbreak areas and field settings.

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29
Real Time RT-PCR for the diagnosis of Nipah virus infection

**a.** **Name of the test/ assay:** Real Time RT-PCR for the diagnosis of Nipah virus infection.

b. **Authors/Inventors:** Dr. PD Yadav and Dr. DT Mourya

c. **Background:**

Nipah virus (*Genus Henipavirus, family Paramyxoviridge*) causes a viral zoonotic disease. Pteropus bats (fruit eating species, popularly known as flying foxes) are supposed to be the natural hosts of the virus. Nipah virus emerged in Malaysia in 1998, resulting in significant morbidity and mortality in both pig and man. In India, the disease was recorded in humans without any involvement of pigs. The NiV genome consists of a negative-sense, single-stranded RNA of approximately 18.2 kb, encoding six structural proteins, nucleoprotein (N), phosphoprotein (P), matrix protein (M), fusion protein (F), attachment glycoprotein (G), and the large protein or RNA polymerase protein (L). We have indigenously developed a Real Time RT-PCR assay that can be used to detect Nipah viral RNA in human and animal specimens.

d. **Principle of the test:**

Qualitative reverse transcription PCR (RT-qPCR) is used when the starting material is RNA. In this method, RNA is first transcribed into complementary DNA (cDNA) by reverse transcriptase from total RNA. One-step assays
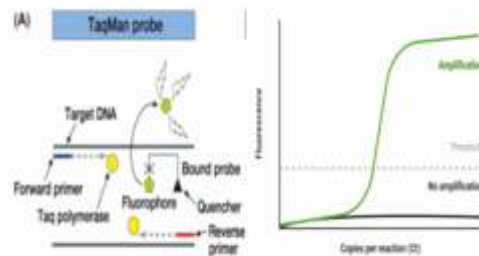
combine reverse transcription and PCR in a single tube containing reverse transcriptase and DNA polymerase. One-step RT-qPCR only utilizes sequence-specific primers for the highly conserved N gene.

e. **Performance characteristics:**

The specificity of the designed real time RT-PCR primers was tested against RNA isolated from influenza virus, respiratory syncytial virus (RSV), dengue virus, KFDV, CCHFV and Tioman virus positive samples.

Influenza and respiratory syncytial viral RNAs were used as both are paramyxoviruses. Dengue, CCHF and KFD viral RNAs were used as outliers and Tioman viral RNA was used as the reservoir host for both Nipah and Tioman viruses is the same. Nipah positive RNA isolated from human and bat samples was also used as positive controls. The primers were therefore found to be 100% specific for Nipah virus. For testing sensitivity of the assay, *in-vitro* transcribed Nipah N RNA was serially diluted from 4×10^9 copies of RNA to 4 copies of RNA per reaction in distilled water. The primers could detect upto four copies of *in-vitro* transcribed RNA in real time.

Intra and inter assay variability (%CV) was 4% and 5% respectively.

f. **External Quality assurance:**

External validation was performed at National Institute for Research in Tribal Health (NIRTH), Jabalpur and King George Medical University, Lucknow, UP and found to be satisfactory.

g. **Intended use:**

This kit is intended for rapid qualitative detection of Nipah viral RNA in the patients presenting with clinical signs of encephalitis.

h. **Translational Status:**

This test is being used within ICMR-NIV laboratories.

i. **Impact:**

This standardized technology is sensitive and reliable which helped in detection of first Nipah outbreak in India and thereafter till recent outbreak in Kerala 2021. This helped in further control measures for outbreak response.

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30
Nipah Virus Point of Care Test

a. **Name of the test/ assay:** Nipah Point of Care test

b. **Authors/Inventors:** Dr. PD Yadav

c. **Background:**

Nipah virus (NiV) is an emerging paramyxovirus observed mainly in Southeast Asia. In India, NiV outbreaks were reported from West Bengal in 2001 and 2007; the threat of NiV in India again came to the forefront with an outbreak report from Kerala in 2018, 2019 and 2021. Considering the need for rapid diagnosis at the laboratory and field settings, a Point-of-Care (PoC) Nipah PCR assay was developed and validated for NiV diagnosis.

d. **Principle of the test:**

The test works on the principle of DNA amplification, for the N gene; hence provides the ability to diagnose disease early in infection because of its excellent sensitivity and specificity.

The Truelab Real Time Quantitative micro PCR System is a compact battery-operated system which has single testing capability and provides the results within 1 hour after collection of sample. Hence, it enables same day reporting and initiation of evidence-based treatment for the patient. It also has real-time data transfer capability (through SMS/E-mail/data push) for immediate reporting of results in emergency cases.

e. **Performance characteristics:**

The test was found to be highly sensitive, specific with detection limit up to 10 copies of NiV RNA. The sensitivity and specificity of the Truenat™ Nipah PCR test were found to be 97% (95% CI 90%-100%) and 100% (95% CI 98%-100%) respectively when compared with NiV real time RT-PCR.

Intra and inter assay variability (%CV) was 3% and 3% respectively.

f. **External Quality assurance:**

Trueprep® AUTO Universal Cartridge Based Sample Prep Device and the Truenat™ Nipah PCR system was installed at the Institute of Epidemiology Disease Control and Research [IEDCR], Dhaka, Bangladesh and samples were checked for system performance. Both the systems were also installed at Government Medical College, Ernakulam, Kerala during 2019 NiV outbreak and two field sites in Punjab under NiV surveillance in bats. A panel of 10 positives and 20 negatives was tested at different sites and the results were found to be concordant.

g. **Intended use:**

This test will enable the rapid detection of NiV in the field during outbreak situations for quick diagnosis and to facilitate containment of the NiV.

h. **Translational Status:**

This test is being used within VRDLs and ICMR-NIV laboratories.

i. **Impact:**

Timely diagnosis of NiV plays very important role in the patient management and curbs the further spread of infection. With the help of Nipah PoC, quick diagnosis was achieved during the recent Nipah outbreaks in Kerala during 2019 and 2021. This helped in saving many lives and reducing the panic.

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31
RT-LAMP technology
for detection of SARS-CoV-2

a. **Name of the test/ assay:** RT-LAMP assay for detection of SARS-CoV-2

b. **Authors/ Inventors:** Dr. SS Nandi, Dr. U Lambe, SA Sawant, T Gohil and Dr. JM Deshpande.

c. **Background:**

The novel coronavirus SARS-CoV-2 that originated in Wuhan, China in December 2019 is the causative agent of the ongoing COVID-19 pandemic. The number of cases increased exponentially during the second wave of infection. The Real Time PCR (rRT-PCR) is being used as a gold standard for the laboratory diagnosis of COVID-19. Diagnostic technologies possessing sensitivity and specificity equivalent to rRT-PCR assays are needed to ramp up testing capacity in most countries. Newer platforms need to be technically less demanding, require minimum equipment and reduce turn-around time for reporting results. Hence, this study was designed to explore the loop mediated isothermal amplification (LAMP) for detection of SARS-CoV-2 and evaluate its performance by comparison with rRT-PCR.

d. **Principle of the test:**

RT-LAMP uses multiple forward and reverse (backward) primers and one or two loop primers. RT-LAMP uses the RTx reverse transcriptase that gets activated above 45°C and modified Bst 2.0 DNA polymerase having strand displacement activity. Therefore, RT-LAMP reactions can be carried out at a single incubation temperature (isothermal). Primers B2 and B3 initiate cDNA synthesis. Forward and Backward Inner Primers (FIP and BIP) are specialized primers consisting of two parts, F2/F1c and B2/B1c, respectively. F2 and B2 bind with the template strand to initiate the amplification process while F1c and B1c sequences serve as overhangs which help loop formation as RT-LAMP reaction continues. The short distance between the F2 and F1c (and B2/ B1c) helps formation of a loop structure within the amplicon. The loop primers increase the number of initiation points for DNA synthesis by binding complementarily to the single stranded loops and increase the pace of amplification. In the in-house assay, novel sets of primers were designed from conserved regions of E and N gene of SARS-CoV-2. The internal control (IC) gene (Beta actin) is also included in the assay. This IC can be used in combination with various other RT-LAMP based diagnostic assays. The IC helps as an indicator for quality check of sample collection, nucleic acid extraction and quality of reaction.

e. **Performance characteristics:**

A sum total of 253 nasal/ throat swabs of suspected COVID-19 patients collected in the virus transport medium (VTM) by various hospitals were received for laboratory diagnosis under COVID-19 surveillance. Samples were tested by real time RT-PCR as per the ICMR standard protocol. The same samples were tested retrospectively by the RT-LAMP assay. The diagnostic sensitivity and specificity of the RT-LAMP assay was 98.46% and 100%, respectively, as compared to the rRT-PCR. The limit of detection of RT-LAMP was calculated to be 40 copies.

Sr. No.	RT-LAMP end point color	Visual observation	Interpretation
1	E N B	Both E and N gene reaction color is pink B-actin reaction color is yellow	Negative for both E and N genes. Negative for SARS-CoV-2
2	E N B	E gene reaction color is pink and N gene reaction color is yellow B-actin reaction color is yellow	Negative for E gene and positive for N gene. Positive for SARS-CoV-2
3	E N B	E gene reaction color is yellow and N gene reaction color is pink B-actin reaction color is yellow	Positive for E gene and negative for N gene. Positive for SARS-CoV-2
4	E N B	Both E and N gene reaction color is yellow B-actin reaction color is yellow	Positive for both E and N genes. Positive for SARS-CoV-2

Table: Interpretation of results for detection of SARS-CoV-2 RNA: The sample is considered as positive for SARS-CoV-2 RNA if it exhibits either E gene or N gene positive or both E and N genes positive in all the above scenarios. The sample is considered as negative for SARS-CoV-2 RNA if both the genes display negative results..

f. External validation:

The external validation was carried out at National Institute of Biologicals (NIB), Noida. A panel of samples was tested using RT-LAMP and the results were compared with rRT-PCR (gold standard). The assay has achieved 100 % diagnostic sensitivity and specificity.

g. Intended use:

The assay can be used for rapid laboratory diagnosis of COVID-19.

h. Translational Status:

The RT-LAMP technology has been transferred to two commercial organizations for scaling up.

Inauguration of RT-LAMP kit by Dr. (Prof.) Balram Bhargava, Secretary DHR and DG-ICMR

Submitted patent application:

Title of the patent "Rapid RT-LAMP assay for detection of SARS-CoV-2"

Inventors: Dr. Shyam Sundar Nandi, Dr. Upendra Lambe, Sonali Ankush Sawant, Trupti Gohil, Dr. Jagadish Mohan Deshpande.

Indian Patent Application Number: 202011023573, June 2020.

PCT Application number: PCT/IN2021/050549, June 2021

i. Impact:

The ICMR-NIV RT LAMP technology will be useful to ramp up national testing capacity (lower level laboratories), Village level, Block Level, Railway Station, Airports, Shopping Malls to diagnose COVID-19 in the country. This assay could enable field testing outside of the diagnostic laboratory. Helping in diagnostics in pandemic situation without any sophisticated instruments (Like PCR or RT-PCR Machine) to perform the assay and no highly qualified technician required to perform the test. The results can be interpreted visually within 30-40 minutes.

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32
Rapid Point of Care kit for early detection of silicosis/silico-tuberculosis

a. **Name of the test/ assay:** Rapid Point of Care (PoC) kit for early detection of silicosis/silico-tuberculosis

b. **Authors/ Inventors:** Dr. SS Nandi, Dr. U Lambe, Dr. K Sarkar (ICMR-NIOH, Ahmedabad), SA Sawant and Dr. JM Deshpande.

c. **Background:**

Club cell protein 16 (CC16) is the most abundant protein in broncho-alveolar secretions. As per the literature data, many chronic pulmonary inflammatory diseases such as anthraco-silicosis, chronic obstructive pulmonary disease (COPD), asthma etc. cause depletion of CC16. Chronic silicosis, the commonest and widely prevalent form of silicosis, is an irreversible occupational ailment of the respiratory system caused by the invasion of lung tissue (parenchyma) due to dust consisting of crystalline silica or silicon dioxide of respirable size. Evidence suggests significant reduction of CC16 in chronically silica dust-exposed workers with no change in respiratory symptoms. Additionally, the patients with silicosis are highly susceptible to tuberculosis because of depleted lung immunity. This condition is also called silico-tuberculosis. Normal chest radiology and lung function tests indicate that serum CC16 could be an early asymptomatic detection tool for silicosis among silica-exposed population at risk. Therefore, development of a PoC assay for detection of silicosis is of primary importance. We therefore developed a PoC, semi-quantitative lateral flow device based on gold nanoparticles for screening of occupational silica dust exposed workers for early detection of silicosis.

d. **Principle of the test:**

The PoC assay can be particularly employed for semi-quantitative estimation of CC16 in human serum samples. This assay can be used periodically to assess the serum CC16 levels among workers with a history of silica dust exposure. The serum CC16 protein migrates by capillary action through the nitrocellulose membrane and reaches the test lines. At the test line a certain amount of serum CC16 is captured by the rabbit anti-CC16 polyclonal antibodies. The excess amount of CC16 protein is trapped by the second test line and the same is followed by the third test line. This is followed by running Gold Nanoparticles (GNPs) conjugated with anti-CC16 monoclonal antibodies (anti-CC16 mAb + GNPs complex) through the Nitrocellulose membrane (NCM) which will produce a red colored band at the test line and the control line. The intensity and the number of red lines developed at the test zone is directly proportional to the concentration of CC16 in the sample semi quantitatively. If the serum CC16 concentration is 6 ng/ml or less, one red colored band is detected. If the serum CC16 concentration is in the range of 6.1 to 9 ng/ml then the assay produces two bands and if the serum CC16 concentration is more than 9 ng/ml, the assay produces three bands. One control band is observed at the control line irrespective of the CC16 concentration present in the serum. The performance evaluation of the assay has been done by testing 104 serum samples in parallel with the lateral flow assay and the commercially available ELISA [Club Cell Protein (CC16) Human ELISA, Biovendor Czech Republic].

Table: Interpretation of semi-quantitative assay for detection of silicosis.

e.   Performance characteristics:

The performance evaluation of the assay has been done by testing 104 serum samples in parallel with the lateral flow assay and the commercially available ELISA [Club Cell Protein (CC16) Human ELISA, Biovendor Czech Republic]. The sensitivity of the assay was 100% while the specificity was 95%.

f.   External validation:

The process for the external validation has been initiated. The geographical areas with highest incidence of silicosis such as parts of Gujrat and Rajasthan are being targeted. The study will be carried out by collecting the serum samples from silicosis positive clinical subjects and a number of healthy volunteers by taking prior ethical approval and consent from the subjects. The serum CC16 value will be tested using lateral flow assay and results will be compared with ELISA.

g.   Intended use:

This assay may be used as a proxy marker for periodic screening and early detection of silicosis in silica dust exposed vulnerable workers. The PoC device will be used for screening of workers exposed to occupational silica dust for early detection of silicosis.

h.   Translational Status:

The technology transfer has been done to two commercial partners for the production and marketing of the kit.

Inauguration Silicosis kit by Dr. (Prof.) Balram Bhargava, Secretary DHR and DG-ICMR

Submitted Patent Application:

Title of the patent "A Point of Care Device, Method and Kit involving Club Cell protein 16 as a marker for Silicosis."

Inventors: Dr. Shyam Sundar Nandi, Dr. Upendra Lambe, Dr. Kamalesh Sarkar, Sonali Ankush Sawant, Dr. Jagadish Mohan Deshpande.

Indian Patent Application No: 202011014266 (31st March, 2020).

International PCT application No. PCT/IN2021/050328 dated 31st March 2021

i.   Impact:

The technology is quite robust and requires a low amount of sample of about 10μl. This is a point of care device and thus can be used without high operational needs/equipment. The time for diagnosis is 10 minutes whereas the conventional tests take a minimum of 4 hours. There is no need of any technical expertise or training to diagnose the patients with the present kit as the diagnosis is based only on visualization of bands. It will be a cost effective technology. This point of care kit for early detection of silicosis/silico-tuberculosis will save many lives in India and abroad.

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33
Rapid LAMP technology for detection of *Corynebacterium*

a. **Name of the test/ assay:** Rapid LAMP technology for detection of Corynebacterium diphtheria

b. **Authors/ Inventors:** Dr. SS Nandi, Dr. S Roy (ICMR-NIE, Chennai), U Lambe, SA Sawant, T Gohil, Dr. M Murekar (ICMR-NIE, Chennai) and Dr. JM Deshpande.

c. **Background:**

Diphtheria is an acute, highly infectious, and potentially lethal disease caused by diphtheria toxin-producing bacterial strains of *Corynebacterium diphtheriae*. Diphtheria is re-emerging in various States of India. Till recently it was known to be primarily a childhood disease. However, increasingly more cases are being reported from adults. Diphtheria is usually diagnosed based on a patient's clinical presentation. Laboratory demonstration of the presence of *Corynebacterium diphtheriae* in the throat swab samples requires culture and isolation or PCR-based tests that require time. This is also expensive, requires expertise and can be performed only in high-end laboratories. The objective of this work was to develop an RT-LAMP assay for the detection of toxigenic genes for differentiation between toxigenic and non-toxigenic species of C. diphtheriae.

d. **Principle of the test:**

RT-LAMP uses multiple forward and reverse (backward) primers and one or two loop primers. RT-LAMP uses the RTx reverse transcriptase that gets activated above 45°C and modified Bst 2.0 DNA polymerase having strand displacement activity. Therefore, RT-LAMP reactions can be carried out at a single incubation temperature (isothermal). Primers B2 and B3 initiate cDNA synthesis. Forward and Backward Inner Primers (FIP and BIP)

are specialized primers consisting of two parts, F2/F1c and B2/B1c, respectively. F2 and B2 bind with the template strand to initiate amplification process while F1c and B1c sequences serve as overhangs which help loop formation as RT-LAMP reaction continues. The short distance between the F2 and F1c (and B2/ B1c) helps formation of a loop structure within the amplicon. The loop primers increase the number of initiation points for DNA synthesis by binding complementarily to the single stranded loops which increases the pace of amplification. For the detection of *Corynebacterium diphtheriae*, two genes have been targeted based on bioinformatics analysis. It is established that tox gene encoding diphtheria toxin is present only in the pathogenic strains of C. diphtheriae. On the other hand, dtxR gene encoding global regulator is a species-specific gene. The C. diphtheriae species can be detected by using species specific gene dtxR. In this in-house assay, novel sets of primers were designed from conserved regions of dtxR and tox genes.

e. **Performance characteristics:**

A significant number of samples are being processed for the calculation of sensitivity and specificity by using known positive and negative clinical samples. The results of LAMP are compared with the results of culture on Tellurite agar and confirmed PCR results.

f. **External validation:**

The validation of this assay is under process. A significant number of clinical samples will be collected by taking prior ethical approval. The samples will be collected from healthy individuals after taking consent from the subjects. The results of the LAMP assay will be compared with results on Tellurite agar culture and PCR.

g. **Intended use:**

This technology can be used as a rapid screening assay for the detection of C. *diphtheriae* and differentiation between toxigenic and non-toxigenic species of C. *diphtheriae*.

h. **Translational Status:**

The technology transfer process will be initiated by ICMR-HQ soon.

Fig: Interpretation of LAMP assay for detection of
***Corynebacterium* diphtheriae**

Submitted Patent Application:

Title of the patent "Development of a colorimetric isothermal assay for detection of *Corynebacterium diphtheriae*"

Inventors: Dr. Shyam Sundar Nandi, Dr. Subarna Roy, Dr. Upendra Lambe, Sonali Ankush Sawant, Trupti Gohil, Dr. Monoj Murekar, Dr. Jagadish Mohan Deshpande. Patent application submitted to Indian Patent Office (Patent Application Number 202111015391, Dated: 31.03.21).

i.   Impact:

It is cost effective and less time consuming technology. This assay could enable point-of-care testing outside of the diagnostic laboratory and can be applied for contact tracing and diagnosis in field conditions. This assay is also able to differentiate between toxigenic and nontoxigenic species of C. *diphtheriae*.

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34
Multiplex SNP technology for identification of host genetic susceptibility markers to Enterovirus A 71 infection

a.   Name of the test/ assay: Multiplex SNP technology for identification of host genetic susceptibility markers to Enterovirus A 71 infection in Indian population.

b.   Authors/ Inventors: Dr. SS Nandi, SA Sawant, T Gohil and Dr. JM Deshpande.

c.   Background:

EV71 infection is endemic in India. However, detection of EV-71 was observed during a mild outbreak of October 2003 in Calicut (Sasidharan et al., 2005). Again during 2012 in Ahmedabad, Gujarat and 2013-2014 in Pune, Maharashtra reported cases of Hand-Foot-Mouth Disease (HFMD) (Ganorkar et al., 2017). Interestingly, there have been no reports of outbreaks of HFMD, encephalitis/ meningitis or acute flaccid paralysis of EV-A71 etiology in the country. The reasons for this are not yet clear. Recent research reports have identified point mutations (SNPs) in a small number of host genes leading to susceptibility to severe EV-A71 infections. Varying genetic susceptibility of the population to EV-A71 infection or pathogenicity of the circulating strains may be the reasons. There are two hypotheses a) the indigenous EV71 genotypes are naturally less virulent and b) the Indian population is genetically less susceptible to diseases caused by EV-71.

We used the prior knowledge of genetic susceptibility to EV-71 to develop a multiplex Single Nucleotide Polymorphisms (SNP) assay to explore mutations in several genes. A total of 15 SNPs in 12 genes have thus been published as host genetic risk factors/ markers for EV-A71. The genes include IFNAR1, SCARB2, OAS, Interferon lambda 4, SELPLG, Interleukin 10, Toll-like receptor 3, Interleukin-17, Retinoic acid-inducible gene I,

Interleukin-6, Interferon gamma (IFN- γ) and Melanoma differentiation-associated gene 5. It would be interesting to understand the genetic contribution to susceptibility to EV71 infection in the Indian population.

d.   Principle of the technology:

The assay combines multiplex PCR amplification with a multiplex primer extension assay to allow targeted detection of all four mutations in one reaction. 15 SNPs were selected from 12 different genes associated with susceptibility towards EV-71 infection. The 12 genes used for the study are: IFNAR1 gene (four SNPs rs1012334, rs2843710, rs1787572, rs113181057), IL-10 (rs1800896), SCARB2 (rs41284767), IL-6 (rs1800796), MDA5 (rs1990760), IFNL4 (rs12979860), SELPLG (rs2228315), IL-17 (rs763780), OAS1 (rs10774671), TLR3 (rs3775291), RIG-1 (10813831) and IFN-G (rs2430561). Totally 15 SNP primers along with corresponding 30 new PCR primers were designed for detection of SNPs in the 12 different genes using NCBI database. Each SNP primer was of different length with the addition of [GACT]$_n$ tail to the 5□-end to provide a unique length for each primer. The SNP primers have been arranged to develop 2 new multiplex SNP detection assays. SNP multiplex assay 1 has 7 SNPs and SNP multiplex assay 2 has 8 SNPs.

e.   Performance characteristics:

The SNP Multiplexed assay developed targeting 15 genetic markers from 12 genes against EVA-71 infection. The 15 SNP primers were divided into two assays –SNP Multiplex assay1 and SNP Multiplex assay2. The SNP Multiplex assay1 consists of 7 SNP gene primers namely- IL-10 rs1800896, SCARB2 rs41284767, IL-6 rs1800796, MDA5 rs1990760, IFNAR1 rs1012334, IFNL4 rs12979860, SELPLG rs2228315. The SNP Multiplex assay2 consists of 8 SNP gene primers namely- IFNAR1 rs2843710, IL-17F rs763780, OASI rs10774671, TLR3 rs3775291, IFNAR1 rs113181057, RIG-I rs10813831, IFN-G rs2430561 (Figure). Both the assays showed distinct SNP peaks with no overlap or ambiguity in interpretation of the results. Thus, the assay provides genotypic information for 15 SNPs involved in EV-71 susceptibility for human population. The peaks observed in the assays were validated using Sanger sequencing as a gold standard method.

f.   Inter laboratory comparison / External validation:

The assay was validated in collaboration with Central Railway Hospital, Kalyan, Ministry of Railways, Govt. of India. 100 blood samples were collected from healthy adult individuals who provided informed consent for participation and volunteered for the study. The data for 100 blood samples have been generated. The polymorphisms observed for each of the 12 selected genes were analyzed and the frequency of the individual alleles were calculated. This data is compared with data available from the Chinese population
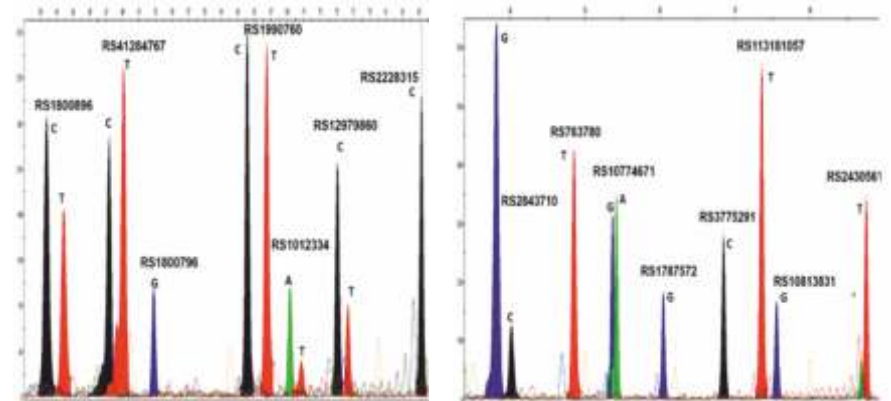
Figure: A) SNP Multiplex Assay 1: The result of a human DNA sample. The 7 SNPs can be read without ambiguity using this assay. B) SNP Multiplex Assay 2: The result of a human DNA sample. The 8 SNPs can be read without ambiguity using this assay.

g.   Intended use:

This assay can be used to carry out a country-wide survey of the genetic markers among Indian subjects and to determine the prevalence of the EV-A71 genetic susceptibility markers in the Indian population. Prevalence of genetic susceptible markers may assist evaluation of risk of EV-A71.

h.   Translational Status:

The patent application has been submitted. "Assay to investigate multiple genes as host genetic risk factors for Enterovirus71 infection severity."

Inventors: Dr. Shyam Sundar Nandi, Sonali Ankush Sawant, Dr. Jagadish Mohan Deshpande. Submitted Indian patent application No: 201811023228. PCT Application Number PCT/IN2019/050468.

i. **Impact:**

This technology can be used for country-wide survey of the genetic markers among Indian subjects. The population-based screening for genetic susceptibility to EV-A71 in Indian population can be achieved with this kind of assay. Prevalence of genetic susceptible markers may assist evaluation of risk of EV-A71.

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Real Time RT-PCR assays No. 35 to 40

Principle: All Real Time RT-PCR assays developed (No. 35 to 40) are based on TaqMan chemistry.

Quantitative PCR (qPCR) or real-time PCR is used for sensitive & specific detection and quantification of nucleic acid targets. TaqMan real-time PCR assays consist of target-specific primers and one or more probes optimized for specific applications. The probe is labeled with two fluorescent moieties. The emission spectrum of one overlaps the excitation spectrum of the other, resulting in "quenching" of the first fluorophore by the second.

The assay uses the 5´–3´ exonuclease activity of Taq polymerase to cleave a dual-labeled probe during hybridization to the complementary target sequence and fluorophore-based detection. As the Taq polymerase reaches the probe, the 5´ to 3´ exonuclease activity of the polymerase degrades the probe during hybridization to complementary target sequence and fluorophore is released from the quencher (fig 1a, 1b). The fluorescence is directly proportionate to amplification and is detected in real time.
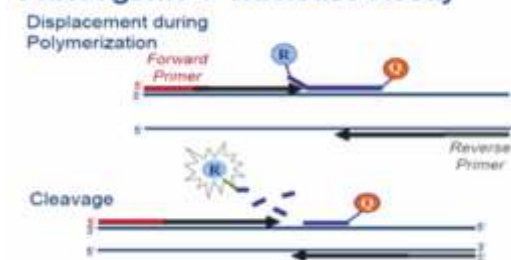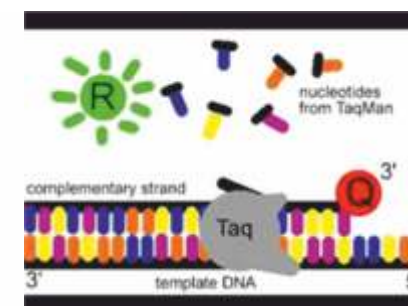

| Figure 1a | Figure 1b |

35
Multiplex single tube Real Time RT PCR assay for detection of SARS CoV-2

a. **Name of the test/ assay:** Multiplex single tube Real Time RT PCR assay for detection of SARS CoV-2.

b. **Authors:** Dr. VA Potdar, Dr. ML Choudhary, Mrs. V Vipat and Mrs. S Kadam

c. **Background:**

Coronaviruses have characteristic club-shaped spikes that project from their surface, which in electron micrographs creates an image reminiscent of the solar corona, hence the name. The genome of SARS CoV-2 is comprised of a single-stranded positive-sense RNA, ~29.9 Kb in size. The genome contains 38% of GC content and 11 protein-coding genes, with 12 expressed proteins. The genetic arrangement of ORFs highly resembles that of SARS CoV-1 and MERS CoV. The ORFs are arranged as replicase and protease (1a–1b) and major S, E, M, and N proteins.

Coronavirus disease 2019 (COVID-19) is a contagious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS CoV-2). The first known case was identified in Wuhan, China, in December 2019. The disease has since spread worldwide, leading to the ongoing pandemic.

Globally, as of 10 January 2022, there have been 305,914,601 confirmed cases of COVID-19, including 5,486,304 deaths. India reported 35,707,727 cases and 483936 deaths due to COVID 19.

The ICMR-NIV, Pune, being an apex laboratory of ICMR, developed a robust diagnostic methodology to detect SARS CoV-2 using Real Time RT PCR method and deployed this test to public health laboratories. In the backdrop of the day-by-day increasing testing strategy, it was necessary to have uninterrupted supply of diagnostic reagents to the laboratories.

The developed kit is a combination of screening and confirmatory assays in a single tube. The target genes used in the kit are E, ORF1ab and RdRp of SARS CoV-2 and β actin as a housekeeping gene. The kit includes a cocktail of in-vitro transcribed RNA for each target in the test as a positive control.

d. **Performance characteristics:**

Sensitivity / limit of detection was determined using 10-fold serial dilutions ($\sim$108 to 5 RNA copies/μl) of in-vitro transcripts (RNA) of E or ORF1ab or RdRp gene of SARS CoV-2. Performance of the assay showed a detection limit of 3.57 copies for E gene, 2.18 copies for ORF1ab and 25 copies for RdRp gene.

e. **External validation:**

This assay has gone through vigorous inter institutional validation. It was validated by 10 VRDL sites and was found to be 98-100% sensitive and 100% specific when compared with the ICMR-NIV two tube assay for SARS CoV-2.

Testing of the kit using the WHO External Quality Assurance Services (EQAS) panel showed 100% concordant results.

f. **Intended use:**

The SARS CoV-2 kit has been used to rule out SARS CoV-2 infection. It has also been used as a gold standard for validation of RT-PCR and antigen detection kits.

The kit was proposed for and is currently being used in surveillance of SARS CoV-2, checking for reinfection, SARS CoV-2 status before and after vaccination and to test the anti-SARS CoV-2 property of the drugs / compounds. During Covaxin Phase 2 and 3 clinical trials, this was used to rule out infection and confirm study enrolment.

g. **Translational Status:**

A provisional patent application for the Multiplex single tube SARS CoV-2 assay has been filed on April 01, 2021 and patent application number is 202111015708.

Multiplex SARS CoV-2 Detection Kit

h.   Impact:

Till September 2020, kit was widely used by all the government laboratories across India. The kit was also used as a gold standard for validation of Make in India RT PCR and antigen kits, environmental studies and during Phase II and III covaxin vaccine trials. A total of 64 lakh reagents were supplied.

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36
Multiplex single tube Real Time RT PCR assay for detection of Influenza A, B and SARS CoV-2

a.   Name of the test/ assay: Multiplex single tube Real Time RT PCR assay for detection of Influenza A, B and SARS CoV-2

b.   Authors: Dr. VA Potdar, Mrs. V Vipat and Mrs. S Kadam

c.   Background:

Influenza viruses are enveloped negative-sense single-strand RNA viruses with a segmented genome. They belong to *Orthomixoviridae* family and have four subtypes A, B, C and D. Influenza virus contains eight RNA segments which encode eleven proteins. The viral glycoproteins haemagglutinin (HA) - which facilitates viral entry, neuraminidase (NA) - which facilitates viral release, viral nucleoprotein (NP), matrix protein (M1) and membrane protein (M2), the non-structural protein NS1 and nuclear export protein (NEP). Influenza A viruses have been classified into diverse subtypes on the basis of antigenic variability of HA (H1 to H16) and NA (N1 to N9) glycoproteins.

Influenza, commonly known as "the flu", is an infectious disease caused by influenza viruses. Symptoms range from mild to severe and often include fever, runny nose, sore throat, muscle pain, headache, coughing, and fatigue. Influenza may progress to pneumonia, which can be caused by the virus or by a subsequent bacterial infection. Other complications of the infection include acute respiratory distress syndrome, meningitis, encephalitis, and worsening of pre-existing health problems such as asthma and cardiovascular disease. Antigenic shift (virus re-assortment) and drift in the virus causes pandemics and epidemics worldwide. In the past 150 years, five pandemics of human influenza have occurred; in 1889 "Russian flu" (H3N8) followed by the 1918 "Spanish flu" (H1N1), the "Asian flu" in 1957 (H2N2), the "Hong-Kong flu" in 1968 (H3N2) and

the most recent 2009 "Swine flu" (H1N1) or Influenza A (H1N1) pdm09.

Globally, in a year, 5–15% of the population get infected by the influenza virus. There are 3–5 million severe cases reported annually, with up to 650,000 deaths, most commonly seen in high-risk groups, including young children, the elderly, and people with chronic health conditions. In India during the 2009 pandemic, 27,236 cases were reported, with 981 deaths. In the year 2018, the Southern states of India contributed to 47.7% of morbidity and 20.2% of mortality due to Influenza A. As per the National Centre for Disease Control (NCDC), India has recorded a total of 6,701 swine flu cases and 226 deaths across the country. The maximum number of cases have been reported from Rajasthan (2,363) followed by Delhi (1,011) and Gujarat (898). Rajasthan reported the highest number of deaths (85), followed by Gujarat (43) and Punjab (30).

Due to annual outbreaks and occasional pandemics, controlling influenza has become a major public health challenge. Vigilance is required to understand the co-circulation of influenza and SARS CoV-2 viruses. Hence, it was necessary to develop a combo assay to detect SARS CoV-2 and Influenza A and B in a single tube.

The combo kit detects all influenza A viruses, Type B virus and SARS CoV-2 in a single tube. The assay uses M gene for detection of Influenza A, NS gene for Influenza B and ORF1ab gene for SARS CoV-2.

d. Performance characteristics:

Sensitivity / limit of detection was determined using 10-fold serial dilutions ($\sim 10^8$ to 5 RNA copies/μl) of *in-vitro* transcripts (RNA) of ORF1ab gene of SARS CoV-2. For influenza A and B viruses, 10-fold dilution of well characterized isolates was used to detect the limit of detection. Performance of each target was as follows: ORF1ab detects 2.18 copies, Influenza A 34 copies, Influenza A (H1N1) pdm09 10 Copies, A(H3N2) 10 copies and Influenza B detects 20 copes (10^{-7} dilution).

e. External validation:

This assay went through vigorous inter institutional validation. It was validated by 5 VRDL sites and was found to be 98-100% sensitive and 100% specific.

Testing of the kit using WHO EQAS panel showed 100% concordant results.

f. Intended use:

- Surveillance of SARS CoV-2 and Influenza
- To check reinfections
- To check the SARS CoV-2 status before and after vaccination
- To test anti - SARS CoV-2 property of drugs / compounds

g. Translational Status:

An expression of interest will be floated to identify suitable vendors for technology transfer.

Multiplex combo kit for Influenza A, B and SARS CoV-2 kit and Influenza Subtyping kit

h. Impact:

The kit is useful to monitor Influenza and SARS CoV-2 simultaneously. The kit has been used by 20 VRDL laboratories under PAN INDIA influenza-like illness (ILI) and severe acute respiratory infections (SARI) Surveillance. During the SARS CoV-2 pandemic it was possible to keep track on influenza circulation using this kit. Total 26 thousand reactions were supplied to VRDLs.

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37
Real Time RT PCR assay for Detection of Human coronaviruses (HCoVs) and Middle East respiratory syndrome coronavirus (MERS CoV)

a. **Name of the test / assay developed:** Real Time RT PCR assay for detection of Human coronaviruses (HCoVs) and Middle East Respiratory Syndrome coronavirus (MERS CoV).

b. **Authors:** Dr. VA Potdar, Mrs. V Vipat and Mrs. S Kadam

c. **Background:**

Human coronaviruses are members of the *Coronaviridae* family, which belongs to the Nidovirales order. These viruses are enveloped, non-segmented, positive-sense, and single-stranded RNA viruses that cause mild or severe respiratory disease-like symptoms in birds and mammals, including humans. Their genome size is about 27-32 kilobases (kb) which consists of non-structural (ORFs) and four structural proteins, membrane (M), envelope (E), spike (S), and nucleocapsid (N) proteins. Corona viruses are classified into four genera on the basis of their antigenic and genetic properties as follows:

1. Alpha coronavirus (αCoV)
2. Beta coronavirus (βCoV)
3. Gamma coronavirus (γCoV)
4. Delta coronavirus (δCoV)

Coronaviruses, HCoVs and MERS CoV can cause respiratory tract infections that range from mild to lethal. Mild illnesses such as common colds are observed in humans and birds. HCoVs can cause life-threatening pneumonia and bronchiolitis especially in the elderly, children and immunocompromised patients. The more lethal HCoVs are SARS, MERS and SARS CoV-2.

Globally, Coronaviruses are the cause of approximately one-third of common cold infections in humans. In 2002, the first lethal Severe Acute Respiratory Syndrome (SARS) was reported with pneumonia-like symptoms with a diffuse alveolar injury which led to Acute Respiratory Distress Syndrome (ARDS). Similarly in 2012, Middle East Respiratory Syndrome was reported due to a novel β-corona virus; MERS-CoV which affected more than 2519 individuals across 27 countries and led to the death of 866 individuals, with a case-fatality rate of 34.3%.

In south western India, the prevalence of HCoVs among Severe Acute Respiratory Infection (SARI) cases was 1.04%. HCoV OC43 was predominantly detected followed by HCoV NL63 and HCoV 229E.

Owing to their ability to recombine, mutate and infect multiple species and cell types, coronaviruses can cause outbreaks in both humans and animals. These viruses have the propensity to jump between species and this can lead to pandemics and epidemics. On the background of SARS CoV-2 it is prudent to have differential diagnosis for known human corona viruses. For this purpose, ICMR-NIV has established the Real Time RT PCR.

The following duplex Real Time PCR assays were developed:

No.	Set of RT PCR	Virus	Gene used in RT-PCR assay
1	Set 1	229E -alpha coronavirus NL63-alpha coronavirus	Nucleocapsid (N) Nucleocapsid (N)
2.	Set 2	OC43-beta coronavirus HKU1-beta coronavirus	Nucleocapsid (N2) ORF1ab
3.	Set 3	MERS CoV- Middle East respiratory syndrome corona virus	E and RdRp

d. **Performance characteristics:**

The assay is 98-100% sensitive and 100% specific.

e. **External validation:**

Testing of the set 1 and 2 assays using EQAS panel provided by quality

control for molecular diagnosis (QCMD) showed 100% concordant results.

Testing of set 3 assay using WHO EQAS panel showed 100% concordant results.

f.   Intended use:

- Surveillance of Coronaviruses

- Differential diagnosis of coronavirus (es) infections

g.   Translational Status:

Currently, the assay is used for research purpose only. In future, an expression of interest will be floated to identify suitable vendors for technology transfer.

h.   Impact:

Against the background of SARS CoV-2 pandemic, using this assay one can provide differential diagnosis of known human corona viruses. The MERS CoV-2 detection was helpful to track the infection if any in the travelers / passengers coming from affected areas. The reagents and training provided to South East Asian countries enhance their capacity to detect MERS CoV-2 with the help of WHO SEARO.

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38
Real Time RT-PCR assay for detection of Respiratory Syncytial Virus

a.   Name of the test / assay: Real Time RT PCR assay for detection of Respiratory syncytial Virus

b.   Authors: Dr. VA Potdar, Mrs. V Vipat and Mrs. S Kadam

c.   Background:

Respiratory syncytial virus (RSV) is ~150 nm in size, mostly spherical enveloped virus belonging to the genus *Orthopneumovirus*. It is a negative-sense, single-stranded linear RNA virus, approximately 15Kb in length, consisting of 10 genes (NS1-NS2-N-P-M-SH-G-F-M2-L) encoding for 11 proteins. RSV is divided into two antigenic subtypes, A and B, based on the reactivity of the F and G surface proteins to monoclonal antibodies. RSV subtype A (RSVA) is considered more prevalent and virulent than RSV subtype B (RSVB), due to higher viral loads and faster transmission rate. F and G proteins are the primary targets for neutralizing antibodies and F protein causes merging of cell membrane and forms large multinucleated syncytia.

'RSV' is the single most common cause of hospitalization of infants due to respiratory disease. It is an important pathogen which causes respiratory infections in all age groups. Infection rates are typically higher during the cold winter months, causing bronchiolitis in infants, common colds in adults, and more serious respiratory illnesses such as pneumonia in the elderly and immunocompromised individuals.

Worldwide, 70% of bronchiolitis cases in children are due to RSV. RSV infection in adults is mild but about 25% of infected adults progress to significant lower respiratory tract infection, such as bronchiolitis. Immune compromised patients of all ages are at a high risk of pneumonia. A systematic review conducted in the year 2013 on global burden of Acute

Lower Respiratory Tract Infections (ALRI) in children described that about 12 million hospital admissions occurred due to severe ALRI and 3 million are due to very severe ALRI.

In India, RSV mainly peaks during winter in North India and some correlation with low temperature has been observed. The rate of RSV detection in various hospital and community based studies, mostly done in children vary from 5% to 54% and from 8% to 15%, respectively. Both genotypes RSV A and RSV B have been detected in India. Acute Lower Respiratory Tract Infections (ALRI) are estimated to cause 75% of all acute illnesses and are the leading cause of hospitalization for infants and young children in India.

WHO launched the Battle against Respiratory Viruses (BRaVe) initiative to prioritize research to gain a better understanding of the epidemiology, pathogenesis, prevention and clinical management of respiratory virus infections across different populations and resource settings. ICMR NIV is a part of WHO RSV study and developed a duplex Real Time PCR for diagnosis of RSV and its subtypes.

The assay uses the nucleocapsid (NC) gene of RSV A and nucleoprotein (NP) gene of RSV B for detection of RSV in a single tube assay.

d. Performance characteristics:

Sensitivity/ limit of detection was determined using 10-fold serial dilutions ($\sim 10^8$ to 5 RNA copies/μl) of in-vitro transcripts (RNA) of nucleocapsid and nucleoprotein genes RSV A and RSV B respectively. Performance of the assay showed detection limit of 12 copies for RSV A and 10 copies for RSV B.

This assay has 98-100% sensitivity and 100% specificity. The assay was used by ten sites under the Global Health Security Agenda (GHSA) and ICMR task force project of pneumonia.

e. External validation:

Using this assay, WHO EQAS panels for the year 2018, 2019 and 2021 were tested with 100% concordance.

f. Intended use:

- Surveillance of RSV
- Diagnosis of RSV

g. Translational Status:

Currently, the assay is used for research purpose only. In future, an expression of interest will be floated to identify suitable vendors for technology transfer.

h. Impact:

The assay is useful to track the mortality and morbidity due to RSV in less than two years children, pediatric and elderly populations. The assay is used in many national programs of national and international fundings, including ICMR Task force project of Pneumonia.

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39
Real Time RT-PCR assay for detection of Respiratory Viruses other than Influenza and RSV

a. **Name of the test / assay:**   Real Time RT-PCR assay for detection of respiratory viruses other than influenza and Respiratory syncytial viruses (RSV)

b. **Authors:** Dr. VA Potdar, Mrs. V Vipat and Mrs. S Kadam

c. **Background:**

Respiratory viruses are the most frequent causative agents of disease in humans, with significant impact on morbidity, mainly in children. There are more than 200 respiratory viruses that infect humans. The viruses other than influenza and RSVs also cause upper and lower respiratory tract infections (URTI, LRTI) with symptoms ranging from   mild rhinorrhea associated with common colds to severe cough, wheezing, bronchiolitis, and pneumonia. Most common of them with their characteristics are listed below in the Table.

	Virus	Family	Genome	No. of genes	No. of proteins expressed	Subtypes and serotypes
1	Human Para influenza viruses (HPIV)	*Paramyxoviridae*	15kb Negative sence linear	6 genes(NP-P-M-F-HN-L)	4 Six protein	4 subtypes PIV1-4
2	Human metapneumo virus (HMPV)	*Pneumoviridae*	13 kb Negative sence linear RNA	8 genes(N-P-M-F-M2-SH-G-L)	eight proteins	2 sub types A (A1,A2) And B(B1,B2)
3	Human Rhinoviruses (HRV)	*Picornaviridae*	7.2 kb Positive sence, linear RNA	Single gene	11 proteins	3 subtypes and >100 serotypes

	Virus	Family	Genome	No. of genes	No. of proteins expressed	Subtypes and serotypes
4	Human Adenoviruses (HAdV)	*Adenoviridae*	Linear (ds) DNA genome, between 26 and 48 Kbp	22 to 40 genes	22 to 40 proteins	Subtypes A-G Subtypes B, C and E for Respiratory Disease
5	Human Bocavirus (HBoV)	Parvoviridae	5.3 kb, Linear ss DNA	5 genes	9	4 Subtyped HBoV1–4, HBov -1 is common for Respirator Disease

The list of respiratory viruses and virus specific genes used in the assay are follows:

No.	Virus	Gene used in RT PCR assay
1	Human Para influenza viruses (HPIV)	haemagglutinin Neuraminidase
2.	Human Metapneumovirus (HMPV)	Nucleiocapsid
3.	Human Rhinoviruses(HRV)	Polyprotein
4.	Respiratory Adenoviruses (HADV)	Hexon
5	Human Bocavirus (HBoV)	NS1 protein

d. **Performance characteristics:** The duplex assay has 98-100% sensitivity and 100% specificity. Performance of each assay showed detection limit of 10 to 15 copies of viral RNA in the assay.

e. **External validation:** The assay was validated using external quality control panel (Quality Control in Molecular Diagnostics Respiratory II panel) with 100% concordant results.

f. **Intended use:** Surveillance and diagnosis of infections caused by respiratory viruses other than Influenza and RSVs.

g. **Translational Status:** Currently, the assay is used for research purpose only. In future, an expression of interest will be floated to identify suitable vendors for technology transfer.

h.   Impact:

The assay provided differential diagnosis of 14 different respiratory viruses in hospitalized SARI and ILI cases. These assays were successfully used in national projects. This assay can confirm one or more viruses (around 55%) in SARI cases, which could reduce the excess use of antibiotics.

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40
Real Time RT-PCR assay for detection of Avian Influenza viruses

a.   Name of the test/ assay: Real Time RT-PCR assay for detection of avian influenza viruses.

b.   Authors: Dr. VA Potdar, Mrs. V Vipat and Mrs. S Kadam

c.   Background:

Avian influenza (AI) viruses are type A influenza viruses, enveloped, negative-sense, single-strand ed RNA viruses with a segmented genome. They contain eight RNA segments and encode for eleven proteins viral glycoproteins haemagglutinin (HA), neuraminidase (NA), viral nucleoprotein (NP), matrix protein (M), membrane protein (M2), the nonstructural protein (NS1) and nuclear export protein (NEP).

Avian influenza refers to the disease caused by infection with avian (bird) influenza (flu) Type A viruses. These viruses occur naturally among wild aquatic birds worldwide and can infect domestic poultry and other bird and animal species. Avian flu viruses do not normally infect humans. However, sporadic human infections with avian flu viruses have occurred. The reported signs and symptoms of avian influenza A virus infections in humans have ranged from mild to severe and included conjunctivitis, influenza-like illness (e.g., fever, cough, sore throat, muscle aches) sometimes accompanied by nausea, abdominal pain, diarrhoea, and vomiting, severe respiratory illness (e.g. shortness of breath, difficulty breathing, pneumonia, acute respiratory distress, viral pneumonia, respiratory failure), neurologic changes (altered mental status, seizures), and the involvement of other organ systems. Avian influenza viruses depending on their genetic makeup and ability to cause severe disease are classified as high pathogenic avian influenza (HPAI) viruses (e.g. H5N1, H5N6, H7N9) and low pathogenic avian influenza (LPAI) viruses (e.g

H7N2, H9N2). The majority of human cases of influenza A (H5N1) and A(H7N9) virus infection have been associated with direct or indirect contact with infected live or dead poultry.

Globally HPAI viruses have potentially high mortality rates (up to 50%) and pandemic potential. From January 2003 to 26 August 2021, there were 863 cases of human infection with avian influenza A (H5N1) virus reported from 18 countries. Of these 863 cases, 456 were fatal (CFR of 53%). WHO reported 916 lab confirmed human cases of H7N9 during 2013-2017 period.

India reported outbreaks of avian influenza A (H5N1) in poultry farms every year since 2006. In January and February 2021, Haryana reported an outbreak of avian influenza A(H5N8) from severely affected poultry in Panchkula district. A human case was reported from India in July 2021.

In view of this data, it is necessary to have laboratory preparedness for early detection of AI viruses in poultry as well as the personnel involved in handling and culling the infected poultry thereby controlling the AI outbreaks to minimize public health risk. Being a WHO National Influenza Centre and an apex body of ICMR, NIV has developed the diagnostic capacity for detection of AI viruses.

Real time RT-PCR for following avian influenza viruses is developed:

No.	Virus	Gene used in RT-PCR assay
1	Influenza A (H5N1)	HA
2.	Influenza A (H7N9)	HA
3.	Influenza A (H9N2)	HA
4	Influenza A (H10N8)	HA

d. Performance characteristics:

All the above assays have 98-100% sensitivity and 100% specificity.

e. External validation:

Testing of the four assays using 18 WHO influenza EQAS panels showed 100% concordance. The WHO panel consists of non-seasonal high and low pathogenic avian viruses.

Testing of all four assays using CDC EQAS panel showed 100% concordant results.

f. Intended use:

Surveillance and outbreak investigation of avian influenza (AI) viruses in human.

g. Translational Status:

Currently the assay is used for research purpose. In future the expression of interest will be floated to identify suitable vendors for technology transfer.

h. Impact:

Development of these assay is the first step towards countries preparedness. These assays are useful to monitor high pathogenic and low pathogenic avian infections in humans during avian outbreaks in poultry & wild birds. Using these assays, identified India's first avain A (H9N2) and A (H5N2) cases.

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Vaccine Candidates

41
Inactivated Kyasanur forest disease virus vaccine

a. **Name of the technology:** Inactivated Kyasanur forest disease vaccine candidate

b. **Authors/Inventors:** ICMR-NIV, Pune Team [Lead person Dr. C. N. Dandawate]

c. **Background:**

Kyasanur forest disease (KFD), a tick-borne viral disease with hemorrhagic manifestations, occurs as seasonal outbreaks. Though KFD was initially restricted to the Shimoga and the neighbouring districts of Karnataka, it showed geographic expansion since 2012 to Tamil Nadu, Maharashtra, Kerala, and Goa covering the Western Ghats region of India. Vaccination with formalin inactivated tissue-culture vaccine is the key strategy for the prevention of the disease in the region.

Prior to the currently used formalin inactivated KFD virus (KFDV) vaccine produced in chick embryo fibroblasts, several vaccines were tested for the control of the disease. Field studies conducted in 1970–71 with the formalized KFDV demonstrated a serological response in 59% of the vaccinees after two doses.

In the previous field evaluation of the vaccine during 1990–92 in Shimoga, Uttar Kannada and Chikamagalur districts, an effectiveness of 79.3% (95% CI: 64.7–87.9) with one dose and 93.5% (95% CI: 87.9–96.6) with two doses was reported.

Based on these findings, a vaccine production unit was established at Shimoga, Karnataka and the indigenously manufactured vaccine was licensed for use in the affected districts. Subsequently, the vaccine production was shifted to the Institute of Animal Husbandry and Veterinary Biologicals, Hebbal, Bangalore. Immunization with this vaccine has remained the key strategy for prevention of KFD in Karnataka since 1990. The focal immunization strategy involves annual rounds of vaccination using formalin inactivated tissue-culture vaccine.

d. **Principle:** A whole virion inactivated KFDV vaccine.

e. **Performance characteristics:**
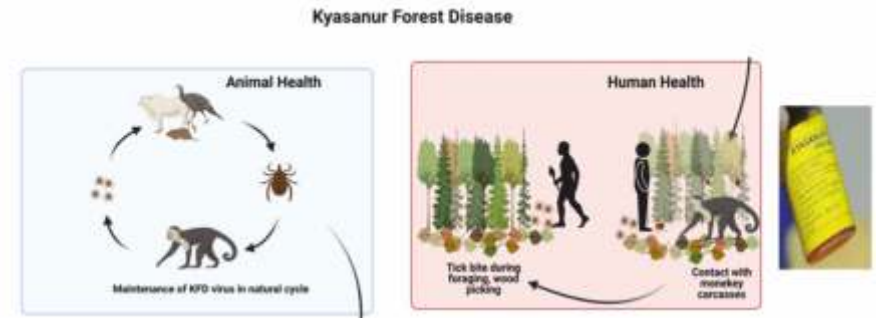
The formalin inactivated KFD vaccine candidates were studied using Presbytis entellus (Langur) model. A preliminary trail of this vaccine was conducted in Presbytis entellus, the black faced langur. A dose of 1ml of vaccine was given each time at an interval of one month to each monkey by subcutaneous route. The monkeys were challenged with KFDV after vaccination. There was no untoward effect in any monkey after inculcation with the KFD vaccine. All monkeys developed neutralizing antibodies to KFDV; one week after the second dose of vaccine haemagglutination inhibition (HI) antibody titers in four monkeys ranged between 1:10 to 1:20. All monkeys showed neutralizing antibodies till upto 15 months after vaccination.

After challenge with KFDV, circulation of the virus was detected in the sera of five monkeys for a shorter duration than in the controls.

Non-human primate studies were performed and demonstrated the safety of this vaccine using live KFDV.

Serological response to KFD vaccine: A field trial of the KFD vaccine was conducted in Shimoga district in 1970-71. Paired serum of 214 vaccinated individuals and 204 controls were tested for antibodies and HI and neutralization tests. A sero conversion of 59.32% was observed

among the vaccinated persons.

f.   **Intended use:**

To vaccinate people in endemic areas against KFDV.

g.   **Translational Status:**

Technology transferred. This vaccine is being used in the current vaccination program in Karnataka state health program.

h.   **Impact:**

Inactivated KFD vaccine was administered in the endemic area for the disease and helped to save the lives of people in KFD affected states as well as researcher working with this virus in field and laboratory.

Publications:

i.   Dandawate, C. N., Desai, G. B., Achar, T. R., & Banerjee, K. (1994). Field evaluation of formalin inactivated Kyasanur forest disease virus tissue culture vaccine in three districts of Karnataka state. The Indian journal of medical research, 99, 152–158.

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iv. Kiran SK, Pasi A, Kumar S, Kasabi GS, Gujjarappa P, Shrivastava A, Mehendale S, Chauhan LS, Laserson KF, Murhekar M. Kyasanur Forest disease outbreak, and vaccination strategy, Shimoga District, India, 2013–2014. Emerg Infect Dis. 2015;21(1):146–49. doi:10.3201/eid2101.141227.

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42
Inactivated Japanese encephalitis disease vaccine

a.   **Name of the technology:** Inactivated Japanese encephalitis disease vaccine

b.   **Authors/Inventors:** Dr. MM Gore, Dr. CN Dandawate, Mr. VM Ayachit, Dr. GN Sapkal and Dr. VP Bondre

c.   **Background:**

Japanese encephalitis (JE) caused by the mosquito vector was first recognized in India in the year 1955. Since then in many rural parts of the country, various major outbreaks were observed predominantly affecting children under 10 years of age with morbidity rate of 0.30 to 1.5 per 100,000 population. The case fatality rates ranged from 10% to 60% and those who recover are left with many neurological and psychiatric defects. Vaccination with inactivated tissue-culture vaccine is the key strategy for the prevention of the disease in the region. ICMR-NIV formulated and developed JENVAC as a single dose inactivated JE vaccine. This Vero cell-derived vaccine is prepared from an Indian strain (Kolar- 821564XY) of the JE virus. The JENVAC technology was transferred to Bharat Biotech Limited (BBIL), Hyderabad; India. It is a safe and highly-effective vaccine that protects against all the known strains of JE with sero-protection efficacy of 98.67%.

d.   **Principle:** A whole virion inactivated JE vaccine.

e.   **Performance characteristics**

f.   In a phase I study, the safety and immunogenicity of BBIL's JENVAC vaccine was established in healthy adult volunteers and the development proceeded to phase II/III study. The phase II/III, randomized, single blinded, active controlled study was conducted to evaluate the

immunogenicity and safety of JENVAC vs. Chinese SA14-14-2 (live attenuated JE vaccine) in healthy volunteers. In this study, the proportion of subjects achieving sero-protection after a single dose of respective vaccine, was significantly higher in JENVAC treatment arm (98.7%) compared to that in the SA-14-14-2 arm (77.6%), 28 days post vaccination. Similarly, a phase IV, open labeled, comparative, randomized, active controlled study was conducted to evaluate the immunogenicity and safety of a single dose of JENVAC vs. SA-14-14-2 vaccine in healthy volunteers. While the proportion of subjects being seronegative or seropositive for JE antibodies was similar in both treatment groups at the baseline, proportion of subjects achieving sero-protection was significantly higher in the JENVAC treatment arm (92.4%) compared to that in the SA-14-14-2 arm (71.4%), 4 weeks after vaccination. Further, the higher sero-protection rate was persistent till 1 year of follow up among the subjects receiving JENVAC vaccine; 81.7% vs 47.9% (p = 0.0001). Sera from JENVAC subjects neutralized JEV genotypes I, II, III, and IV equally well. It was thus concluded that JENVAC elicits long-lasting, broadly protective immunity.

g. **Intended use:** To vaccinate people in endemic areas against JE.

h. **Translational Status:** Technology transferred to BBIL, Hyderabad. This vaccine contributed to the universal vaccination program of India.

| Milestones on JENVAC development	
1. Request sent to ICMR on collaboration with BBIL	13/03/2007
2. Draft MOU sent to ICMR	24/05/2007
3. MOU signed between NIV and BBIL	25/11/2008
4. MTA signed	18/12/2008
5. Kickoff meeting NIV and BBIL representative	03/02/2009
6. JE vaccine candidate handed over to BBIL	17/02/2009
Process Development for Antigen Production / Formulation Development / Pre-clinical Studies at BBIL(NIV supplied reagents and technical support for sera testing)	
1. Phase—I Clinical Study	2010
2. Phase II/III Clinical Study	2011
3. Document submission to DCG (I)	2012
4. DCGI approved the manufacturing and marketing licensure	2013

Publications:

i.   Singh A, Mitra M, Sampath G, Venugopal P, Rao JV, Krishnamurthy B, Gupta MK, Sri Krishna S, Sudhakar B, Rao NB, Kaushik Y, Gopinathan K, Hegde NR, Gore MM, Krishna Mohan V, Ella KM. A Japanese Encephalitis Vaccine From India Induces Durable and Cross-protective Immunity Against Temporally and Spatially Wide-ranging Global Field Strains. J Infect Dis. 2015 Sep 1;212(5):715-25. doi: 10.1093/infdis/jiv023. Epub 2015 Jan 18. PMID: 25601942.

ii. Vadrevu KM, Potula V, Khalatkar V, Mahantshetty NS, Shah A, Ella R. Persistence of Immune Responses With an Inactivated Japanese Encephalitis Single-Dose Vaccine, JENVAC and Interchangeability With a Live-Attenuated Vaccine. J Infect Dis. 2020 Oct 1;222(9):1478-1487. doi: 10.1093/infdis/jiz672. PMID: 31858116; PMCID: PMC7529014.

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43
Inactivated SARS-CoV-2 vaccine

a. Name of the technology: Inactivated SARS-CoV-2 vaccine candidate

b. Authors/Inventors: ICMR-NIV, Pune team and BBIL team [Lead from ICMR-NIV, Dr. Pragya Yadav]

c. Background:

The COVID-19 pandemic is a global health crisis that has severely affected mankind and posed a great challenge to the public health system of affected countries. Inactivated viruses have been traditionally used for vaccine development and such vaccines have been found to be safe and effective for the prevention of many diseases. Indian Council of Medical Research partnered with Bharat Biotech International Limited (BBIL) to develop an inactivated vaccine for SARS CoV-2. Three whole virion inactivated vaccine candidates BBV152A [3μg+ Aluminium hydroxide (Algel)-Imidazoquinoline (IMDG)], BBV152B (6μg+Algel-IMDG) and BBV152C (6μg+Algel) were developed by BBIL, Hyderabad in collaboration with ICMR-NIV, Pune using β-propiolactone (BPL) inactivation method. The BBV152 vaccine candidate along with aluminum hydroxide adjuvant alone or with aluminum hydroxide chemisorbed with imidazoquinoline was found to be immunogenic and safe in the preclinical studies on laboratory mice, rats and rabbits. Evaluation of the immunogenicity and protective efficacy of the three vaccine formulations (BBV152A, BBV152B, BBV152C) in Syrian hamsters and rhesus macaques was undertaken. Rhesus macaques and Syrian hamsters appears to be quite promising animal models for SARS-CoV-2.

d. Principle:

A whole virion inactivated SARS-CoV-2 vaccine.
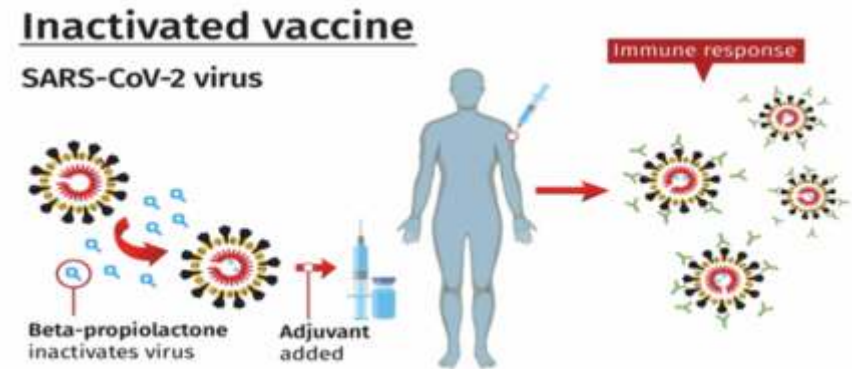
e. Performance characteristics:

Safety and Immunogenicity of the vaccine candidates were studied in Syrinan hamster and Rhesus Macaque models. The protective response was observed with increasing SARS-CoV-2 specific IgG and neutralizing antibody titers from 3rd-week post-immunization. Viral clearance was observed from bronchoalveolar lavage fluid, nasal swab, throat swab, and lung tissues at 7 days post-infection in the vaccinated groups. No evidence of pneumonia was observed by histopathological examination in vaccinated groups, unlike the placebo group which showed features of interstitial pneumonia and localization of viral antigen in the alveolar epithelium and macrophages by immune histochemistry. Non-human primate studies were performed and demonstrated the safety and efficacy of this indigenous vaccine using live SARS CoV-2. These studies established the safety and efficacy of this indigenous vaccine which is presently in phase-3 clinical trials. As a part of the regulatory guidelines, all the data were submitted to the DCGI and CDSCO. On January 2, 2021, DCGI recommended the grant of permission for restricted emergency use of COVAXIN™ after the meeting of Subject Expert Committee of CDSCO. The development and demonstration of the efficacy of this indigenous vaccine in such a short period is one of the major achievements for the country towards self-reliant India, public health, and the war against COVID-19.

f. External Quality Assurance:

The vaccine candidate BBV152 has completed Phase I/II clinical trials in

India and is presently in phase III. The data of this study substantiates the immunogenicity and protective efficacy of the vaccine candidates. Phase III trial indicated 78% clinical efficacy for this vaccine. The vaccine is approved by World Health organization which has provided Emergency use licensing on 3rd November 2021 for this vaccine.

g. Intended use:

To vaccinate people against the current pandemic of SARS-CoV-2

h. Translational Status:

Technology transferred. This vaccine is being used in the current vaccination program.

i. Impact:

Covaxin an Inactivated Severe acute respiratory syndrome corona virus 2 (SARS-CoV-2) vaccine candidates approved by World health Organization for EUL has played very crucial role in this pandemic. Due to vaccination many lives were saved, hospitalization was reduced and vaccine has shown good efficacy against various circulating strains of SARS CoV-2.

Publications:

i. Yadav, P.D., Ella, R., Kumar, S. et al. Immunogenicity and protective efficacy of inactivated SARS-CoV-2 vaccine candidate, BBV152 in rhesus macaques. Nat Commun 12, 1386 (2021). https://doi.org/10.1038/s41467-021-21639-w

ii. Brunda Ganneru, Harsh Jogdand, Vijaya Kumar Daram, Dipankar Das, Narasimha Reddy Molugu, Sai D. Prasad, Srinivas V. Kannappa, Krishna M. Ella, Rajaram Ravikrishnan, Amit Awasthi, Jomy Jose, Panduranga Rao, Deepak Kumar, Raches Ella, Priya Abraham, Pragya D. Yadav, Gajanan N. Sapkal, Anita Shete-Aich, Gururaj Deshpande, Sreelekshmy Mohandas, Atanu Basu, Nivedita Gupta, Krishna Mohan Vadrevu,Th1 skewed immune response of whole virion inactivated SARS CoV 2 vaccine and its safety evaluation. iScience, Volume 24, Issue 4, 2021,102298, ISSN 2589-0042,https://doi.org/10.1016/ j.isci.2021.102298.

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v. Yadav PD, Sapkal GN, Abraham P, Ella R, Deshpande G, Patil DY, Nyayanit D, Gupta N, Sahay RR, Shete AM, Panda S, Bhargava B, V. Krishna Mohan. Neutralization of variant under investigation B.1.617 with sera of BBV152 vaccinees, Clinical Infectious Diseases, 2021;, ciab411, https://doi.org/10.1093/cid/ciab411

vi. Yadav PD, Sapkal GN et al. Neutralization against B.1.351 and B.1.617.2 with sera of COVID-19 recovered cases and vaccinees of BBV152. Journal of travel medicine. taab104, https://doi.org/ 10.1093/jtm/taab104

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xi.   Murhekar, M. V., Bhatnagar, T., Selvaraju, S., Saravanakumar, V., Thangaraj, J., Shah, N., Kumar, M. S., Rade, K., Sabarinathan, R., Asthana, S., Balachandar, R., Bangar, S. D., Bansal, A. K., Bhat, J., Chopra, V., Das, D., Deb, A. K., Devi, K. R., Dwivedi, G. R., Khan, S., … ICMR Serosurveillance Group (2021). SARS-CoV-2 antibody seroprevalence in India, August-September, 2020: findings from the second nationwide household serosurvey. The Lancet. Global health, 9(3), e257–e266. https://doi.org/10.1016/S2214-109X(20)30544-1

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Miscellaneous

44
CRISPR Cas9 based development of poliovirus receptor gene (CD155) knockout RD cell line

a. **Name of the technology:** CRISPR Cas9 based development of poliovirus receptor gene (CD155) knockout RD cell line for handling potentially infectious materials in virology laboratories

b. **Authors/ Inventors:** Dr. SS Nandi, S Sawant, T Gohil and Dr. JM Deshpande.

c. **Background:**

Destruction of all poliovirus containing materials, safe and secure handling of retained polioviruses for vaccine production and research will be obligatory to eliminate facility-associated risks. Polioviruses and poliovirus potentially infectious materials (PIM) include fecal or respiratory samples requiring containment have been defined in WHO-GAPIII documents. Non-polio laboratories culturing viruses from PIM are most affected as cell cultures of human and monkey origin are also poliovirus permissive. Poliovirus containment may seriously affect storing and handling historical collections of samples, virus isolates and future PIM in respiratory and enteric diseases laboratories like those involved in enterovirus, hepatitis A and E, rotavirus, measles and rubella virus surveillance, diagnostics and research.

d. **Principle of the technology:**

A cell line which is permissive to most non-polio enteroviruses and non-permissive to poliovirus has been developed. This would be advantageous to laboratories whose major field of work is non-polio enteric viruses. This cell line will ensure that the poliovirus does not grow in these laboratories. CRISPR technology is widely used for gene editing. In this technology, poliovirus non-permissive cells derived from the RD cell line by knocking out the poliovirus receptor (CD155) gene using the CRISPR/Cas9 system has been reported.

e. **Performance characteristics:**

626 stool samples were tested prospectively in L20B (murine cell line), RD (Rhabdomyosarcoma) and RD-knock out cells (RD-SJ40) using the standard virus isolation algorithm. Testing of more than 600 stool samples of AFP cases confirmed that the RD-knock out cells (RD-SJ40) did not support growth of poliovirus. All non-polio enterovirus (NPEV) types isolated in parental RD cells were also isolated in RD-SJ40. Thus, RD-SJ40 cells are safe for NPEV isolation from poliovirus PIM without derogating GAPIII containment requirements.
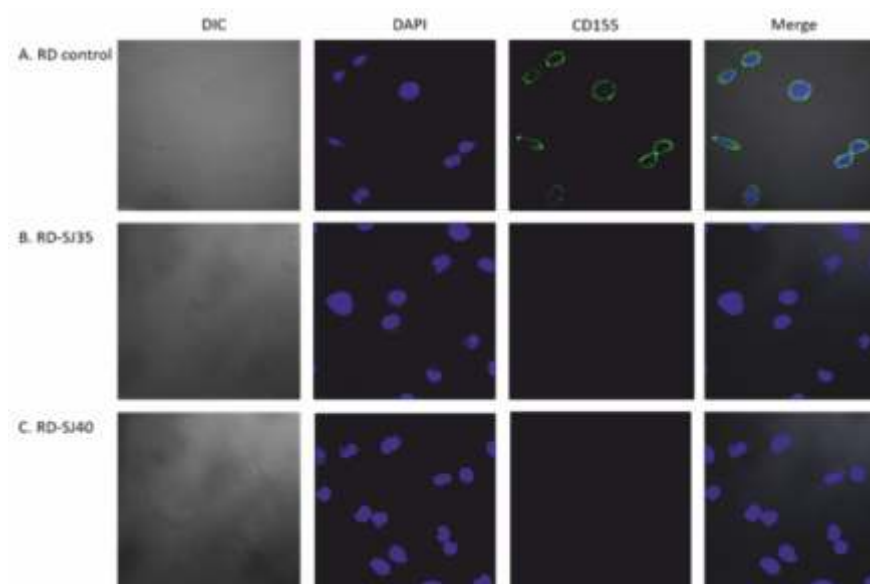
Figure: Detection of CD155 cellular receptor by immunofluorescence - Poliovirus non-permissive RD cells (RD-SJ40) did not express CD155-specific surface immunofluorescence. Differential interference contrast (DIC) microscopy was performed and 4',6-diamidino-2-phenylindole(DAPI) fluorescent stain was used to stain the cell's DNA.

f. **Inter laboratory comparison / External validation:**

The cells were supplied to three National Polio Laboratories supporting Acute Flaccid Paralysis (AFP) surveillance in the country. Field testing of poliovirus non-permissive RD cells was done in three national polio laboratories. As expected, RD-knock out cells (RD-SJ40) did not support

growth of poliovirus and all NPEV types could be isolated in RD-SJ40.

g. Intended use:

CD155 (poliovirus receptor) gene knockout cells (RD-SJ40) can be widely used for virus culture of NPEVs. The CD155/PVR knockout RD-SJ40 cells will find wide applications in laboratories worldwide. It is envisaged that the WHO Global Polio Laboratory Network will also support the use of CD155 knockout RD-SJ40 cells for enterovirus work in the 146 network laboratories all over the world. The CD155/PVR knockout RD-SJ40 cells can be used safely in all non-polio laboratories wanting to grow NPEVs/ enteric viruses from clinical samples (stool or respiratory secretions) for diagnostic purposes and research without the fear of poliovirus growth as inadvertent contamination.

h. Translational Status:

The cell line is successfully submitted to ATCC (American Type Culture Collection), USA. A MoU will be signed between ATCC, USA and Director General (DG), ICMR for commercialization of the newly developed cell line.

The patent application entitled "Poliovirus receptor (PVR/CD155) knockout cells derived from RD (human rhabdomyosarcoma) cell line by CRISPR" has been submitted,

Inventors: Dr. Shyam Sundar Nandi (PI), Sonali Ankush Sawant, Dr. Jagadish Mohan Deshpande. Indian Patent Application No: 201811034727. International PCT application No. PCT/IN2019/050671.

i. Impact:

The CD155/PVR knockout RD cells will find wide applications in laboratories worldwide as requirement of the WHO Global Action Plan III. The CD155/PVR knockout cells RD can be used safely in all non-polio laboratories wanting to grow non-polio enteroviruses/ enteric viruses from clinical samples (stool or respiratory secretions) for diagnostic purposes and research without the fear of poliovirus growth as inadvertent contamination.

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Instrument free nucleic acid extraction technology

a. Name of the test/ assay developed: Instrument free nucleic acid extraction technology

b. Authors/ Inventors: Dr. SS Nandi, Dr. U Lambe, Ms. SA Sawant, Dr. JM Deshpande.

c. Background:

Molecular diagnostic assays have been proven better in terms of sensitivity and specificity than other diagnostic assays. For a molecular assay to function efficiently, the first pre-requisite is a good quality of nucleic acid for the test for which a laboratory well equipped with sophisticated instruments is required. Further, even though the molecular assays are simple to perform, due to the complicated nucleic acid extraction method, they cannot be used as a point-of-care diagnostic. Based on these considerations, it was conceived to design a nucleic acid extraction procedure. . The objective of this study was to standardize a method for extraction of nucleic acids using syringe filter and its comparative evaluation with the commercially available RNA extraction kit.

d. Principle of technology:

The centrifugation step was replaced by a filtration step through a combination of nylon and Polyvinylidene difluoride (PVDF) membrane filter. The current method does not require a sophisticated bio-safety cabinet as the molecular transport medium (MTM) lyses the viral capsid and can be handled without fear of being infected. RNA binds specifically to the nylon and PVDF membrane while the contaminants pass through. Inhibitors such as divalent cations and proteins are removed in two efficient wash steps, leaving pure viral nucleic acid. This nucleic acid is

eluted in nuclease free water. The methodology can be useful for extraction of RNA from throat or nasal swab samples of COVID-19,

e. Performance characteristics:

To confirm the efficiency of this method, thirty throat swab samples were extracted by the syringe filter method and QIAamp Viral RNA Mini kit QIAGEN (manufacturer's protocol). The extracted nucleic acid was subjected to the inhouse developed RT-LAMP assay for detection of SARS-CoV-2. The results were comparable in both the methods. Also the same nucleic acid samples were subjected to real time PCR (rRT-PCR). The Ct values of samples extracted by syringe filter method was comparable with the nucleic acid extracted by QIAGEN kit method.
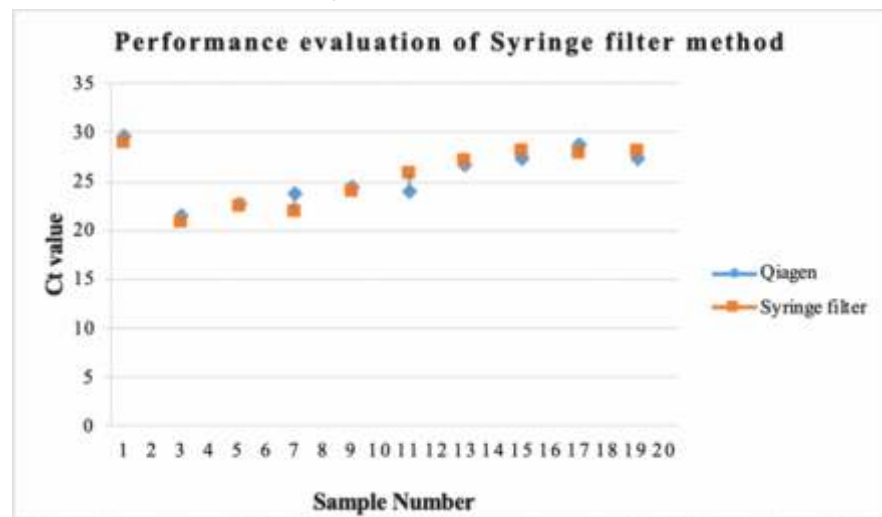
Figure : Comparison of RNA extraction method by Qiagen kit and syringe filter method by real-time PCR analysis. The Ct values of RdRp gene obtained by subjecting RNA extracted by Qiagen as well as syringe filter method are shown in the graph.

f. External validation:

External validation is in progress. A number of samples will be subjected to nucleic acid extraction by using commercially available extraction kit and syringe filter method. The nucleic acid extracted by both the methods will be subjected to Real-Time PCR. The analysis will be done by comparing the Ct values obtained from each method.

g. Intended use:

This method can be employed for extraction of nucleic acid in block and village level, airports, railway stations etc. for diagnosis of COVID-19.
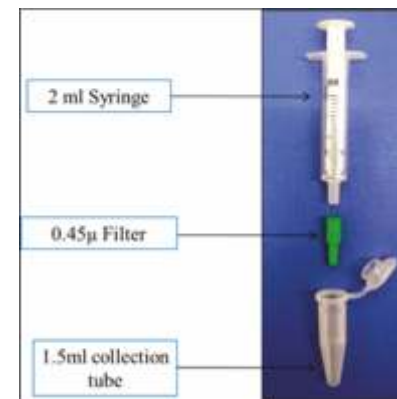
Figure: Assembly of syringe and filter for the extraction of nucleic acid using a user-friendly method.

h. Translational status:

Title of patent "Instrument free nucleic acid extraction method".

Inventors: Dr. Shyam Sundar Nandi, Dr. Upendra Lambe, Ms. Sonali Ankush Sawant, Dr. Jagadish Deshpande. Patent application accepted by IPR Cell, ICMR HQ for submission to Indian Patent Office, August 2021. Indian Patent Application No: 202111043228.

i. Impact:

This technology does not require a sophisticated bio-safety cabinet as the molecular transport medium (MTM) lyses the viral capsid and can be handled without fear of being infected. This methodology is modified for extraction of nucleic acids from throat or nasal swab samples which can be useful as substitute technology for RNA extraction without any instrument like High Speed Centrifuge. This instrument free nucleic acid extraction technology may be used for any point of care kind technology development in future.

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Acronyms:

- AIIMS: All India Institute of Medical Sciences
- BBIL: Bharat Biotech International Limited
- BCIL: Biotech Consortium India Limited
- BJMC –Ahmadabad: Byramjee Jeejeebhoy Medical College Ahmedabad
- BSL-4: Biosafety level 4
- CCHF: Crimean Congo Hemorrhagic Fever
- CDC: The Centers for Disease Control and Prevention
- CHP: Chandipura
- CI: Confidence interval
- CMC- Vellore: Christian Medical College, Vellore
- CRME: Center for Research in Medical Entomology
- CSF: Cerebrospinal Fluid
- Ct: Cycle threshold
- CV: Coefficient of Variation
- DBT: Department of Biotechnology
- DHR: Department of Health Research
- ELISA: Enzyme-linked immunoassay
- ERC: Enterovirus Research Center
- EQAS: External quality assurance scheme
- geq/ml: genome equivalents per milliliter (1 geq/ml = 1 copy/ml)
- HAV: Hepatitis A virus
- HEV: Hepatitis E virus
- HFMD: Hand-Foot-Mouth Disease
- HRP: Horseradish Peroxidase
- ICMR: Indian Council of Medical Research
- ICMR-NIE: Indian Council of Medical Research – National Institute of Epidemiology
- ICMR-NIRTH: Indian Council of Medical Research – National Institute of Research in Tribal Health
- ICMR-NIOH: Indian Council of Medical Research – National Institute of Occupational Health
- IgG: Immunoglobulin G
- IgM: Immunoglobulin M
- IISc-Bangalore: Indian Institute of Science, Bangalore
- JE: Japanese Encephalitis
- KFDV: Kyasanur Forest Disease Virus
- KIPM – Chennai: King Institute of Preventive Medicine & Research, Chennai
- LAMP: Loop mediated isothermal amplification
- ng/ml: Nanograms per milliliter
- NIHSAD: National Institute of High Security Animal Diseases
- NVBDCP: National Vector Borne Disease Control Program
- pfu: Plaque-forming unit
- PGIMER- Chandigarh: Postgraduate Institute of Medical Education and Research, Chandigarh
- PoC: Point of care
- PRNT: Plaque Reduction Neutralization Titration
- RD: Rhabdomyosarcoma
- RMRC: Regional Medical Research Center
- RSV: Respiratory Synctial Virus
- RT-PCR: Reverse transcription polymerase chain reaction
- SNP: Single nucleotide polymorphisms
- TCID50: Median Tissue Culture Infectious Dose
- TMB/H_2O_2: Tetramethyl benzidine/Hydrogen Peroxide
- VRDL: Virus Research and Diagnostic Laboratory network
- WHO: World Health Organization

Glossary:

- **Acute onset:** A sudden, rapid, or unanticipated development of a disease or its symptoms.

- **Chromogenic substrate:** A colourless chemical that is converted by an enzyme into another, coloured, chemical.

- **Dead-end host:** A host that shelters an organism but is unable to transmit the organism to a different host.

- **Endemic:** regular occurrence of a disease among particular people or in a certain area.

- **Epidemic:** a widespread occurrence of an infectious disease in a community at a particular time.

- **Inter-assay CV:** It is a measure of the variance between sample replicates ran on different runs.

- **Intra-assay CV:** It is a measure of the variance between sample replicates ran within the same run.

- **Seroconversion:** Development of specific antibodies in the blood serum as a result of infection or immunization

- **Sensitivity:** The sensitivity of a test (true positive rate) refers to the proportion of those who have the condition (when judged by the 'Gold Standard') that received a positive result on the newly developed test.

- **Specificity:** The specificity of a test (True Negative Rate) refers to the proportion of those who do not have the condition (when judged by the 'Gold Standard') that received a negative result on the newly developed test.

- **Sporadic:** occurring occasionally, singly, or in irregular or random instances.

- **Turnaround time:** The amount of time taken to complete a process.

- **Viremia:** Presence of virus in body.

- **Zoonotic:** A disease that can be transmitted from animals to human.

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