'Support to Indian Institutes for imparting training' to the Faculty of Medical Colleges/ Research Institutes under Human Resource Development Scheme of Department of Health Research

1. Area of Training:

Molecular virological techniques for Dengue and chikungunya (Modern Biology)

2. Name of the Institution and contact details:

National Institute of Virology, 20A Dr Ambedkar Road, Pune-411001

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3. (a) Name of the Principal Investigator and contact details

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(b) Name of the Co- Investigators and contact details

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4. Training Module
Programme **-**Duration of the training- 1 month

I. Introduction:

Presently, Dengue is one of the most important mosquito borne infections in India. Chikungunya virus, transmitted by the same *Aedes aegypti* mosquito, also causes annual outbreaks in different parts of India. Both viruses are known to co-circulate. Diagnosis is one of the key factors in management of dengue and chikungunya. The epidemiological data for both viruses is scarce and for better policy formulation the true burden of the two diseases is required.

II. Aim of the program

The aim is to provide training to representatives from various branches of health care. It will enable the candidates to undertake studies on dengue and chikungunya. With the expertise gained they can undertake projects on defining prevalence, incidence, molecular characterization of the circulating viruses, disease burden, etc. The training provided in ELISAs, cell culture, virus isolation and molecular techniques will strengthen their capacity to take up studies on other viruses too.

III. Existing faculty members, their details, positions, available with the institution for imparting training programme.

S. No.	Faculty - Name and designation	Research interest of faculty
1	Dr. Cecilia Dayaraj, Scientist F	Molecular Virology, Cell Biology & Immunology
2	Dr. Paresh Shah, Scientist E	Molecular Virology, Arbo virus diagnostics
3	Dr. Deepti Parasher, Scientist 'D'	Molecular Virology, Antivirals
4	Dr. K.Alagarasu, Scientist 'C'	Immunology, Immunogenetics,
5	Dr Jayati Mullick, Scientist E	Molecular Virology, Immunology, Avian Influenza, Biosafety
6	Dr Pragya Yadav	Bio-safety, Emerging viral diseases
7	Dr. B.V. Tandale, Scientist 'E'	Epidemiology
8	Dr Anuradha S Tripathy, Scientist 'E'	Immunology

9	Dr. Yogesh Gurav, Scientist 'D'	Epidemiology
10	Dr. Mangesh D Gokhale, Scientist 'C'	Entomology
11	Dr. A.B. Sudeep, Scientist 'D'	Entomology and Cell Culture
12	Dr. Pratip Shil, Scientist 'C'	Bio-informatics
13	Mr Walimbe, Technical Officer B	Biostatistics &Bio-informatics
14	Dr. Kanjan Patil, Scientist B	Diagnostics
15	Dr. Jayashri A Patil, Technical Assistant	Molecular Phylogeny
16	Mr Santosh M Jadhav	Biostatistics & Bioinformatics

IV.   Available infrastructure facilities
        Cell culture Facilities
        Sequencer
        Real Time PCR machine
        Thermal Cyclers
        Automated RNA Extractor
        Confocal Microscope
        Equipments for Molecular Biology

V.   Training schedule with elaborate details day wise or week wise along with the topic.

DAY -1	Topics	Lecture/Practical
9.30 - 10.30 am	Inauguration Introduction	
11.00 – 12.00	Bio-safety-Over view	Lecture
12.00– 1.00 pm	Assessment of Candidates	Written test
2.00-3.00 pm	Orientation	
3.30-4.30 pm	Basic Virology	Lecture
4.30 – 5.30pm	Bio-safety	Lecture

DAY -2	Topics	Lecture/Practical
9.30 -10.30 am	Introduction to viral diagnosis	Lecture
11.00– 12.00 pm	Epidemiology of Dengue in India	Lecture
12.00– 1.00 pm	Epidemiology of Chikungunya in India	Lecture
2.00-3.00 pm	Viral Hemorrhagic Fevers	Lecture
3.30 – 4.30 pm	Washing & sterilization	Practical
4.30-5.30 pm	Allocation of assignments	

DAY -3	Topics	Lecture/Practical
9.30 - 10.30 am	Sample collection, Transportation, registration, processing and storage	Lecture
11.00– 12.00	VIRUS LIMS : Registration and reporting	Lecture
12.00– 1.00 pm	VIRUS LIMS : Registration and reporting	Demonstration
2.00– 5.30 pm	Reagent (PBS, cell cutlure medium) preparation	Practical

DAY-4	Topics	Lecture/Practical
9.30 - 10.15 am	Dengue- clinical features and classification of disease -WHO	Lecture
10.45-11.15 am	Chikungunya- clinical features	Lecture
11.15– 12.00	Virus isolation & propagation methods - *In vitro*	Lecture
12.00– 1.00 pm	Virus isolation & propagation methods - *In vivo*	Lecture
2.00– 5.30 pm	Cell culture - Sub culturing of cell lines & preparation of 24-well plates	Demonstration

DAY 5	Topics	Lecture/Practical
9.30-10.30 am	Assays for titration of viruses	Lecture
11.00– 1.00 pm	Infection of cell lines with virus – titration of virus by PFU assay	Demonstration
2.00-5.30 pm	Field trip for mosquito collection	Practical

DAY 6	Topics	Lecture/Practical
9.30-10.30 am	Primary and secondary cell lines and cell culture techniques	Lecture
11.00 – 12.00	Cryopreservation and revival of cell lines	Lecture
12.00-1.00 pm	Dengue pathogenesis	Lecture
2.00 – 5.30 pm	Cryopreservation and Revival of cryopreserved cells	Demonstration

Day -7	Topics	Lecture/Practical
9.30- 10.30 am	MAC & GAC –ELISA (DENV &CHIKV)	Lecture
11.00– 1.00 pm	Chikungunya GAC-ELISA	Practical
2.00-5.30 pm	DEN MAC ELISA	Practical

DAY 8	Topics	Lecture/Practical
9.30 – 10.30 am	Immunoflourescence assay	Lecture
11.00-1.00 pm	Termination and determination of PFU titre	Demonstration
2.00 - 5.30 pm	Immunoflourescence assay	Practical

DAY 9	Topics	Lecture/Practical
9.30 - 10.30 am	Nucleic acid extraction and Reverse Transcription	Lecture
11.00 – 1.00 pm	RNA extraction	Practical
2.00-4.30 pm	Reverse transcription (DENV)	
4.30 – 5.30 pm	Polymerase chain reaction (PCR) and its variants	Lecture

DAY 10	Topics	Lecture/Practical
9.30 - 10.30 am	RT-PCR - DENV and CHIKV	Lecture
11.00 to 1.00 pm	Multiplex RT-PCR- DENV(1st cycle)	Demonstration
2.00 – 5.30 pm	Multiplex RT-PCR DENV serotyping (2nd cycle)-	Demonstration

DAY 11	Topics	Lecture/Practical
9.30-10.30 am	Real time RT-PCR	Lecture
11.00-1.00 pm	RT- PCR DENV –gel electrophoresis -analysis	Demonstration
2.00 -5.30 pm	Real time RT-PCR (for DENV/CHIKV)	Practical

DAY 12	Topics	Lecture/Practical
9.30 - 10.30am	Sequencing	Lecture
11.00– 1.00 pm	Purification of amplicon by Gel extraction	Practical
2.00– 5.30 pm	Sequencing reaction and purification	Practical

DAY 13	Topics	Lecture/Practical
9.30 - 1.00 am	Sequence analysis – BLAST	Demonstration
2.00-3.00 pm	Basic Bioinformatics I	Lecture
3.00-5.30 pm	Basic Bioinformatics (CLUSTAL)	Demonstration

DAY 14	Topics	Lecture/Practical
9.30 - 10.30 pm	Molecular Phylogenetics	Lecture
11.00-1.00 pm	Molecular Phylogenetics	Demonstration
2.00 – 5.30 pm	Molecular Phylogenetics	Practicals

DAY 15	Topics	Lecture/Practical
9.30 - 10.30 am	Molecular epidemiology of Dengue	Lecture
11.00-12.00	Molecular epidemiology of Chikungunya	Lecture
12.00– 1.00 pm	Dengue vaccines	Lecture
2.00– 5.30 pm	Cell culture - Sub culturing of cell lines & preparation of 24-well plates	Practical

DAY 16	Topics	Lecture/Practical
9.30 - 10.30 am	Antivirals for Dengue /CHIKV	Lecture
11.00-12.00	Host genetics – DENV/CHIKV	Lecture
12.00– 1.00 pm	CHIK pathogenesis	Lecture
2.00– 5.30 pm	Infection of cells Titration in 24 well plate	Practical

DAY 17	Topics	Lecture/Practical
9.30 - 10.30 am	CHIK vaccines	Lecture
11.00-12.00	Outbreak investigations	Lecture
12.00– 1.00 pm	RNA Extraction - CHIKV RT reaction -CHIKV	Practical
2.00– 5.30 pm	1st PCR CHIKV	Practical

DAY 18	Topics	Lecture/Practical
9.30 - 10.30 am	Study designs for DEN/CHIK epidemiology	Lecture
11.00-12.00	Termination of titration plate and calculation of titres	Practicals
12.00– 1.00 pm	2nd PCR CHIKV	Practical
2.00– 5.30 pm	Gel electrophoresis and analysis	Practical

DAY 19	Topics	Lecture/Practical
9.30 - 10.30 am	Basic biostatistics	Lecture
11.00-1.00 pm	Sequence analysis – BLAST	Practical
2.00– 5.30 pm	Basic Bioinformatics (CLUSTAL)	Practical

Day 20	Lab visits at NIV, Ambedkar Rd and time for assignment
Day 21	NIV/MCC, Pashan and time for assignment

DAY 22	Topics	Lecture/Practical
9.30 - 12.30 pm	Assessment	Written test & Presentation of Assignment
2.00-4.30 pm	Wrap up –Discussion, Difficulties and Feed back	Group discussion
4.30 pm	Valedictory function	

VI.   Relevance in public health

In 2015, there were almost a million confirmed dengue cases and 27,000 confirmed Chikungunya cases reported by NVBDCP. This data was generated by the 527 sentinel centres and 15 apex referral centres of the programme. Considering the number of centres, there is an obvious underreporting of cases. This calls for the strengthening the capacity of many more medical colleges and health centres for viral diagnosis. The training which is provided in this workshop enables the serological as well as molecular diagnosis of dengue and chikungunya viruses. It also imparts training in virus isolation and molecular characterization of viruses. The training imparted to health care associated personnel from all over India will ensure that data on dengue and chikungunya epidemiology can be generated from different regions. The holistic picture of the disease status will help the health authorities to decide and formulate policies for intervention and control.

The training acquired can be put to use in the diagnosis and detection of any virus that may emerge in future. The need for such strengthening of capacity has been underlined by the recent spectre of Zika virus.

5.Eligibility Conditions : Permanent employees of the state or central government institutions as well as government medical colleges or hospitals (minimum qualification Bachelor's Degree in any Life Science faculty /MBBS).