Expansion of the measles and rubella laboratory network, India

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Objective
To expand the measles and rubella laboratory network of India by integrating new laboratories.

Methods
In collaboration with the World Health Organization (WHO), the Indian government developed a 10-step scheme to systematically expand the number of laboratories performing serological and molecular testing for measles and rubella. The Indian Council of Medical Research and WHO identified suitable laboratories based on their geographical location, willingness, preparedness, past performance and adherence to national quality control and quality assurance mechanisms. The 10-step scheme was initiated with training on measles and rubella diagnostic assays followed by testing of both measles and rubella serology and molecular unknown panels, cross-verification with reference laboratories and ended with WHO on-site accreditation.

Findings
After extensive training, technical support, funding and monitoring, all six selected laboratories attained passing scores of 90.0% or more in serological and molecular proficiency testing of measles and rubella. Since 2018, the laboratories are a part of the measles and rubella network of India. Within 12 months of initiation of independent reporting, the six laboratories have tested 2287 serum samples and 701 throat or nasopharyngeal swabs or urine samples.

Conclusion
The process led to strengthening and expansion of the network. This proficient laboratory network has helped India in scaling up serological and molecular testing of measles and rubella while ensuring high quality testing. The collaborative model developed by the Indian government with WHO can be implemented by other countries for expanding laboratory networks for surveillance of measles and rubella as well as other infectious diseases.

Introduction
Countries in the World Health Organization (WHO) South-East Asia Region have extended their targets to eliminate the vaccine-preventable infections, measles and rubella, by 2023.1 These infections lead to substantial childhood morbidity and mortality.2 Measles mortality is particularly high in malnourished children residing in areas with poor health-care systems3,4 and rubella infection contracted during pregnancy can lead to miscarriage and congenital rubella syndrome.5,6

India is facing multiple challenges to meet these elimination targets. In 2019, the Indian government reported 10 430 measles cases and 3404 rubella cases to WHO.6 Active field surveillance along with a strong laboratory network is pivotal to the success of disease elimination programmes.7–9 In view of this requirement, field surveillance in India was strengthened and expanded, switching from outbreak to case-based surveillance and by 2019 all states had completed the transition. In the new surveillance approach, health workers collect serum and throat or nasopharyngeal swab or urine samples from individuals with suspected measles for laboratory confirmation and virus genotyping.10

To meet the increased testing demand due to the new surveillance approach, the Indian government made focused efforts to broaden the scope of several of the virus research and diagnostic laboratories in the country. This expansion would also strengthen and expand the national measles and rubella network, allow for a wider geographical coverage, improvements in turn-around time for testing and reduced shipment costs.

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The WHO-coordinated global measles and rubella laboratory network was established to provide high-quality, standardized measles and rubella surveillance in six WHO regions in 2000. Being a part of the South-East Asia Region, the Indian measles and rubella laboratory network becomes an integral part of the WHO global laboratory network. To ensure testing uniformity across the network, laboratories need to follow the network's testing requirements. An on-site annual accreditation review is conducted for each network laboratory to recognize them as being proficient.

In 2017, the Indian Council of Medical Research and WHO partnered to expand the measles and rubella laboratory network by integrating some of the virus research and diagnostic laboratories of the Indian Council of Medical Research and Department of Health Research, Government of India. Here, we describe the systematic 10-step scheme undertaken to integrate these laboratories into the measles and rubella laboratory network. We also describe challenges faced and lessons learnt during the expansion.

Methods
The Institutional Ethical Committee of the Indian Council of Medical Research–National Institute of Virology approved the study (NIV/IEC/2018/November/D-38).

Study setting
The measles and rubella laboratory network of India, initiated in 2003, consisted of 11 national and two reference laboratories in 2017. The King Institute of Preventive Medicine, Chennai, hosts the reference laboratory for serology and the Indian Council of Medical Research–National Institute of Virology, Mumbai Unit hosts the reference laboratory for molecular testing and sequencing. In 2019, the laboratories processed 18 000 serological tests and 5 000 molecular tests (WHO India, personal communication, 27 January 2020).

The Indian government collaborates with WHO National Public Health Support Programme, which is involved in various vaccine-preventable disease surveillance activities, such as polio, measles and rubella. Field units in the programme collect samples from suspected measles and rubella cases, and these are sent to a laboratory in the network. This testing follows the WHO-India recommended test algorithm.

In India, there were 106 virus research and diagnostic laboratories in 2019 with capability for serological and molecular testing of 20–25 medically important viruses. These laboratories are supported by the government for infrastructure, equipment, human resources and consumables for diagnosis. A small number of these laboratories can also perform genomic sequencing.

Selection of laboratories
To expand the national measles and rubella network, in 2017 the Indian Council of Medical Research and WHO selected six virus research and diagnostic laboratories based on the following criteria: (i) areas having high caseload but no measles and rubella laboratories, fully operational laboratory with good track record; (ii) proven capacity for serological and molecular diagnosis of any virus; (iii) good turn-around time for testing (≤ 72 hours); (iv) presence of trained human resources; (v) willingness to participate; (vi) good connectivity by air, rail and road; (vii) participation of these laboratories in other national programmes; and (viii) availability of computer and internet connection.

The laboratories were further evaluated for satisfactory compliance with the external quality assurance programmes for arboviruses (dengue, chikungunya, Japanese encephalitis) and human influenza viruses, presence of operational equipment and annual maintenance contracts of equipment, having detailed standard operating procedures for diagnosis of different viruses in place and understanding of corrective preventive actions.

Obtaining WHO proficient status
Selected laboratories went through a 10-step scheme (Box 1), developed by the WHO country office for India and Indian Council of Medical Research. This scheme was developed to ensure compliance with WHO recommended protocols and quality assurance requirements. The scheme commenced with assessment of selected laboratories for enrolment in the network and ended with attainment of WHO proficient status by the successful laboratories.

Step 1
To evaluate the laboratories’ readiness to undertake additional work of increased measles and rubella testing, the Indian Council of Medical Research undertook an on-site pre-assessment of each laboratory. This assessment included evaluation of available infrastructure, equipment, human resources, existing laboratory quality management systems, willing responsible officer and areas where strengthening and improvement was required.

Step 2
Laboratories ensured personnel’s willingness to participate in the network, improved infrastructure and bought equipment if needed, by using Indian government funds received when they integrated under the virus research and diagnostic laboratories scheme.
Step 3
At the National Institute of Virology, Mumbai Unit, WHO and the reference laboratories provided a 5-day practical training to the laboratories. Training covered how to perform the enzyme-linked immunosorbent assay (ELISA) and the conventional reverse transcription polymerase chain reaction (PCR) method as per the WHO recommended protocols. WHO also invited trainers from the National Institute of Health, Thailand, for this training. The Indian Council of Medical Research facilitated the training by providing funds, facility, facilitators and all logistics support for in-country participants.

Step 4
The Indian Council of Medical Research–National Institute of Virology, Mumbai Unit provided the laboratories with practice panels. The serology practice panel comprised of 10 samples containing 400 µL serum each of measles and rubella IgM positive and negative sera. These were tested both for measles and rubella IgM. Sera for practice panels had been received through the surveillance network and tested at the National Institute of Virology, Mumbai Unit. We selected sera with optical density values of 0.4–1.0 and of good quality (no haemolysis, non-lipolytic). WHO country office for India provided the reagents: Enzygnost* Anti-Measles-Virus/immunoglobulin (IgM) and Enzygnost* Anti-Rubella-Virus/ IgM ELISA kits (Siemens Diagnostics Products, Marburg, Germany). Laboratories were requested to submit their reports to the Indian Council of Medical Research–National Institute of Virology, Mumbai Unit, within 30 days of receipt of panel for review and scoring.

Step 5
After completion of practice panels, the two reference laboratories sent the laboratories unknown samples for testing. The unknown panel comprised of 40 serology and 10 molecular testing samples, prepared as mentioned above. Laboratories achieved a passing score of 90.0% or more (concordance of ≥ 36/40 samples in serology panel and ≥ 9/10 samples in molecular panel) were then allowed to receive samples directly from the WHO National Public Health Support Programme.

Step 6
To validate the laboratories, the serology reference laboratory performed parallel testing by instructing the laboratories to make two aliquots of the first 50 serum samples received from the WHO National Public Health Support Programme. If the sample number was lower than 50, 10.0% positive and 10.0% negative of the total samples was used. One aliquot was for their own testing and the other was to be referred to the serology reference laboratory.

Simultaneously the WHO National Public Health Support Programme trained the laboratories on data management on the WHO India measles laboratory information system software.

Step 7
To be allowed to report their results independently to the national surveillance programme, the laboratories needed to achieve concordance of 90.0% or more of the panel results (as per the WHO accreditation checklist; available in the data repository) in the parallel testing.

In April 2019, WHO and the Indian Council of Medical Research with CDC organized a training on sequence analysis using Informer technologies Inc., Los Angeles, USA and submission of sequences in WHO measles and rubella nucleotide surveillance MeaNS and RubeNS database.

Step 8
The laboratories enrolled in an external quality assurance scheme and participated in a global proficiency test. All panels were procured by WHO. The Victorian Infectious Diseases Reference Laboratory, Melbourne, Australia provided serology panels, which comprised of 20 measles and rubella IgM positive and negative sera. Results were reported to the Victorian Infectious Diseases Reference Laboratory website within 14 days of receipt of the panel. The Viral Vaccine Preventable Diseases Branch, CDC, Atlanta, USA, provided five spotted cards as a molecular panel. Laboratories submitted the results in CDC’s reporting format within six weeks of panel receipt. The laboratories submitted the sequences to MeaNS/RubeNS proficiency test panel portal for review.

Step 9
International experts supported by WHO assessed laboratories on-site as per the WHO measles and rubella accreditation checklist (available in the data repository). During the assessment, experts comprehensively evaluated: laboratory layout; implementation of good laboratory practices; laboratory techniques, staff training and staff vaccination records; biosafety and biosecurity aspects; laboratory quality management systems; and documentation, including logbooks, equipment calibration and standard operating procedures. The experts provided recommendations for further improvement, such as biosafety and good laboratory practices, documentation and regular supervision of activities involved.

Step 10
In the final step, WHO designated laboratories with a ≥ 80.0% score on the WHO accreditation checklist as a WHO proficient laboratory and included them in the national measles and rubella network.
Results

During the first phase of expansion, the Indian Council of Medical Research and WHO selected six laboratories for obtaining proficiency status and inclusion in the network (Table 1).

During on-site assessment in 2017–2018, all six laboratories expressed willingness to accept additional workload of measles and rubella surveillance within existing human resources. A biosafety cabinet was provided to one laboratory as part of system strengthening.

For the measles practice panel, two laboratories scored 100.0% (10/10) while four scored 90.0% (9/10). In the rubella practice panel, one laboratory scored 90.0% (9/10) while the rest scored 100.0% (10/10). The Indian Council of Medical Research–National Institute of Virology, Mumbai Unit carried out trouble shooting with laboratories to identify areas for improvement. The laboratory received information about important factors that can affect the final results of serology assay such as maintaining desired room temperature, pipetting techniques and incubation periods. As a result, five laboratories achieved a 100.0% (40/40) score in the unknown panel for measles and rubella serology, while one laboratory scored 97.5% (39/40) for measles and one laboratory 95.0% (38/40) for rubella (Fig. 1). In the molecular panel, all laboratories scored 100.0% (10/10) on the measles testing, while five laboratories achieved a 100.0% (10/10) score on the rubella testing and one laboratory 90.0% (9/10; Fig. 2).

After successful completion of step 5, the laboratories’ staff members attended data management training. Step 6 also covered parallel testing for which all six laboratories achieved a passing score of 90.0% or more in serology (Fig. 1). Subsequently, WHO country office for India instructed the field units of the WHO National Public Health Support Programme in catchment areas of the new laboratories to send samples directly to the laboratories for testing. Considering their overall performance, all laboratories initiated independent reporting to the national surveillance programme through the information system under Step 7. Simultaneously, the laboratories initiated submission of positive PCR products to the reference laboratory for sequencing. The training on analysing sequences of measles and rubella enabled the laboratories to

| Laboratory Location | Type of test | No. (%) of positive tests | Measles | Rubella | Measles | Rubella |
|---------------------|--------------|--------------------------|---------|---------|---------|---------|
|                      | Serum sample |                         |         |         |         |         |
|                      | Urine sample or throat swab |                     |         |         |         |         |
|                      | Due to geographical location the laboratory has very low workload |                     |         |         |         |         |

Table 1. Measles and rubella diagnosis by the newly integrated laboratories in the Indian measles and rubella laboratory network, 2018–2019
identify genotype and independently submit data in the WHO MeaNS and RubeNS database. In Step 8, as part of the external quality assurance scheme, the laboratories participated for the first time in the WHO global proficiency test panel for both serology and molecular tests. All six laboratories successfully achieved passing scores of 90.0% or more in both the panels.

Four of the six laboratories also underwent on-site WHO accreditation review by international experts between September and October 2019. Two laboratories achieved passing scores of 90.0% or more, whereas the other two achieved more than 80.0% scores (further information available in authors’ data repository). Due to the unforeseen coronavirus disease 2019 (COVID-19) pandemic, the on-site assessment of the remaining two laboratories (one in Hassan and one in Port Blair) had to be postponed. However, these two laboratories submitted an internal audit report to the WHO National Public Health Support Programme for review and will undergo the WHO accreditation process once the pandemic situation improves.

With the successful implementation and completion of the 10-step scheme, the six laboratories were integrated as subnational laboratories into the Indian measles and rubella laboratory network. The entire activity of inclusion of the laboratories in the network took approximately 24 months. Even after initiation of independent testing, these laboratories were closely monitored for their performance indicators, results of cross-verification for serology and timeliness of result reporting. Within 12 months of initiation of independent reporting, the six laboratories have tested a total of 2287 sera and 701 throat or nasopharyngeal swabs or urine samples (Table 1). In future, each laboratory is expected to process a minimum of 3000 serology and 750 molecular testing samples per year. However, these numbers may differ among laboratories.

During a 12-month period, all laboratories except one have maintained 90.0% or more reporting timeliness for serology (Fig. 3). Three of the six laboratories were successful in maintaining the reporting timeliness of 90.0% or more for molecular testing. The remaining laboratories could not maintain the timeliness due to repurposing of human resources and consumables for COVID-19 diagnostic testing.

**Fig. 1. Serology testing performance of newly integrated laboratories in the Indian measles and rubella laboratory network, 2018–2019**

**Fig. 2. Molecular testing performance of newly integrated laboratories in the Indian measles and rubella laboratory network, 2018–2019**
For quality assurance the laboratories participated in cross-verification or confirmatory testing of sera with the serology reference laboratory in 2018–2019 (Table 1). During cross-verification, each laboratory had to send 50 samples annually or 10.0% positive and 10.0% negative of the total samples tested for serology. All six laboratories have achieved >90.0% cross-verification scores for both measles and rubella, which has helped them in achieving WHO proficient status and integration into the global measles and rubella laboratory network.

**Discussion**

Regional elimination of measles and rubella to achieve eradication is one of the top global public health priorities. To meet targets for elimination, WHO has adopted a strategic plan for elimination of measles and rubella, containing five objectives: (i) achieving and maintaining high population immunity with ≥ 95.0% two-dose measles and rubella vaccination coverage in all parts of each country; (ii) developing and sustaining a sensitive and timely case-based surveillance; (iii) developing and maintaining a proficient laboratory network; (iv) ensuring adequate outbreak preparedness and response; and (v) strengthening support and linkages. While the WHO National Public Health Support Programme, India along with the Indian government are addressing all of the objectives, here we only describe how they have addressed objective (iii) in the strategic plan. To do so, six virus research and diagnostic laboratories were engaged in a 10-step scheme developed by WHO and the Indian Council of Medical Research. This process aimed to ensure seamless integration to the national measles and rubella laboratory network while initiating high quality independent testing through laboratories obtaining WHO proficiency status.

Implementation of the scheme was challenging. Selecting appropriate laboratories within the required catchment areas, fulfilling the requirements of the inclusion checklist, having willing responsible officers, engaging international trainers, developing practice and external quality assurance panels were burdensome. However, the biggest challenge was to re-orient the laboratories to align the laboratory systems, quality assurance and controls requirements and data entry as per WHO global network requirements. Before the laboratories’ inclusion in the network, they were already testing for measles and rubella in samples referred to them from the Integrated Disease Surveillance Programme, which is not a part of the WHO global network mechanism. They therefore had a completely different channel of data entry and quality assurance and quality controls before joining the network. Realigning them to a different mechanism for the WHO global network proved to be difficult but was achieved through rigorous follow-ups and trainings. Also, shortage of serology kits due to discontinuation of Siemens kits because of unforeseen problems with the manufacturer mandated the need to standardize testing with Euroimmun kits (Euroimmun AG, Lübeck, Germany). This shortage delayed the timelines of operationalization of the new laboratories. Meeting the sudden increase in demand for molecular testing reagents was also challenging for the laboratories. However, coordinated efforts by the Indian Council of Medical Research and the Ministry of Health and Family Welfare helped in overcoming the crisis by supporting the provision of serology diagnostic kits, while WHO provided molecular diagnostic reagents. The Indian Council of Medical Research and the Ministry of Health and Family Welfare also earmarked separate budgets for repeated trainings, on-site visits and shipment of clinical specimens. Existing resources at the laboratories in terms of human resources, equipment and infrastructure were used for undertaking the extra workload of the measles and rubella network. The Indian Council of Medical Research and WHO incurred additional costs, such as trainings and travel, panel preparation, diagnostic and consumables procurement and shipment. The approximate cost incurred on these requirements was 0.23 million United States dollars.

Overall, the 10-step scheme had several advantages in addition to laboratories obtaining WHO proficient status. The process helped in raising awareness of laboratories towards the importance of strict adherence to test protocols along with good laboratory practices, highlighting the weaknesses of some laboratories and pointing out the areas requiring improvement and strengthening, instilling confidence in laboratories which repeatedly achieved passing scores. This exercise also helped the laboratories in overall improvement of quality in all other testing areas, such as arboviruses, respiratory viruses and enteric viruses.

Following the successful integration, the Indian Council of Medical Research and WHO have identified nine more virus research and diagnostic laboratories. These laboratories are in...
Successful measles and rubella laboratory strengthening in India has helped in improving the turn-around time of testing and guiding field surveillance teams to take appropriate public health interventions for case management and curtailing transmission, thus aligning India's efforts towards regional measles and rubella elimination (Ministry of Health and Family Welfare, Government of India, unpublished data, 31 December 2021). The expansion also supported the transition to a more sensitive method of measles surveillance. After a successful pilot in 2018 in three states, where the measles case definition no longer included the symptoms coryza, conjunctivitis and cough, and instead used fever and rash surveillance, the entire country transitioned to such a method of surveillance in 2021. In the future, the expanded network will also help in meeting the key surveillance performance indicators of at least two non-measles, non-rubella discarded fever and rash cases per 100 000 population at national level.26,27

The 10-step scheme presented here can serve as a guiding tool for countries planning to enhance national laboratory capacity, following global standards. While the scheme can be deployed for measles and rubella laboratory expansion, it can also be used for strengthening and expanding laboratory networks for surveillance and early detection of pathogens of public health importance.

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Résumé

Expansion du réseau de laboratoires d’analyse de la rougeole et de la rubéole en Inde

Objectif Étendre le réseau de laboratoires d’analyse de la rougeole et de la rubéole en Inde en y intégrant de nouveaux établissements.

Méthodes En collaboration avec l’Organisation mondiale de la Santé (OMS), le gouvernement indien a mis au point un programme en 10 étapes destiné à accroître systématiquement le nombre de laboratoires effectuant des tests sérologiques et moléculaires de détection de la rougeole et de la rubéole. Le Conseil indien de la recherche médicale et l’OMS ont identifié une série de laboratoires éligibles en raison de leur situation géographique, de leur volonté d’y participer, de leur niveau de préparation, de leurs performances antérieures et de leur respect des mécanismes nationaux de contrôle et d’assurance qualité. Ce programme en 10 étapes a commencé par une formation aux analyses diagnostiques pour la rougeole et la rubéole, suivie de la réalisation de tests sérologiques et moléculaires pour détecter la rougeole et la rubéole sur des échantillons inconnus, une vérification croisée avec les laboratoires de référence et enfin, une accréditation OMS sur site.

Résultats Après avoir reçu une formation complète, un soutien technique, un financement et fait l’objet d’une surveillance, les six laboratoires sélectionnés ont tous réussi, obtenant une note égale ou supérieure à 90,0% dans les tests d’aptitude au dépistage sérologique et moléculaire de la rougeole et de la rubéole. Depuis 2018, ces laboratoires font partie du réseau d’analyse de la rougeole et de la rubéole en Inde. Douze mois après l’établissement des premiers rapports indépendants, les six laboratoires avaient testé 2287 échantillons de sérum et 701 écouvillons nasopharyngés, prélèvements de gorge ou échantillons d’urine.

Conclusion Le programme a permis de renforcer et d’étendre le réseau de laboratoires compétents. L’Inde a ainsi pu développer un système de tests sérologiques et moléculaires de haute qualité pour dépister la rougeole et la rubéole. Le modèle collaboratif adopté par le gouvernement indien avec l’OMS pourrait s’appliquer à d’autres pays qui souhaitent étendre leur réseau de laboratoires chargés de surveiller la rougeole et la rubéole, mais aussi d’autres maladies infectieuses.

Resumen

Ampliación de la red de laboratorios para el diagnóstico del sarampión y la rubéola en la India

Objetivo Ampliar la red de laboratorios para el diagnóstico del sarampión y la rubéola en la India mediante el establecimiento de nuevos laboratorios.

Métodos En colaboración con la Organización Mundial de la Salud (OMS), el gobierno de la India desarrolló un programa de 10 pasos para ampliar de manera sistemática el número de laboratorios que realizan pruebas serológicas y moleculares para detectar el sarampión y la rubéola. El Consejo Índico de Investigación Médica y la OMS identificaron los laboratorios adecuados según sus características geográficas, su disposición, su preparación, sus resultados anteriores y su adhesión a los mecanismos nacionales de control y garantía de calidad. El programa de 10 pasos se inició con la formación sobre las pruebas de diagnóstico del sarampión y la rubéola, seguida de la realización de pruebas serológicas y moleculares para el sarampión y la rubéola en grupos desconocidos, la verificación cruzada con los laboratorios de referencia y finalizó con la acreditación in situ a cargo de la OMS.

Resultados Luego de un extenso entrenamiento, apoyo técnico, financiamiento y seguimiento, los seis laboratorios seleccionados alcanzaron puntajes de aprobación del 90,0 % o más en las pruebas de aptitud serológica y molecular para el sarampión y la rubéola. Desde 2018, los laboratorios forman parte de la red contra el sarampión y la rubéola de la India. En los 12 meses posteriores al inicio de la presentación de informes independientes, los seis laboratorios han analizado 2287 muestras de suero y 701 exudados faríngeos o nasofaríngeos, o muestras de orina.

Conclusión El proceso condujo al fortalecimiento y la expansión de la red. Esta competente red de laboratorios ha ayudado a India a ampliar las pruebas serológicas y moleculares para el sarampión y la rubéola, garantizando al mismo tiempo la alta calidad de las pruebas. El modelo
con el fin de vigilar los casos de sarampión y rubéola, así como otras enfermedades infecciosas.

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