

Investigator Studies Program (MISP) Clinical Protocol Template	
Requirements for Submitting a Full Clinical Protocol	
Section #1 - MISP Protocol Identification	
Study Title:	<i>A Prospective Cohort Study To Compare The Burden Of Medically Attended and Community RSV, hMPV, SARS-CoV-2 and influenza LRTI In Infants And Children Under 5 Years Of Age and adults (16-60 years) In Rural India</i>
Brief title	<i>Burden of respiratory viruses in India.</i>
Request Date:	7 February 2025
Institution Name	MAHAN Trust
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Protocol ID	MAHAN RSV 2025 1

Section #2- Core Protocol	
2.1 Objectives & Hypotheses	<i>2.1 List the objectives.</i> This proposal addresses the following topic of the MISP: Towards pan-respiratory viral prevention: <i>Burden of hMPV, SARS-CoV-2 and influenza in relation to RSV</i> We propose the following main objectives : Primary: To determine the population-based epidemiology of respiratory syncytial virus (RSV) lower respiratory tract illness (LRTI) in infants and children less than 5 years of age and adults, elderly people in rural India. Secondary: To determine the population-based epidemiology of human metapneumovirus (hMPV), <i>SARS-CoV-2</i> and <i>influenza</i> LRTI in infants and children less than 5 years of age and adults, elderly people in rural India, in relation to RSV. 2.1.1 Sub-objectives. 2.1.1.1 Primary - To prospectively determine the clinical features and mortality from RSV lower respiratory tract illness in a cohort of infants and children under 5 years and adults, elderly people in 30 villages, over 3 years, and all newborns entering the cohort during the 3 years of the study. - To determine the incidence (per 1000 child years and adults, elderly people of observation) of the following classifications of RSV LRTI, using the WHO classification of disease: non-severe pneumonia, severe pneumonia, very severe pneumonia, RSV hospitalization, RSV mortality. - To evaluate the RSV subgroup specific (RSV A and RSV B) burden of illness and to compare severity and incidence based on subgrouping. 2.1.1.2 (Secondary) - To prospectively determine the clinical features and mortality from influenza A and B, hMPV and <i>SARS-CoV-2</i> lower respiratory tract illness in a cohort of infants and children under 5 and adults, elderly people in 30 villages, over 3 years, and all newborns entering the cohort during the 3 years of the study - To generate incidence data [per 1000 child, adult and elderly years of observation) for other respiratory viruses (influenza A and B, hMPV and <i>SARS-CoV-2</i>) over the same time, describing the same endpoints above namely non-severe pneumonia, severe pneumonia, very severe pneumonia, hospitalization, mortality, attributable for each of the viruses - to evaluate the hMPV subgroup specific (hMPV A and hMPV B), SARS-CoV-2 subgroup specific and influenza subgroup (Influenza A H1N1 pdm2009, H3N2 and Influenza B) burden of illness, and to compare severity based on subgrouping. <i>2.1.1 List the clinical hypotheses.</i> This is primarily a descriptive observational study. However, we have proposed null hypotheses in the statistical methods, addressing each of the objectives and sub objectives
2.2 Background & Rationale, Significance of Selected Topic & Preliminary Data	Respiratory syncytial virus (RSV) is the most common cause of infection of the upper and lower respiratory tract in infants and young children worldwide and is a major public health burden.^{1 2} It has been estimated that globally in 2019 there were 33.0 million episodes of RSV acute lower respiratory tract infections (LRTI) in children less than 5 years of age resulting in about 3.6 million hospitalizations, with 6.6 million episodes occurring in infants < 6 months of age and 1.4 million RSV associated ALRI hospitalizations³. It was estimated that India bears a fifth of the global burden of illness (6.2 million episodes of RSV LRTI).³ With very few recent studies on the burden of RSV illness in rural communities in lower-middle income countries (LMIC), where most children live⁴⁻¹⁰ and given the imminent availability of monoclonal antibodies¹¹⁻¹³ and maternal vaccines to prevent them^{14,15}, there is

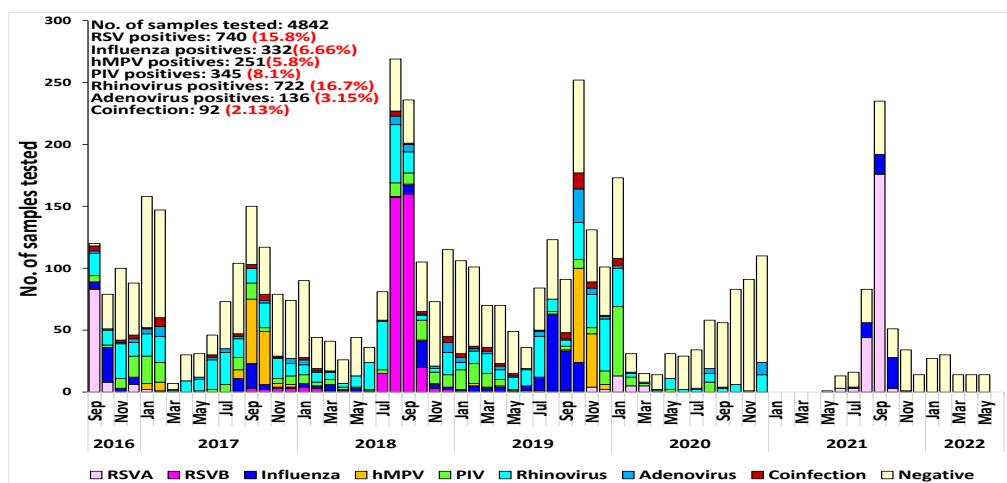
a need to understand the burden of disease in these rural communities, especially those with high infant mortality, such as those proposed here.¹⁶

Prof Simoes has been involved for many years with hMPV¹⁷⁻²¹, influenza²²⁻²⁹ and more recently SARS CoV2 burden of disease studies^{28,30-33} and global estimates^{20,21,34-38} as well as vaccine trials of maternal influenza vaccines³⁹⁻⁴¹ and Pediatric SARS CoV2 vaccine trials⁴²⁻⁴⁴ and adult monoclonal antibody studies⁴⁵. During the Bill and Melinda Gates foundation funded studies in Melghat, in addition to RSV, we measured hMPV and influenza as well as SARS CoV2 in children with severe or very severe LRTI and hospitalized children, as well as those who died.^{1,16} However these did not reflect the true burden of LRTI since we did not measure the burden in those with non-severe pneumonia. Gates foundation was focused on mortality estimates, which those studies were geared towards.

There are several lacunae in the BMGF studies. They were focused on estimating the burden of mortality due to RSV, and hence the recruitment strategies focused on identifying those with severe and very severe disease in the community and in the hospital. However we were unable to identify the less severe infections, that contribute a significant burden of disease. In addition, the focus being on RSV, though we collected information on other viruses, those were not a priority of the foundation. We have some preliminary data, however it is all pre pandemic. As we are aware, the pandemic has changed the political age groups and presentations of RSV, as we and others have described in recent publications. This has not been well described for hMPV and influenza, and certainly not in comprehensive studies of community and hospital-based burden of disease, that we propose in this study.

Preliminary Data: Under the auspices of the Bill and Melinda Gates Foundation, we conducted a study in Melghat, Central India with the primary goal of capturing the burden of RSV as a cause of mortality in rural infants and children under 2 years of age. This was an active surveillance of children under two years of age in 93 villages 5 primary health centers and 3 hospitals serving these villages, conducted between Sept 2016 and Dec 2020. Village health workers, and counselors at the health facilities monitored all lower respiratory tract infections (LRTI) in consented subjects. Children with severe, or very severe LRTI and all who died, had nasopharyngeal swabs collected for detection of RSV by molecular methods including RSV A and B.

Results of Virology testing: In the 12 134 subjects, there were 5308 episodes of non-severe LRTI 2481 episodes of severe LRTIs and 1808 of very severe LRTIs. 4842 NP swabs were collected and tested by Real time RT-PCR for 16 respiratory viruses including RSV subtyping. During 2021 and 2022 additionally the samples were tested for SARS CoV 2. Out of 4842 almost 55% samples i.e., 2637 were positive for one or more respiratory viruses. RSV was detected in 740 cases (360 RSV A and 380 RSV B) followed by rhinovirus in 722 cases, PIV was detected in 345, Influenza 332, hMPV in 251, Adenovirus detected in 136. SARS CoV 2 was detected in 19 cases. RSV was detected in alternate year outbreaks with a dominance of RSV A or RSV B subtypes. In 2016 RSV A was predominantly in circulation where as in 2017 and 2018 RSV B was exclusively in circulation. In 2021 RSV A predominated. (Figure 1) ***RSV and hMPV alternate and Influenza occurs annually.***



Please note that in 2021-22 Testing was done only for RSV, Influenza and SARS CoV2

RSV Mortality: Fifteen of 16(94%) children with RSV died of LRTI, 14 in the community and 1 in the hospital. The case fatality ratios for severe RSV LRTI in the first 6 months of life were 3/52(7.1%) and 1/36(2.8%) in the community and hospital respectively. Of those with very severe LRTI in the community, 17.6% died. There were no very severe RSV LRTI hospital deaths. The adjusted RSV LRTI mortality rates ranged from 1.0 – 3.0/1000 child years (CY) overall, and 2.0 – 6.1/1000 CY, accounting for 20% of the LRTI deaths and 10% of the post neonatal infant mortality.¹⁶

Pre and post pandemic RSV burden: RSV + severe and very severe LRTI in the community and hospital were compared in the cohort of subjects from the 33 villages before (2016-2020) and after the major SARS CoV 2 outbreak in India. Population based rates, of severe and very severe RSV LRTI measured over the RSV seasons, were compared during the 2 periods using incidence rate ratios (IRR) with 95% CI.

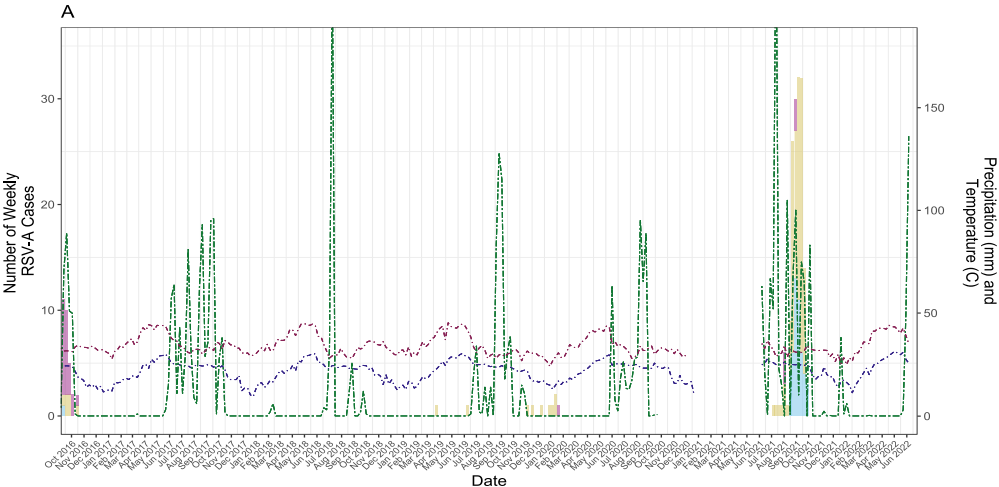
The incidence of severe and very severe RSV increased in all age groups in the post pandemic period. In the 0 to 90 days and 91 to 180 days age groups there was an almost 3-fold increase, with a IRR of 2.8 (1.4-5.2) and 2.8 (1.4-5.0) respectively.

Incidence Rate Ratios for Severe and Very Severe RSV Associated Lower Respiratory Tract Infections in 33 Villages

Sep 2016 to Dec 2020 Jun - Dec 2021

Age	Incidence	Incidence	IRR
Days	/1000 C-Y	/1000 C-Y	(95% CI)
Overall			
0-90	107.6	304.2	2.8 (1.4-5.2)
91-180	140.0	385.2	2.8 (1.4-5.0)
181-270	107.9	259.9	2.4 (1.2-4.6)
271-364	131.0	254.3	1.9 (1.0-3.6)
365-547	67.9	168.0	2.5 (1.2-4.7)
548-730	53.6	145.0	2.7 (1.4-4.9)

Please note that for the BMGF study, since Mortality was the primary goal, we only tested Severe and very severe LRTI for RSV. In the proposed study, to truly understand the burden of disease we are proposing testing ALL LRTI in the community or medically attended (non severe, severe, very severe, outpatient, hospitalized infants and children and all those that die from 33 villages This is a much more intensive look as a smaller group

	During the pandemic post pandemic period, we only found RSV A circulating, but we also tested children with non-severe RSV LRTI. Please see figure below to illustrate the ratios of non-severe: severe/very severe RSV A immediately post pandemic. A 
2.3 Study Design	Melghat is a hilly, forested area spread over >4000 km² in Maharashtra state, Central India, with population of 2,80,000 scattered over 320 villages, distributed in 2 blocks. The government primary health care delivery is through sub-center with paramedical workers for 2-5 villages, a Primary Health Center (PHC) with a doctor and nurses for every 30 villages, a rural sub-district hospital (RH) and 2 district hospitals 1 for mother and children and the other for adults. MAHAN hospital, where the study is based is a charitable trust hospital that provides free or low-cost care to the community. We propose an active surveillance observational study in Melghat of a cohort of all newborns, infants and children under 5 years, and adults in age group of 16-60 years, for 36 months, of LRTI detected at home, primary health centers or hospitals in the catchment area of 30 villages under surveillance. The age range for the adult participants is 16-60 years. In Melghat as per our study from 1998 to 2024, the death rate of people in economically productive age group, which is 16-60 years is very high, >500 per 1,00,000 population of that age group i.e. 2.3 times that of rest of India. The death of person in this earning age group is great loss to the family, community and nation. The major causes of mortality are respiratory diseases. Hence, we included this age group in our study. But if MSD, India intends to add adults above the age of 60 years, then MAHAN trust is ready to include elderly people also. Active surveillance for LRTI cases in the community will be performed through weekly home visits by village health workers (VHWs) The VHWs are local women living in and accepted by the community. They have been trained using standard WHO materials, translated into Hindi with a refresher course every 6 months. A nasopharyngeal swab will be obtained from children and adults (16-60 years) with non-severe, severe or very severe LRTI and those who die. A counselor is posted at every hospital and PHC who will capture every hospitalization of children under 5 and adults from the 30 villages, will identify any children from the 30 villages who visit the OPD with an LRTI and collect a swab from them. Children/adult with any lower respiratory illness will have a swab collected at admission and from any child/adult who dies. Samples (2000) will be tested for a panel of respiratory viruses first with a screening PCR(RSV, hMPV, SARS CoV 2 and Influenza) followed by subtyping when positive (RSV A and RSV B; hMPV A and hMPV B; Influenza A H1N1 pdm2009, H3N2 and Influenza B and SARS CoV 2) at the National Institute of Virology, Pune.

2.4 Diversity & Inclusion	The study is based on a poor rural population in India, where all of the participants are of Indian origin/descent. Since India is the single most important country contributing to the burden of morbidity and mortality of respiratory illness globally, and most of this occurs in parts of rural India where infant mortality and adult mortality still remains high, this population is the exact population where studies on the viral epidemiology and burden need to be conducted. Hence this population though not diverse, is a highly relevant population whether burden of disease occurs, but is really unstudied till our preceding study referenced in the text.
2.5 Patient Engagement	<i>Please explain how the study design, recruitment and conduct will be informed by a patient or patient advocate.</i>
2.6 Study Flowchart	Attached separately
2.7 Study Procedures	Obtaining Clearances: Prior to any research procedures, clearances will be obtained from the Government of Maharashtra health department, the National Institute of Virology (located in Pune, India), and MAHAN Institutional Review Board Clearance. We will obtain clearance and consent to do the study from the Village Head and the Council of Elders of each of the 30 villages. Study Population: Previous to the BMGF study we conducted a census of all 93 villages. Starting in April 2016 trained VHWs and one supervisor per 10 villages conducted a census of all households in the 30 villages. Subsequent to that all births, deaths and migrations were captured, and entered into the census database. All houses in the 93 villages and the roads have also been geocoded. All adults and children in the 93 villages have a unique ID number (UID number). We are currently conducting a house-to-house census, for all the houses in the 30 villages (all of which were in the original 2016 census) which will be completed in the next three months. During the census we will update data that was collected in the 2016 census in these same villages. This will allow us to collect household information on socio-economic factors, and respiratory risk factors that will be used for our analysis of the proposed study. It will also provide a denominator for the study. At the start of the study All children under the age of five years and adults (16-60 years) will be recruited for prospective observation for LRTI, after obtaining informed consent from the parent/caretaker. Subsequently using pregnancy monitoring, all parents of live-born neonates will be approached for consent to follow-up their children for the next 3 years. Subjects will be followed until they reach the age of 5 years or 60 years, migrate permanently out of the 30 villages, withdrew consent, or expire. Health worker training: All VHWs, and their supervisors and health center/hospital based counselors will be re-trained using a modified World Health Organization (WHO) methodology, in the recognition of WHO defined Pneumonia⁴⁶, and its recording on paper case report forms as well as how to collect nasopharyngeal (NP) swabs in infants and children, and adults (16-60 years). WHO classifications include non-severe, severe and very severe categories of pneumonia. VHWs will be re-trained in survey taking, as well as in filling out weekly reports on the health of subjects, pregnancy monitoring, and monitoring for subject deaths. Counselors will be re-trained in filling out case report forms for hospital admissions, and supervisors will be re-trained in grief counseling and conducting verbal autopsies using a standardized semi-structured form. Subject monitoring in the community: Our experience is that VHWs develop a keen rapport with the families who they follow with weekly home visits, specifically following the respiratory health of the subjects. Any child or adult with an LRTI, will have a NP swab collected for pathogen detection. Treatment will

follow Govt. of Maharashtra guidelines that permits VHW's to prescribe amoxicillin for pneumonia/ within 24 hours of the assessment, supervisors will visit the subject and conduct a respiratory assessment as well. All subjects with LRTI who are hospitalized, will be followed up at home one and two weeks later. (Follow up form)

VHWs are also informed of community deaths and, after grief counseling is offered to the family, either by a supervisor or local trained traditional healers or nurses, with informed consent, NP swabs will be obtained from the deceased child whenever feasible. (the community is used to this)

Subject monitoring in the health centers and hospitals:

Counselors working in the 14 government facilities will monitor the outpatient and conduct a daily census of all new admissions, and after obtaining informed consent, (from children and adults from the 30 villages) will administer a short questionnaire and collect a NP sample from children (0-5 years) and adults (16-60 years) with LRTI or sepsis, as well as all who die. (if they have not been already collected by the VHW in the village) for an acute illness visit. Total samples to be collected 2000.

Nasopharyngeal swab collection and sample processing:

NP swabs will be collected using flocked swabs and transported in viral transport medium to MAHAN hospital where they will be stored at 4-8°C. Batches are transported to the ICMR - National Institute of Virology Pune for pathogen testing. The samples will be tested in a 2-step manner (screening and subtyping) as outlined previously by real time PCR using standardized protocols^{47,48}.

Verbal Autopsy:

A supervisor and a VHW from the community will conduct a verbal autopsy (VA) within 2 weeks of the death. The VA uses standardized Indian government protocols modified for use in Maharashtra incorporating local terminology^{49,50}. The narratives are read by two independent trained physicians, and where there is discordance, a third Senior pediatrician adjudicates the difference.

Lower Respiratory Tract Infection: Cause of Death

A child or adult with an LRTI classified either by a VHW or supervisor in the Village or Physician in a hospital who dies within 15 days of the diagnosis is considered an LRTI death. In the absence of an examination, a verbal autopsy classified as Pneumonia codifies the death as an LRTI death.

Data entry:

Data entry operators enter data into an access database and the data manager checks data quality daily. Incomplete data forms are sent back to the field for correction.

How will we achieve our objectives with this study design?

Since this is a prospective longitudinal study, with weekly home visits to every child or adult enrolled as well as every newborn, we have exact measurements on every child or adult in the study, and can calculate the **denominator of child/adult years of observation**, as we have done in our previous publications in the same population.

Our staff are well trained in recognizing non-severe, severe, and very severe LRTI, and all children and adults identified with LRTI are examined by a supervisor within 24 hours. Our unpublished observation from the previous study, shows a kappa of 0.85, which is excellent agreement. In addition, village health workers receive, refresher training every six months. All outpatient, and inpatient visits of children/ adults are monitored at the primary health centers and the hospital by study-based counselors. Finally, all deaths are monitored. This robust system allows us to capture the entire spectrum of LRTI (**Numerator**) for all infants and children less than 5 years of age and adults (16-60 years) in the 30 villages. **LRTI classification** has also been well established. Monitoring also allows complete medically attended LRTI (**MA-LRTI**) data capture.

	Pregnancy monitoring (monthly visits to every household of women of childbearing age, by female VHW's) to determine whether they had a period within the last four weeks. For those who do not, the date of the LMP is captured at this visit. This allows gestational age determination with great accuracy, and in fact has been shown to be as accurate as ultrasound. This allows the classification of gestational age at birth for all infants in the study. All pathogen testing is done at India's premier virology Institute, National Institute of Virology, which is the reference laboratory for all viral testing in India. It is the equivalent of the CDC and the US. This allows for detection of all respiratory viruses outlined in the study, which we have been doing successfully for the last five years. Since every child is monitored at home, as well as captured in the hospital, and then followed up at home (an ideal epidemiologic active surveillance model with built-in capture-recapture of all MA-LRTI) we know the outcomes of every LRTI. Hence this active surveillance methodology allows us to ensure that we will be able to address all of the proposed objectives. We recognize that RSV epidemics occur once in two years in this population alternating with hMPV, which is why we plan a three-year study															
2.8 Study duration and study timelines	Total study period : 4 Years The actual study i.e. surveillance will be conducted over three years. Initially all children under five years of age and adults (16-60 years) will be consented and recruited at the start of the study. Subsequently all newborn babies born in the 30 villages will be followed prospectively till the end of the study (36 months after the start of the study) or till they reach five years of age whichever comes first. The study will proceed in four major phases over a 4-year implementation period: <table><tr><th>Phase</th><th>Duration</th><th>Activities</th></tr><tr><td>Phase 1: Preparatory</td><td>Months 0–3</td><td>Ethics approvals, staff recruitment & training, village selection, community sensitization</td></tr><tr><td>Phase 2: Baseline & Enrolment</td><td>Months 4–6</td><td>Household census, cohort enrolment, baseline respiratory screening, GIS tagging</td></tr><tr><td>Phase 3: Surveillance & Follow-Up</td><td>Months 7–42 (Year 1–3)</td><td>Weekly active surveillance, ALRTI identification, sample collection, follow-up</td></tr><tr><td>Phase 4: Analysis & Dissemination</td><td>Months 43–48</td><td>Data cleaning, statistical analysis, publication, translation, dissemination workshops</td></tr></table>	Phase	Duration	Activities	Phase 1: Preparatory	Months 0–3	Ethics approvals, staff recruitment & training, village selection, community sensitization	Phase 2: Baseline & Enrolment	Months 4–6	Household census, cohort enrolment, baseline respiratory screening, GIS tagging	Phase 3: Surveillance & Follow-Up	Months 7–42 (Year 1–3)	Weekly active surveillance, ALRTI identification, sample collection, follow-up	Phase 4: Analysis & Dissemination	Months 43–48	Data cleaning, statistical analysis, publication, translation, dissemination workshops
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2.9 Implementation Strategy: Ensuring Consistent and High-Quality Data Collection	This plan outlines the strategy and operational procedures to ensure the standardized and rigorous collection of data for the prospective cohort study across 30 villages in rural India. The strategy is built on the foundation of the provided clinical protocol and focuses on training, supervision, data management, and quality control. 1. Centralized Training and Standardization To ensure every team member collects data uniformly, we will conduct a robust, standardized training program. • Standardized Training Curriculum: ○ A comprehensive training manual which was developed by MAHAN trust with the help of University of Colorado, Denver and Gates foundation, USA which is based on methodology developed by international experts, will be used. This manual is time tested over 6 years and which has shown the one of the best results in world.															

- The manual has been translated into Hindi to ensure clarity for all Village Health Workers (VHWs).

- **Key Training Modules will include:**

1. **Clinical Assessment:** In-depth training on using the WHO classification to identify and categorize Lower Respiratory Tract Infections (LRTI) as non-severe, severe, and very severe pneumonia. This will involve video examples, case studies, and practical demonstrations.
2. **Specimen Collection:** Hands-on training on the correct technique for collecting nasopharyngeal (NP) swabs from infants, children, and adults.
3. **Data Recording:** Detailed instruction on accurately completing all paper-based case report forms. Also, we are trying for data collection on tablets if we get sufficient funding.
4. **Ethical Conduct:** Training on the informed consent process, grief counselling, and maintaining participant confidentiality.
5. **Verbal Autopsy:** Specialized training for supervisors on conducting standardized semi-structured verbal autopsies as per protocol developed by MAHAN trust based on SEARCH and WHO model.

- **Training Schedule:**

- An intensive initial training workshop will be conducted for all VHWs, supervisors, and hospital/PHC counsellors during first 6 months of the study.
- Mandatory two-monthly refresher courses will be held to reinforce knowledge, address challenges, and introduce any protocol updates.

2. Field Operations and Supervision

A clear structure for field operations and active supervision is critical for maintaining data quality.

- **Team Structure:**

- **Village Health Workers (VHWs):** Local trained semiliterate well experienced tribal women residing in the study villages, will be responsible for weekly home visits, identifying LRTI cases, and collecting initial data and swabs. They have very good community rapport since 2016 as they are providing free

	health services to people of these villages and conducted similar study for 5 years. ○ Supervisors: One supervisor assigned per 10 villages, responsible for verifying LRTI cases, providing secondary assessment, overseeing VHWs, and conducting verbal autopsies. ○ Counsellors: Stationed at primary health centres and hospitals to capture all medically attended LRTI cases from the study villages. • Data Collection Workflow: 1. Weekly Home Visits: VHWs will visit every enrolled household weekly to monitor the respiratory health of subjects. 2. Immediate Reporting: Upon identifying a potential LRTI case/death, the VHW will immediately report it to their supervisor. 3. Supervisor Verification: The supervisor will visit the subject within 24 hours to conduct a secondary respiratory assessment and confirm the classification. This dual-check system ensures high accuracy. But even if the supervisor cannot come in 24 hours or the patient is serious, then the VHW will collect the NPS with all due precautions. 4. Hospital Monitoring: Counsellors will screen all outpatients and new admissions daily to identify and enrol subjects from the 30 villages. 5. Follow-up: All subjects with LRTI who are hospitalized will be followed up at home one and two weeks later to capture the complete outcome. 6. Program manager, paediatrician and the project director regularly monitor the sample and data collection process. • 3. Data Management and Quality Control A robust data management system will ensure the integrity and accuracy of the collected information. • Data Entry and Verification: ○ Well experienced data entry operators will enter all data from paper forms into a centralized Access database. ○ The data manager will perform daily quality checks on the entered data, looking for inconsistencies, missing values, or errors. ○ Any incomplete or erroneous forms will be immediately sent back to the respective supervisor for correction in the field. • Sample Management:
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	○ A strict chain of custody will be maintained for all NP swabs. ○ Swabs will be collected, placed in viral transport medium, and stored at MAHAN hospital at 4-8°C. ○ Batches will be transported in a temperature-controlled environment (dry ice packs) to the National Institute of Virology in Pune for testing. • Quality Assurance Measures: ○ Regular Audits: Field supervisors/manager/ consultants will conduct random spot-checks on VHWs to observe their assessment and data collection techniques. ○ Inter-rater Reliability: Periodically, two supervisors / program manager will independently assess the same patient to calculate the kappa score for agreement, ensuring that the LRTI classification remains consistent across the team. ○ Data Audits: The principal investigators will conduct periodic audits of the database to ensure data quality and adherence to the protocol. 4. Community Engagement and Ethical Oversight Building and maintaining trust with the community is paramount for the success of this long-term study. • Initial Engagement: Before the study begins, meetings will be held with the Village Head and the Council of Elders in each of the 30 villages to explain the study's purpose and obtain their formal consent and support. • Informed Consent: A clear and standardized process for obtaining written informed consent will be followed for every participant. VHWs and counsellors are well trained to explain the study in the local dialect and answer any questions from the families. • Sensitive Procedures: For post-mortem swab collection, supervisors or trained local counsellors will provide grief counselling to the family before seeking consent, ensuring the process is handled with the utmost sensitivity and respect.
2.10 Statistical Analysis and Sample Size Justification	Statistical Analysis Plan All data will be entered into a RedCap database and exported for analysis. All statistical analyses will be done by the Biostatistician Dr Raje, who is part of the study team, Statistical methods The demographic and socio-economic status of each subject included in the cohort will be summarized according to scale of measurement. Continuous variables will be expressed in terms of mean and standard deviation, while categorical variables will be summarized in terms of frequencies and percentages.

	<u>Primary objective</u> a) To prospectively determine the burden (clinical features and mortality) of RSV LRTI in a cohort of infants and children under 5 and adults (16-60 years) in 30 villages, over 3 years, and all newborns entering the cohort during the 3 years of the study. The ratio of total number of RSV positive cases to the total number of respiratory specimens tested during the study period from the catchment area will be obtained as a measure of burden of RSV related LRTI illness in the community. 95% confidence intervals for the ratios will be obtained. This will be done for each of the different classifications of RSV LRTI (non-severe, severe, very severe, mortality) and by location (community or first level health facility /hospital) Results will be stratified by sex, age groups and wealth score quintiles. b) To determine the incidence (per 1000 child years/adult years of observation) of the following classifications of RSV LRTI, using the WHO classification of disease: non-severe pneumonia, severe pneumonia, very severe pneumonia, RSV hospitalization, RSV mortality. The RSV LRTI cases will be categorized using the WHO classification system into five categories, and in each category, the incidence will be obtained as the ratio of number of cases in the category to the total children/adults years of observation, expressed in terms of 1000 child/adults years. The 95% confidence intervals will be obtained for each incidence rate, in each class. The hypothesis of interest will be: there are no differences in the incidence rates of RSV LRTI between different socioeconomic classes. The pair wise comparison of rates between any two classes will be performed using z-test, assuming that the difference in the rates is normally distributed. Results will be stratified by sex, age groups and wealth score quintiles. c) To evaluate the RSV subgroup specific (RSV A and RSV B) burden of illness and to compare severity based on subgroups. The genotype for each case will be determined using PCR. A test based on normal approximation for testing statistical significance of difference in k proportions ($k > 2$) will be used. The null hypothesis tested will be: That there is no association of specific genotype and phenotype. The distribution of cases corresponding to each genotype will be tested for statistical significance across severity-based clinical categorization. Multivariate analyses will correct for any confounding risk factors found in prior stepwise analyses. <u>Secondary objective</u> a) To prospectively determine the burden (clinical features and mortality) of (influenza, SARS COV2 and hMPV) in a cohort of infants and children under 5 and 16-60 years in 30 villages, over 3 years, and all newborns entering the cohort during the 3 years of the study. The ratio of total number of each of the viruses positive cases to the total number of respiratory specimens tested during the study period from the catchment area will be obtained as a measure of burden of influenza, SARS CoV2 and hMPV related LRTI illness in the community. 95% confidence intervals for the ratios will be obtained. This will be done for each of the different classifications of LRTI (non-severe, severe, very severe, mortality) and by location (community or first level health facility /hospital) Results will be stratified by sex, age groups and wealth score quintiles. b) To generate incidence data (per 1000 child years/ adult years of observation) for other respiratory viruses (influenza, SARS CoV2 and hMPV) over the same time, describing the same endpoints above namely non-severe pneumonia, severe pneumonia, very severe pneumonia, hospitalization, mortality, attributable for each of the viruses. The incidence rate for each type of infection will be obtained as the ratio of number of infected cases with particular infection to the total child-year, expressed in terms of 1000 child-years/ adult-years. The hypothesis tested will be: The hypothesis of interest will be: there are no differences in the incidence rates of each specific virus causing LRTI between socioeconomic classes, and will be tested using Z-test for differences in k proportions ($k > 2$). For each infection type, proportion of cases with severity of illness will be obtained and compared using the above test. Individual burden of disease will be compared to that of RSV
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and stratified using the established categories of age group, sex, wealth quintile, GA and disease severity. Differences will be subjected to multivariate analyses to test for significance.

c) To evaluate the Influenza, SARS CoV2 and hMPV subgroup specific (Influenza A and B, and hMPV A and B) burden of illness and to compare severity based on subgroups. The genotype for each case will be determined using PCR. A test based on normal approximation for testing statistical significance of difference in k proportions ($k > 2$) will be used. The null hypothesis tested will be: That there is no association of specific genotype and phenotype. The distribution of cases corresponding to each genotype will be tested for statistical significance across severity-based clinical categorization. Multivariate analyses will correct for any confounding risk factors found in prior stepwise analyses.

Power/Sample Size:

We plan to recruit a **cohort of 22,121 individuals**, comprising:

- 2,726 children aged 0–5 years (U5C)
- 19,395 adults aged 16–60 years,
- Including follow-up of new births and individuals newly entering this age bracket during the 3-year observation period.
- The estimates are based on updated demographic profiles of the selected tribal villages, population and local birth rates.

Expected Case Yield Based on Prior Surveillance Studies:

- From previous longitudinal studies (e.g., 9,600 LRTI cases identified in a cohort of 12,000 over 4 years), we project:
- ~550 cases of Acute Lower Respiratory Infection (ALRI) per year among children under 5.
- Total of ~1,636 ALRI specimens from U5C over 3 years (assuming >90% follow-up and sample collection)

Virological Yield Estimates (based on prior positivity rates from 4,842 specimens):

- Respiratory Syncytial Virus (RSV):
 - $(740/4,842) \times 1,636 \approx 250$ expected RSV-positive cases
- Influenza viruses:
 - $(332/4,842) \times 1,636 \approx 112$ expected influenza-positive cases
- Human Metapneumovirus (hMPV):
 - $(251/4,842) \times 1,636 \approx 85$ expected hMPV-positive cases

Adult Cohort (16–60 years):

- Anticipated 155 pneumonia cases annually, totalling 465 adult cases over 3 years as per our data of last 24 years.

	- Expected nasopharyngeal specimen (NPS) collection rate >90%, i.e., ~418 adult NPS samples. Contingent on additional funding, we aim to scale sample collection to 2,000 NPS from symptomatic children and adults, and dead children and adults over the 3-year period. These sample sizes are powered to detect statistically significant differences ($\alpha = 0.05$, power = 80%) in virus-specific respiratory disease incidence between subgroups (e.g., age, nutrition status, tribal subpopulation) and to analyse long-term sequelae patterns in RSV-positive vs. RSV-negative individuals using lung function tests.														
2.11 Specific Drug Supply Requirements	No Drug requested.														
2.12 Adverse Experience Reporting	No AE reporting as no drug being used, except as relates to study related to mid turbinate swab collection, which are reported to the IRB. SAEs are not relevant for this epidemiological study and not required to be reported to the IRB in Maharashtra/India, if not a drug/vaccine/device study														
2.13 Itemized Study Budget	<i>Please see itemized budget as attached excel sheet.</i> Detailed budget allocation for specimen collection and laboratory analysis. We will do the multiplex Real time -PCR (qRT-PCR) RTPCR for influenza, SARS CoV 2 and RSV and the subtyping for influenza and RSV at National Institute of Virology, Pune, an ICMR center. It which will cost around 2000 INR per sample. <table border="1"> <thead> <tr> <th>Particular</th><th>Amount per sample (INR)</th></tr> </thead> <tbody> <tr> <td>Nasal swab stick and viral media Expenses</td><td>800.00</td></tr> <tr> <td>Nasal swab collection fee</td><td>500.00</td></tr> <tr> <td>Nasal swab travelling expenses from villages to MAHAN centre</td><td>150.00</td></tr> <tr> <td>Nasal swab travelling expenses from MAHAN centre to NIV Pune</td><td>300</td></tr> <tr> <td>NIV charges for RTPCR per sample</td><td>2000</td></tr> <tr> <td>Total expenses for one NPS</td><td>3750</td></tr> </tbody> </table>	Particular	Amount per sample (INR)	Nasal swab stick and viral media Expenses	800.00	Nasal swab collection fee	500.00	Nasal swab travelling expenses from villages to MAHAN centre	150.00	Nasal swab travelling expenses from MAHAN centre to NIV Pune	300	NIV charges for RTPCR per sample	2000	Total expenses for one NPS	3750
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2.14 References	Satav A, Crow R, Potdar V, et al. The Burden of Respiratory Syncytial Virus in Children Under 2 Years of Age in a Rural Community in Maharashtra, India. <i>Clinical Infectious Diseases</i>. 2021;73(Supplement 3):S238-S247.														

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2.15 Publication Plan	We plan to publish at a minimum four papers from this study 1. The burden of RSV in rural India (covering specific objectives 1 a,b,c) 2. The burden of hMPV, SARS CoV2 and influenza in rural India and its relation to RSV (covering specific objectives 2 a,b,c) 3. The pathway of care from community to first level facility (PHC) or hospital and severity of LRTI for RSV, SARS CoV2, hMPV and influenza. 4. Risk factors for the development of severe or very severe LRTI for RSV, hMPV, SARS CoV2 and influenza. Abstracts will be presented at International and local meetings 1. RSV meeting 2. ID Week and or WSPID 3. PEDICON (Pediatric meeting India), APICON
2.16 Plan for Policy Translation	To ensure the study's findings translate into tangible improvements in public health, a proactive dissemination and policy translation plan will be implemented. • Continuous Stakeholder Engagement: ○ From the project's inception, the MAHAN team will establish and maintain regular interactions with key officials in the government health system, academicians, institutes and relevant policymakers.

- A stakeholder advisory group, including public health officials, will be formed to provide input and stay updated on project progress and outcomes. This fosters a sense of ownership and rapport.
- **Evidence-Based Advocacy:**
 - The study's data on the causes of morbidity and mortality will be used to create compelling arguments for policy changes aimed at reducing the burden of respiratory diseases.
 - We will conduct targeted advocacy meetings with policymakers to present findings and discuss their implications for public health planning, resource allocation, and preventative strategies.
- **Targeted Dissemination of Findings:**
 - **Policy Briefs:** Concise, actionable policy briefs will be created to summarize key findings and provide clear, evidence-based recommendations for policymakers.
 - **Academic Publications:** Results will be published in high-impact national and international medical journals to contribute to the global scientific knowledge base.
 - **Conferences and Meetings:** Findings will be presented at national medical conferences (e.g., PEDICON, APICON), government health meetings, and international forums to reach a wide range of clinical and policy audiences.
 - We will also put public interest litigation to courts for policy implementation.
- **Public and Community-Level Communication:**
 - Simplified summaries, infographics, and reports in local language will be created and shared with community leaders and participants to ensure the local community understands the research outcomes and their importance.
 - We will engage with local media to disseminate key findings to the broader public, raising awareness about the burden of respiratory illnesses.
 - Both of the above activities will act as pressure group over government to form government policies for RSV and generate demand.

MAHAN has experience of last 20 years of translating research into policies. We could successfully modify >30 state level policies to reduce childhood malnutrition and deaths in Maharashtra.

2.17 Local Stakeholder Engagement	Strategy for Engaging Local Stakeholders To maximize the study's relevance, impact, and long-term sustainability, this plan outlines a dedicated strategy for the early and continuous engagement of local health authorities and policymakers. 1. Initial Partnership Building and Alignment • Objective: To establish a formal collaboration with key health system players before the study launch, ensuring alignment with local health priorities from the outset. • Actions: ○ We will conduct formal meetings with District Health Officers (DHO), ADHO, Taluka medical Officers (TMO), and local representatives from the state's Department of Health and Family Welfare and ICDS. ○ We will present the study's objectives, methodology, and expected outcomes, clearly linking them to existing public health goals and challenges in the region. ○ We will formalize the partnership through a Memorandum of Understanding (MoU) or a letter of support to solidify the collaborative relationship. 2. Formation of a Stakeholder Advisory Group • Objective: To create a formal body for ongoing strategic guidance, ensuring the research remains relevant and responsive to local needs and operational realities. • Actions: ○ We will invite key stakeholders to form an advisory group. Members will include local health officials, influential policymakers, and respected community leaders. ○ We will schedule quarterly meetings to review project progress, discuss implementation challenges, and solicit input on the interpretation of findings. ○ We will utilize this group as a forum to co-develop strategies for translating research findings into practical, locally-acceptable health interventions. 3. Regular and Transparent Communication • Objective: To build trust and maintain engagement by ensuring a consistent flow of information to all relevant government and health system partners.

	• Actions: ○ We will distribute concise, quarterly progress reports summarizing key activities, preliminary findings, and any challenges encountered. ○ We will establish a clear point of contact within the MAHAN team for all stakeholder communications. ○ We will try to organize semi-annual review meetings to present more detailed updates and facilitate in-depth discussions with the broader group of health officials. 4. Integration with Existing Health Systems • Objective: To enhance the study's impact and demonstrate its value by embedding its activities within existing government health programs. • Actions: ○ We will work with local health authorities to align the study's surveillance activities with the work of ASHA workers and other community health initiatives, and hospital counsellors. ○ We will share data on disease burden and trends directly with district health teams to aid in their resource planning and outbreak response efforts. ○ We will try to explore pathways for the long-term integration of successful study protocols (e.g., simplified LRTI screening) into the routine functions of the primary health system.
2.18 Challenges	1) Logistical Challenges: Access to Melghat is complicated, the scarcity of transport facilities, poor road conditions, heavy rains that isolate villages during the rainy seasons, and the lack of communication systems make the transportation of NPS from villages to the laboratories difficult. Furthermore, the irregular electricity supply hampers storage of the samples for prolonged periods. • Mitigation Strategy: ○ Use of Prime Store MTM: All NP samples will be collected in Prime Store Molecular Transport Medium, which stabilizes viral RNA at room temperature, reducing dependence on cold chains. ○ VHWs will use own motorbikes or supervisors will use MAHAN's motorbikes to bring the NPS from villages to MAHAN hospital if bus service is not available. ○ Local Refrigeration Hub: Samples will be temporarily stored in 2-4 °C refrigerators at MAHAN Hospital, which has solar-

	powered backup to maintain cold storage during power failures. ○ Batch Transport: We will send consolidated periodic (15-30 days a month) shipments of NPS in ice packs to the National Institute of Virology (NIV), Pune, using reliable courier services or MAHAN's own vehicle/ by train or bus when needed. ○ Solar + Inverter Systems: We will use generator, solar panels and inverters in MAHAN Hospital to maintain refrigerator functionality. 1) Deaths that occurred outside the village, deaths that occurred late at night, funerals that were conducted in the early morning, and refusal by parents hamper sample collection. Mitigation: ○ Community Alert System: VHWs, ASHAs, and tribal leaders will be oriented and motivated to immediately report all deaths. ○ Family Rapport: We will improve our reputation and community participation and relationship with the family so that the family members will inform about the death at the earliest before funeral. MAHAN will enhance family engagement through consistent healthcare support and grief counselling, improving early reporting. Incentives: Small non-monetary incentives or social support (funeral assistance, health service vouchers) may be explored to encourage timely notification. 2) Cultural and Religious Challenges More than 84% of the population belongs to different tribal communities with different dialects and beliefs. More than 60% of the population is illiterate or semi-illiterate. In this context, communication regarding study procedures is challenging. Mitigation: To overcome these challenges, we trained our village healthcare workers and supervisors, established procedures for tracing deaths in the study area, educated the population, and established a bond of trust to ensure the rapid reporting of deaths. In addition, we provide grief counselling to parents and families. We will provide free treatment to all children aged <5 years and all youths and adults in age group of 16-60 years. • Culturally-Sensitive Communication: All health education material and consent processes will be translated into local dialects and explained orally.
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- **Tribal Health Workers:** VHWs are drawn from the same tribal communities and trained to communicate effectively within cultural contexts.

3) Community Staff Challenges

An important challenge for our study was the enrollment of qualified field staff. Qualified healthcare workers are reluctant to work in Melghat mainly due to the harsh terrain.

Mitigation: Hence, we have trained local tribal and often semiliterate youth to conduct the study procedures in the villages, including collection of NP samples.

- **Capacity Building of Local Youth:** We will continuously invest in training semi-literate tribal youth as VHWs and data collectors.
- **Multi-Sectoral Engagement:** We will try to leverage existing human resources—integrated child development schemes staff, accredited social health activists, auxiliary nurse midwives, traditional birth attendants, and traditional faith healers—for helping VHWs and follow-up.
- **Long-Term Career Pathways:** We will try to collaborate with public health institutions to offer accreditation, stipends, and future educational/career pathways for trained tribal workers.

We will also use our trained local tribal youth counsellors at all study hospitals for collection of NPS, grief counselling, and other study procedures.

4) Distrust and Reluctance to Participate

- **Problem:** Historical marginalization contributes to fear or scepticism of medical studies, particularly surrounding death and sample collection.
- **Mitigation Strategy:**
 - **Trust Building Through Services:** Free healthcare for children <5 and adults 16–60 years will continue to improve acceptance.
 - **Grief Counselling Services:** will be provided to bereaved families to build a compassionate connection and increase willingness to participate in post-mortem sample collection.
 - **Community Leadership Involvement:** We will engage Gram Sabha, village elders, and traditional healers as cultural gatekeepers to endorse the study.

2.19 Curriculum Vitae	<i>Investigator should upload dated curriculum vitae in English with a listing of references to Visiontracker at:</i> https://mrk.envisionpharma.com/ienv_mrk/visiontracker/portal/login.xhtml?pgm=ISR&windowId
2.20 Protocol Submission for Investigator-Initiated Studies	<i>U.S. protocols should be submitted by US investigators directly to Visiontracker at</i> https://mrk.envisionpharma.com/ienv_mrk/visiontracker/portal/login.xhtml?pgm=ISR&windowId <i>Non U.S. protocols should be submitted to the MSD office by the investigators.</i>