APPENDICES

APPENDIX I

Enrollment Consent Form

Consent form for the study of surveillance of certain newly described respiratory viral infections in peri-urban, rural and tribal communities in Vellore district in Tamilnadu in an area served by Sri Narayani Hospital and Research Centre (Indian Council of Medical Research, New Delhi)

Study Number:

ICMR sanction No.

Protocol Date:

Sponsor: Indian Council of Medical Research, New Delhi

Principal Investigator: Dr. Sathish Sankar

Enrollment visit for: Study on acute respiratory infection in children

Introduction: Respiratory infections are a major problem in children. It is estimated in children up to 5 yrs of age, there may be 4 to 6 attacks of respiratory tract infections every year. Repeated respiratory infections could cause failure to thrive and loss of school days for school-going children. Some children could become very sick requiring hospitalization. It is important to know the cause of these infections as appropriate therapy could be ascertained.

Purpose of the study:

Since there is very little information on viral respiratory infections in rural and peri-urban populations of India especially Tamilnadu, we would like to find out the role of the newly described respiratory viruses in acute respiratory infections among children.

Study procedure:

Throat swabs will be collected from the children with signs and symptoms of acute respiratory infections. This procedure will be painless and will be carried out by medical doctor and trained nurses. Detailed studies may also reveal whether any novel virus is circulating or whether the respiratory infection is related to the two viruses under study (hMPV/HBoV).

Possible risks:

Generally, when throat swabs are collected by trained professionals there is no risk perceived or anticipated for the child.
Benefits:

The child’s participation in this study will help us in assessing the problem of new respiratory viruses in the community and in this region. During our next visit we will explain the general findings to our community and may advise on some of the control measures to health officers who could then take appropriate steps to reduce the spread of these viruses in your region. If you personally need treatment / medication we will offer advice and consultations for referral to appropriate medical authorities or specialists.

New findings:

This study will give information for the first time on the role of new respiratory viruses in the community in causing childhood morbidity. Any significant information obtained during this study will be communicated to the scientific community and health authorities.

Confidentiality of the records:

The medical records will be kept confidential as required by the law. The records will be identified by hospital number and will not be released to any unauthorized persons. Consent form will have your name, address and signature. It will be kept separately under lock and key.

Compensation for participation:

No monitory compensation will be given for the participation in this study.

Study related questions:

If you have any study related questions you may contact:

Dr. Sathish Sankar Ph.D.
Division of Biomedical Research
Sri Narayani Hospital and Research Centre
Thirumalaikodi, Sripuram, Vellore 632 055
Tamilnadu, India
Phone: +91-0416-2271584/2271844/2271202
Fax no: +91-0416-2270099
Email: sathish3107@gmail.com

Right to participate

If you ward does not participate in this study, he/she will be given the same treatment.

Declaration:

I (Parent/Guardian) have read / the contents of this Consent form have been explained to me. I have understood all the terms and conditions and the information given about the child’s rights in relation to the enrollment procedure for this study. I volunteer on behalf of the child participation in this study.

The results of these tests may or may not be of benefit me or my ward directly. It has also explained to me that the collection of samples is not detrimental to health. If any
result is obtained from the study aiding the diagnosis of illness, appropriate treatment will be given.

Name of the volunteer/guardian

Date
Signature
Countersignature of the doctor
APPENDIX II

Clinical proforma for the study of surveillance of certain newly described respiratory viral infections in a peri-urban, rural and tribal communities in Vellore district in Tamil Nadu in an area served by Sri Narayani Hospital and Research Centre

Hospital No.
Name:                        Age:                        Sex:
Address:                    D.O.B:
Date examined by the doctor:
Respiratory rate ............
Abnormal chest x-ray Yes/No X-ray findings for LRI .................
Duration of hospitalization..............
Illness symptoms:
Fever: .............°F    Duration of fever:..............
LRI    /   URI
<table>
<thead>
<tr>
<th>Symptom</th>
<th>Yes/No</th>
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<tbody>
<tr>
<td>Rash:</td>
<td></td>
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<tr>
<td>Vomiting:</td>
<td></td>
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<td>Fever with exanthema:</td>
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<tr>
<td>Photophobia</td>
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<tr>
<td>Vesicular eruptions</td>
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<tr>
<td>Conjunctivitis</td>
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<td>Influenza illness in family contact</td>
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<tr>
<td>Febrile seizures</td>
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<tr>
<td>Coryza</td>
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<tr>
<td>Upper respiratory symptoms</td>
<td></td>
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<tr>
<td>Enlarged liver</td>
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<tr>
<td>Otitis media</td>
<td></td>
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<tr>
<td>Diarrhea</td>
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<td>Crepitations</td>
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<td>Wheezing</td>
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<td>Asthma exacerbation</td>
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<tr>
<td>Acute bronchiolitis</td>
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<tr>
<td>Pneumonia</td>
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<tr>
<td>Rhinitis</td>
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<td>Sore throat</td>
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<td>Malaise</td>
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<td>Lethargy</td>
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<td>Unproductive cough</td>
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<td>Sternal pain</td>
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<td>Poor air entry</td>
<td></td>
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<tr>
<td>Cough</td>
<td></td>
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<td>Sputum</td>
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<td>Dyspnea</td>
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<td>Vomiting</td>
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<tr>
<td>Breathlessness</td>
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<tr>
<td>Head ache</td>
<td></td>
</tr>
<tr>
<td>Body ache</td>
<td></td>
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</tbody>
</table>
Past history
Vaccination (Oral/Injection) ..............................................................
Similar illness .............................................................................
Recovery ......................................................................................
If yes, number of previous episodes in one year ..............
Family members/Village/Town affected: .................................

Research study investigation result:
............................................................................................

Signature of the physician
APPENDIX III

Lower limit of detection calculation for hMPV

No of bases in plasmid = 3931
No of bases in hMPV = 1185
Total no of bases = 5116

Wt of each base = 330 for ssDNA, 660 for dsDNA

Total molecular wt = 5116X660 = 3376560

Molecular wt of plasmid = 3376560/ (6.023X10^{23})
= 560611X10^{-23} g
= 5.606X10^{-18} g
= 5.606X10^{-12} µg

Spectrometric reading for hMPV (10^{-3} dilution) = 0.005

0.005X30X50 = 7.5µg/ml [30- Dilution factor; 50- constant for DNA, to obtain µg/ml]

= 7.5/ (5.606X10^{-12})
= 1.338X10^{12}/ml in 10^{3} dilution

Conc. of plasmid in 10^{-3} dilution = 1.338X10^{9}/µl

No of plasmid in 5µl (in 10^{-3} dilution) = 1.338X5 = 6.69 X10^{9}

Lower limit of detection (in 10^{-7} dilution) = 6.69X10^{5} plasmid copy/ reaction input.
**APPENDIX IV**

**Lower limit of detection calculation for HBoV**

No of bases in plasmid = 3000  
No of bases in HBoV = 5299  
Total no of bases = 8299  
Wt of each base = 330 for ssDNA, 660 for dsDNA  
Total molecular wt = 8299X660 = 5477340  
Molecular wt of plasmid = 5477340/ (6.023X10^{23})  
= 909403.9515X10^{23}  
= 9.094X10^{18}g  
= 9.094X10^{12}µg  

Spectrometric reading for HBoV (10^{-3} dilution) = 0.007  
0.007X30X50 = 10.5µg/ml  
= 10.5/(9.094X10^{12})  
= 1.154X10^{12}/ml in 10^{-3} dilution  

Conc. Of plasmid in 10^{-3} dilution = 1.154X10^9 plasmid/µl  
No of plasmid in 5µl (in 10^{-3} dilution) = 1.154X5 = 5.77x 10^9  
Lower limit of detection (in 10^{-8} dilution) = 5.77x10^3 plasmid copy/ reaction input.
APPENDIX V

Media and Reagents

Preparation of TBE buffer (10X):

Tris base (HiMedia, Mumbai, India) – 107.81g/L (0.89M)
Disodium EDTA (HiMedia, Mumbai, India) – 7.44g/L (0.02M)
Boric acid (HiMedia, Mumbai, India) – 55g/L (0.89M)

Mix the ingredients well in sterile distilled water and store at room temperature. To prepare the working concentration (1X), dilute the 10X stock solution and use.

Preparation of 0.5µg/ml of ethidium bromide

It was prepared from 10 µg/ml of the stock solution (Sigma, MO, USA). 2µL of the stock solution is added to 40ml of 2% agarose gel to get a final concentration of 0.5µg/ml of ethidium bromide.

Agarose gel preparation:

Agarose (Sigma, MO, USA) was weighed (2%) and dissolved in TBE buffer and melted in microwave oven. The molten agarose was cooled in running tap water to 40ºC before adding ethidium bromide, mixed well and poured in gel casting tray with comb. After solidification the comb was removed, the gel immersed in the gel electrophoresis unit (Tarsons, Mumbai, India) with power supply appropriately.
APPENDIX VI

Paper published based on the work carried out for the thesis
