Annexure 1

Buffers and Solutions

(a) 1X Phosphate buffer saline (PBS) pH 7.4

\[ \text{Na}_2\text{HPO}_4 \] 1.44 g  
\[ \text{KH}_2\text{HPO}_4 \] 0.24 g  
NaCl 8.00 g  
KCl 0.20 g  
dH\text{\textsubscript{2}}\text{O} (distilled water) 1000 ml  

The solution was autoclaved and stored at room temperature.

(b) Sodium bicarbonate (\text{NaHCO}_3) solution 7.5\% (w/v)

\[ \text{NaHCO}_3 \] 7.5 g  
Autoclaved dH\text{\textsubscript{2}}\text{O} 100 ml  

The solution was filter sterilized through 0.22 \text{\mu m} pore size filter and stored at 4\textdegree C.

(c) Glutamine solution 3\% (w/v)

\[ \text{L-glutamine} \] 3 g  
Autoclaved dH\text{\textsubscript{2}}\text{O} 100 ml  

The solution was filter sterilized through 0.22 \text{\mu m} pore size filter, aliquoted and stored at -20\textdegree C.

(d) 100X Antibiotic solution

1 vial of benzyl penicillin 10000 U/ml  
1g of streptomycin sulphate 10000 \text{\mu g}  
dH\text{\textsubscript{2}}\text{O} 100 ml  

The solution was filter sterilized through 0.22 \text{\mu m} pore size filter, aliquoted and stored at -20\textdegree C.

(e) DEPC (Diethyl pyrocarbonate) treated water

DEPC 0.1 ml  
dH\text{\textsubscript{2}}\text{O} 99.9 ml  

The solution was autoclaved and stored at 4\textdegree C.

(f) Heat inactivation of FBS

Sterile, mycoplasma free, virus tested commercially available FBS was inactivated at 56\textdegree C for 30 min in a water bath and stored at -20\textdegree C.

(g) Growth medium (GM)

DMEM (Dulbecco’s Modified Eagle medium) 85.4 ml  
\text{NaHCO}_3 \text{ solution} 2.6 ml  
Antibiotic solution 1 ml  
Glutamine solution 1 ml  
FBS 10 ml  

The solution was filter sterilized through 0.22 \text{\mu m} pore size filter and stored at 4\textdegree C. The medium was resupplemented with glutamine and antibiotics after every 15 days.
(h) **Viral transport medium (VTM)**

- Hank’s medium: 96.4 ml
- NaHCO₃ solution: 2.6 ml
- BSA: 1 g
- Antibiotics: 1 ml

The solution was filter sterilized through 0.22 µm pore size filter, dispensed in 2-3 ml aliquots and stored at 4°C.

(i) **TPCK-trypsin stock solution (0.1%)**

- TPCK-trypsin: 10 mg
- Autoclaved dH₂O: 10 ml

The solution was filter sterilized through 0.22 µm pore size filter, dispensed in 1 ml aliquots and stored at -20°C.

(j) **Virus growth medium (VGM)**

- DMEM: 95.4 ml
- NaHCO₃ solution: 2.6 ml
- Antibiotics: 1 ml
- Glutamine solution: 1 ml
- BSA: 0.6 g
- TPCK-trypsin solution (final concentration 0.5-1 µg/ml)

The solution was prepared fresh.

(k) **Freezing medium**

- Growth medium: 80 ml
- FBS: 10 ml
- DMSO: 10 ml

The solution was prepared fresh and used in ice-cold state.

(l) **Heat inactivation of Fetal bovine serum (FBS)**

Sterile, mycoplasma free, virus tested commercially available FBS was inactivated at 56°C for 30 min. in a water bath. Heat inactivated FBS stored at -20°C.

(m) **Alsever’s solution**

- Dextrose: 20.5 g
- Sodium citrate dihydrate: 8.0 g
- NaCl: 4.2 g
- Citric acid: 0.55 g

This solution was filter sterilized through 0.22 µm membrane filtration and stored at 4°C.

(n) **GT lysis buffer**

- Guanidine thiocyanate: 4 M
- Sarcosyl: 0.5% (v/v)
- 2-mercaptoethanol: 1% (v/v)
- Sodium citrate: 25 mM
- DEPC water: final volume to 100 ml
The solution was filter sterilized and stored at 4°C.

(o) **DEPC saturated phenol**

100 g of Phenol was dissolved in 100 ml DEPC water by incubating the solution at 50°C. The upper aqueous layer was aspirated and the solution was stored at 4°C.

(p) **Chloroform/isoamyl alcohol (49:1)**

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloroform</td>
<td>49 ml</td>
</tr>
<tr>
<td>Isoamyl alcohol</td>
<td>1 ml</td>
</tr>
</tbody>
</table>

The solution was saturated with equal amount of DEPC treated water and stored at 4°C.

(q) **50X TAE buffer (pH 8.2-8.4)**

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tris buffer</td>
<td>242 g</td>
</tr>
<tr>
<td>Glacial acetic acid</td>
<td>57.1 ml</td>
</tr>
<tr>
<td>EDTA (0.5 M)</td>
<td>100 ml</td>
</tr>
</tbody>
</table>

The total volume of the solution was made 1 litre with dH$_2$O and stored at room temperature.

(r) **Ethidium bromide (EtBr) 10mg/ml**

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>EtBr</td>
<td>50 mg</td>
</tr>
<tr>
<td>Milli-Q water</td>
<td>5 ml</td>
</tr>
</tbody>
</table>

The solution was stored at 4°C in dark conditions.

(s) **6X Gel loading buffer**

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bromophenol blue</td>
<td>25 mg</td>
</tr>
<tr>
<td>Xylene cyanol</td>
<td>25 mg</td>
</tr>
<tr>
<td>Ficoll Type 400</td>
<td>1.5 g</td>
</tr>
<tr>
<td>Milli-Q water</td>
<td>10 ml</td>
</tr>
</tbody>
</table>

The solution was stored at room temperature.
Nucleotide sequences of influenza isolates submitted to NCBI

> KY888297.1 Influenza A virus (A/Haryana/018/2015(H1N1)) segment 4 hemagglutinin (HA) gene, partial cds
TGGTAGCCCCATGTGATTTGGGTAATGTAACATTTGTGGCTGGATCCTGGGAATCCAGAGTGTGATCACATTGCTGCTCTACATTGGAAACATCTAATTCCAGACAATGGAAAGTGTTACCCAGGAGATTTCATCAATTATGAGGAGCTAAGAGCAATTGAGCTGCAACAAAGTCTCTATCAGAATGCAGATGTGCTTTTGTGGGGACATCAAGATACAGCAAGAAGTTCAAGCCGGAAATAGCAATAAGACCCCAAGTGGAGATCAAAGGGAGAATTGAACTATTACTGGGACAGTGAGCCCAGGAA

> KY888298.1 Influenza A virus (A/Haryana/019/2015(H1N1)) segment 4 hemagglutinin (HA) gene, partial cds
TGGTAGCCCCATGTGATTTGGGTAATGTAACATTTGTGGCTGGATCCTGGGAATCCAGAGTGTGATCACATTGCTGCTCTACATTGGAAACATCTAATTCCAGACAATGGAAAGTGTTACCCAGGAGATTTCATCAATTATGAGGAGCTAAGAGCAATTGAGCTGCAACAAAGTCTCTATCAGAATGCAGATGTGCTTTTGTGGGGACATCAAGATACAGCAAGAAGTTCAAGCCGGAAATAGCAATAAGACCCCAAGTGGAGATCAAAGGGAGAATTGAACTATTACTGGGACAGTGAGCCCAGGAA

> KY888299.1 Influenza A virus (A/Haryana/020/2015(H1N1)) segment 4 hemagglutinin (HA) gene, partial cds
TGGTAGCCCCATGTGATTTGGGTAATGTAACATTTGTGGCTGGATCCTGGGAATCCAGAGTGTGATCACATTGCTGCTCTACATTGGAAACATCTAATTCCAGACAATGGAAAGTGTTACCCAGGAGATTTCATCAATTATGAGGAGCTAAGAGCAATTGAGCTGCAACAAAGTCTCTATCAGAATGCAGATGTGCTTTTGTGGGGACATCAAGATACAGCAAGAAGTTCAAGCCGGAAATAGCAATAAGACCCCAAGTGGAGATCAAAGGGAGAATTGAACTATTACTGGGACAGTGAGCCCAGGAA

> KY888300.1 Influenza A virus (A/Haryana/024/2015(H1N1)) segment 4 hemagglutinin (HA) gene, partial cds
TGGTAGCCCCATGTGATTTGGGTAATGTAACATTTGTGGCTGGATCCTGGGAATCCAGAGTGTGATCACATTGCTGCTCTACATTGGAAACATCTAATTCCAGACAATGGAAAGTGTTACCCAGGAGATTTCATCAATTATGAGGAGCTAAGAGCAATTGAGCTGCAACAAAGTCTCTATCAGAATGCAGATGTGCTTTTGTGGGGACATCAAGATACAGCAAGAAGTTCAAGCCGGAAATAGCAATAAGACCCCAAGTGGAGATCAAAGGGAGAATTGAACTATTACTGGGACAGTGAGCCCAGGAA

> KY888301.1 Influenza A virus (A/Haryana/040/2015(H1N1)) segment 4 hemagglutinin (HA) gene, partial cds
TGGTAGCCCCATGTGATTTGGGTAATGTAACATTTGTGGCTGGATCCTGGGAATCCAGAGTGTGATCACATTGCTGCTCTACATTGGAAACATCTAATTCCAGACAATGGAAAGTGTTACCCAGGAGATTTCATCAATTATGAGGAGCTAAGAGCAATTGAGCTGCAACAAAGTCTCTATCAGAATGCAGATGTGCTTTTGTGGGGACATCAAGATACAGCAAGAAGTTCAAGCCGGAAATAGCAATAAGACCCCAAGTGGAGATCAAAGGGAGAATTGAACTATTACTGGGACAGTGAGCCCAGGAA
>KY888302.1 Influenza A virus (A/Haryana/043/2015(H1N1)) segment 4 hemagglutinin (HA) gene, partial cds

>KY888303.1 Influenza A virus (A/Haryana/015/2015(H3N2)) segment 4 hemagglutinin (HA) gene, partial cds

>KY888304.1 Influenza A virus (A/Haryana/016/2015(H3N2)) segment 4 hemagglutinin (HA) gene, partial cds

>KY888305.1 Influenza A virus (A/Haryana/029/2015(H3N2)) segment 4 hemagglutinin (HA) gene, partial cds
>KY888306.1 Influenza A virus (A/Haryana/032/2015(H3N2)) segment 4 hemagglutinin (HA) gene, partial cds

CAAGCCTACAGCAACTGTTACCCTATGATGTGCCGGATTATGCTCCCTTAGGTCACTAGTTG
CCTCATCCGGCACAAGTGAGTTAACAATGAAAGCTTCAATTGGGCTGGAGTCACTCAAAACG
GAACAAGTTCTGCTTGCATAAGGGGATCTAATAGTAGTGGTTCTTTAGTAGATGATGAT
CCACTCAAACCTCAAATACCCAGCAGTTGACTATGCAACAAACATTGAAACTTTAGACAA
ATTGTCACATTGAGGAGGTCAACCACCAGGTACGGACAGGACAAATCTTCCTCTTGCTCA
ATCATCAGGAAGAATCAAGTATCTATCCCAAATAGGAAAGCCCAAAGGTCGTAATCCGAGATATCG
GATCTAGAAACCAGATAGGAATACCTCTAGCAGAAATAAGCATCTATGGGACAAATTAGATGAAAA
CCGGGAGACATACTTTTGATTACAGCACAGGGGAATCTAAATTGGTCCTAGGGGTACTTCAAA
ATACGAAAGTGCGAAGGACTCAATAATGAGATCGAT
Annexure 3

CLINICAL PROFORMA

Rapid Detection and Typing of Influenza A and B viruses by Reverse Transcriptase- Loop Mediated Isothermal Amplification (RT-LAMP)

A) Clinical data Sheet

Date of collection:   

CR. No.   

Collected by   

Locality:   

Hosp/OPD/Filed:   

Age in Years:   Age in mo (< 1 year):   

Episode Number:   Sex M/F:   Post illness day:   

Specimen: Nasal Swab/Throat swab/Nasopharyngeal aspirate/other……………..

B) History of patient

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Yes</th>
<th>No</th>
<th>Duration</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Sudden symptoms &lt;12 hours</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>2. Fever</td>
<td></td>
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<tr>
<td>3. Chills and rigor</td>
<td></td>
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</tr>
<tr>
<td>4. Nasal Discharge</td>
<td></td>
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<tr>
<td>5. Ear Discharge</td>
<td></td>
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</tr>
<tr>
<td>6. Cough</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>7. Sore Throat</td>
<td></td>
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<tr>
<td>8. Breathlessness</td>
<td></td>
<td></td>
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<tr>
<td>9. Expectoration</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>10. Headache</td>
<td></td>
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<tr>
<td>11. Body ache</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>12. Fatigue</td>
<td></td>
<td></td>
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<tr>
<td>13. ARI in family in last 2 weeks</td>
<td></td>
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<tr>
<td>14. Concomitant illness</td>
<td></td>
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<tr>
<td>15. Vomiting</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>16. Diarrhea</td>
<td></td>
<td></td>
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<tr>
<td>17. Seizure</td>
<td></td>
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</tbody>
</table>
18. ILI in the family
19. Recent visit outside India
20. Influenza immunization

Temperature: [ ] [ ] Heart Rate: [ ] Respiratory Rate: [ ]

C) Physical Examination

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Yes</th>
<th>No</th>
<th>Duration</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Aural Discharge</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Nasal Discharge</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>3. Air entry</td>
<td></td>
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<tr>
<td>4. Crepitation</td>
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<tr>
<td>5. Wheezing</td>
<td></td>
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</tr>
<tr>
<td>6. Bronchial Breathing</td>
<td></td>
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</tbody>
</table>

D) Antibiotics Therapy: Yes/No/Unknown

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<thead>
<tr>
<th>Antibiotics group</th>
<th>Yes</th>
<th>No</th>
<th>Oral</th>
<th>Parenteral</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Amoxi/Clav</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Penicillin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Azithro/Erthromycin</td>
<td></td>
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<tr>
<td>4. Cephalosporin</td>
<td></td>
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</tr>
<tr>
<td>5. Levo/Gatifloxacin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. Aminoglycosides</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7. Unknown</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

E) Outcome within a week:

Improved [ ] Cured [ ] Not Improved [ ] Expired [ ]

F) Laboratory Data

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Virus Isolation</td>
<td></td>
</tr>
<tr>
<td>2. RT-PCR</td>
<td></td>
</tr>
<tr>
<td>3. Real Time RT-PCR</td>
<td></td>
</tr>
<tr>
<td>4. RT-LAMP</td>
<td></td>
</tr>
</tbody>
</table>

G) Signature:

a). Principal Investigator
   b). Co-investigator
PATIENT INFORMATION FORM

Title of Research: “Rapid Detection and Typing of Influenza A and B Viruses by Reverse Transcriptase- Loop Mediated Isothermal Amplification (RT-LAMP)”

Principal Investigator: Dr. Samander Kaushik, Assistant Professor, Centre for Biotechnology, M. D. University, Rohtak.

Name of Co-Investigator: Prof. Dhruba Chaudhry, Senior Professor and Head, Pulmonary & Critical Care Medicine, PGIMS, Rohtak.

Why this research study is being done?
Influenza viruses are the most important cause of winter time respiratory morbidity throughout the world. Rapid diagnosis is important not only for timely therapeutic intervention but also for the identification of a beginning of influenza outbreak. This study aims to develop a rapid, accurate, sensitive and cost effective diagnostic assay for influenza viruses.

Risks and Discomforts
Potential risks are minimal. The physical risks are related to the simple and routine procedure of collecting nasal and throat samples. Collection of the samples from the nose by aspiration or by swabbing may provoke coughing or sneezing. There a slight risk of mild injury to the lining of the nose that might result in nose-bleed. There will be no other complications or trauma to the children/adult from this study. We do not expect psychological, social, legal, or other risks. The information collected will be maintained confidentially.

Benefits
You will not personally benefit except the laboratory diagnosis of influenza virus from your participation in this research; however, your participation may provide valuable information to the medical community about the human influenza viruses as a cause of respiratory tract infections.

Alternatives
No treatment is being provided as part of this research. Your clinical care will be determined by your doctor based on the severity of the illness and usual standards of care. You have the alternative of not participating in the study. This will not alter your care by your doctor.

**Confidentiality**
All records will be kept confidential. These records will not be available to the public or any other person not connected with the project. The information gathered during this study will be kept confidential to the extent permitted by law. However, Ethics Committee will be able to inspect your records. The results of the treatment, including laboratory tests may be published for scientific purposes; however, your identity will not be revealed.

**Withdrawal without Prejudice**
You are free to withdraw your consent from this study. Your participation is voluntary and if you do not wish to participate can withdraw your name any time. There will be no penalty of any kind on you or your family. The withdrawal of you from study will not affect the treatment. For the minor consent of parent will be must before participation.

**Significant New Findings**
Any significant new findings that develop during the course of the study that may affect your willingness to continue in the research will be provided to you by Prof. Dhruva Chaudhry, PGIMS, Rohtak.

**Cost of Participation**
There will be no costs to you for participation in this study.

**Payment for Participation in Research**
There will be no payment for participation in this study.

**Questions**
If you have any questions about this project, have questions or desire for further information to research participant’s rights and please contact:
Dr. Samander Kaushik, Assistant Professor, Centre for Biotechnology, M.D. University, Rohtak.
Prof. Dhruva Chaudhry, Senior Professor and Head, Pulmonary & Critical Care Medicine, PGIMS, Rohtak.
Legal Rights
You are not waiving any of your legal rights by signing this consent form.

Storage of Specimens

Please initial your choice(s) below:

-----------------

I agree to allow my samples to be preserved for future research on infections.

I do not agree to allow my samples to be preserved for future research on infections.

I wish to be notified if my samples are going to be used for future research on infections.
CONSENT FORM

Title: Rapid Detection and Typing of Influenza A and B viruses by Reverse Transcriptase- Loop-Mediated Isothermal Amplification (RT-LAMP)

Principle investigator: Dr. Samander Kaushik, Assistant Professor, Centre for Biotechnology, Maharshi Dayanand University, Rohtak

Co investigator: Dr. Dhruva Chaudhry, Senior Professor and Head, Pulmonary & Critical Care Medicine, PGIMS, Rohtak

Patient identification number:____________________.

The contents of the information sheet that was provided have been read carefully by me/explained in detail to me, in a language that I comprehend, and I have fully understood the contents. I confirm that I have had the opportunity to ask questions. The nature and purpose of the study, its potential risks/ benefits, the expected duration of the study, and other relevant details of the study have been explained to me in detail. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, and without my medical care or legal right being affected.

I understand that the information collected about me from my participation in this research and sections of any of my medical notes may be looked at by responsible individuals from PGIMS, Rohtak or from regulatory authorities. I give permission for these individuals to have access to my records.

I agree to take part in the above study.

(Signature/ Left Thumb Impression)                                      Date:

Place:

Name of the participant:_____________________________________________

Son/ Daughter/Spouse of:_____________________________________________

Complete postal address: __________________________________________

________________________________________
This is to certify that the above consent has been obtained in my presence.

1) Witness-1

……………………………….
Signature
Name:
Address:

Signature of the Principal Investigator

Date: / Place:

2) Witness-2

……………………………….
Signature
Name:
Address:
Annexure 4

Research papers


Conferences/Workshops

1. National Conference cum Workshop on “Emerging Infectious Diseases” organized by Centre for Biotechnology, Maharshi Dayanand University Rohtak, Haryana, India on November 29, 2017.

2. International Conference on “Microbes for Health and Wealth” organized by Department of Microbiology, Maharshi Dayanand University Rohtak, Haryana, India on November 14, 2017.


4. Authors Workshop organized by Vivekananda Library in association with Elsevier at Maharshi Dayanand University Rohtak, Haryana, India on November 18, 2016.

5. National Conference on “Genetic Diversity and Therapeutic Potential of Natural Products” organized by Department of Genetics, Maharshi Dayanand University Rohtak, Haryana, India on September 17, 2016.
6. The 103rd Indian Science Congress held at University of Mysore, Mysuru, India from January 3-7, 2016.


8. International Research Colloquium on “Interdisciplinary Scope of Microbiology: Present and Future Directions” organized by Department of Microbiology, Maharshi Dayanand University Rohtak, Haryana, India on January 31, 2015.


12. International Workshop on “Epidemiology and Control of Influenza” organized by Asia Pacific Alliance for Control of Influenza (APACI) held at Vallabhbhai Patel Chest Institute, University of Delhi, New Delhi, India from November 7-8, 2014.


14. International Symposium on “Frontier Discoveries and innovative in Microbiology and its Interdisciplinary Relevance” 54th Annual Conference of Association of Microbiology of India (AMI), organized by Department of Microbiology, Maharishi Dayanand University, Rohtak-124001, Haryana, India from November 17-20 2013.

15. National Seminar on “Novel Antimicrobials: An Alternative to Clinical Antibiotics” organized by Department of Genetics, Maharshi Dayanand University Rohtak, Haryana, India on September 14, 2013.

16. International Research Colloquium on “Advances in Microbial Biotechnology: Future prospects” organized by Department of Microbiology and Association of Microbiologists of India-Rohtak Unit at Maharshi Dayanand University Rohtak, Haryana, India on November 20, 2012.

17. “VIRUS-12, Arboviral infections of Public Health Concern: Indian Scenario” one day CME organized by Department of Virology, Postgraduate Institute of Medical Education and Research, Chandigarh, India on September 3, 2012.