Acute Respiratory Infection (ARI) is one of the most severe problems for public health in South East Asia. Most of ARI are infections by Influenza virus, Respiratory Syncytial Virus (RSV), Parainfluenza virus (PIV), Rhinovirus, Enterovirus, human MetaPneumo virus (hMPV) and human Coronavirus. Although many techniques have been developed, such as Direct Immunofluorescence Assay (IFA), RT-PCR, cell culture and microarray, it is still a time and money consuming process to identify respiratory viruses in specimens from ARI patients.

The multiplex RT-PCR, developed by F.Freymuth et al. 2005, can detect 15 prevalent RNA respiratory viruses in 4 RT-PCR reactions with specific primers mix. Institut Pasteur of Shanghai (IPS) developed the 5th multiplex RT-PCR to identify Adenovirus (ADV) and human Bocavirus (hBoV), two prevalent DNA respiratory viruses (Table 1). This technique was validated by detecting 179 children respiratory specimens collected in Nanxiang Hospital, Shanghai, during October 2006 to March 2007. It is also applied in Institut Pasteur of Cambodia (IPC) by group of Dr. Philippe Buchy.

In February 2007, the 1st meeting of SISEA workshop decided to set up a regional respiratory virus surveillance network, and the technique of multiplex RT-PCR should be standardized and transferred to all members in the network. IPS would organize a training course for this technique.

In August 27-28, 2007, 10 researchers and technicians coming from Vietnam, Laos and IPC, participated in the training course in IPS (Annex 1, photo). The course took two days and contained two parts, lectures and practice course of multiplex RT-PCR. Pr.Vincent Deubel, Director of IPS, M.D. MSc.Wei Wang and MSc. Lili Hou (Unit of Emerging Viruses, IPS), and Dr. Mardy from IPC gave lectures. Wei WANG, Mardy SEK, Lili HOU and Peijun REN were supervisors for the practical course. (Annex 2, schedule)

The participants were divided into 4 groups. Each group practiced the whole program of Mutiplex RT-PCR, including viral RNA extraction by using QiAMP viral RNA minikit and QiaCube, auto nucleic extractor (Qiagen), preparation of reaction mix in hood, running Mutiplex RT-PCR (M1-M4) and electrophoresis, with a discussion of result interpretation in the end. We prepared RNA transcripts that were taken as positive controls of RT-PCR and cell supernatants from cells infected by PIV3, Influenza B, OC43 or Rhinovirus and distribute them to each group.

In this training course, the participants were familiarized with the whole protocol of multiplex RT-PCR, its Quality Control applying in IPS and the cautions about working in hood. Each group had good results in their experiments (Annex 3, Results). A warmly solidarity was observed in this course. All of the participants will deep the collaboration and exchange their experiences in multiplex RT-PCR application in future.
Acknowledgements
Agence Development of France financed the course.
Special thanks to Ms. CHEN Fang, IPS, for her kindly assistants in administration

Table 1, Viruses detected in Multiplex RT-PCR

<table>
<thead>
<tr>
<th></th>
<th>Viruses</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>Influenza A, Influenza B, RSV, HMPV</td>
</tr>
<tr>
<td>M2</td>
<td>PIV1, PIV2, PIV3, PIV4</td>
</tr>
<tr>
<td>M3</td>
<td>Influenza C, Enterovirus, Rhinovirus</td>
</tr>
<tr>
<td>M4</td>
<td>OC43, 229E, NL63, HKU-1</td>
</tr>
<tr>
<td>M5</td>
<td>ADV, hBOV</td>
</tr>
</tbody>
</table>

Annex 1. Group Photo
Annex 2

**2007 SISEA 1st network Training Course**

**Application of Multiplex RT-PCR in Respiratory Virus Diagnosis**

**Schedule**

- **1st day:**
  - 8:30-9:30: Introduction of Respiratory Virus
    - Prof, PhD. Vincent Deubel, IPS
  - 9:30-10:30: Introduction of Respiratory Infection
    - MD, MS. Wei Wang, IPS
  - 10:30-11:00: Coffee Break
  - 11:00-11:30: Introduction of Current Techniques used in Virus Diagnosis
    - MS. Lili Hou, IPS
  - 11:30-13:00: Lunch
  - 13:00-14:00: Practice Course Presentation
    - MD, MS. Wei Wang, IPS
  - 14:00-14:30: *Presentation of Qiacube*
    - Qiagen
  - 14:30-15:30: RNA Extraction
    - IPS, IPC
  - 15:30-17:30: Run of Multiplex RT-PCR
    - IPS, IPC
  - 17:30-18:30: *Multiplex Real-Time PCR in diagnosis (Resplex)*
    - Qiagen

*19:00* Welcome Dinner and visit of city

- **2nd day:**
  - 8:30-10:30: Run of Multiplex nested PCR
    - IPS, IPC
  - 10:30-11:00: Coffee Break
  - 11:00-11:30: Agarose Gel Preparation
    - IPS, IPC
  - 11:30-12:30: *Roche PCR Workflow System*
    - Roche Diagnosis
  - 12:30-13:30: Lunch
  - 13:30-14:00: Gel Loading and Electrophoresis
    - IPS, IPC
  - 14:00-15:00: Presentation of RNA Transcription in vitro
    - Dr. Mardy SEK, IPC
  - 15:00-15:30: Coffee Break
  - 15:30-17:30: Result Analysis and Discussion
    - IPS, IPC

*18:00* Dinner
Annex 3
Results of Group 1 and Group 2

Results of Group 3 and Group 4