MULTIPLEX RT-PCR DIAGNOSIS SHOWS HIGH PREVALENCE OF RESPIRATORY SYNCITIAL VIRUS AND BOCAVIRUS AND OF CO-INFECTIONS IN CHILDREN WITH COMMON COLD

WEI WANG1, JUN SHENG2, PEIJUN REN1, JING ZHANG1, XIN LIN1, LILI HOU1, YONGJIN WANG1, FRANÇOIS FREYMUTH3, PHILIPPE BUCHY4, VINCENT DEUBEL1
1Institut Pasteur of Shanghai, Chinese Academy of Sciences, Shanghai Institute of Biological Sciences, 2Shanghai Nanxiang Hospital, pediatric department; 3CHU Caen, Laboratoire de Virologie; 4Institut Pasteur du Cambodge, Phnom Penh, Cambodia

Introduction:

Several strategies have been developed for simultaneous testing of viruses causing acute respiratory infections (ARIs). In order to identify new virus genotypes responsible of ARI, we have developed a sensitive and rapid 5-tubes multiplex RT-PCR assay to identify and characterize 17 viruses, allowing a high throughput screening of respiratory viruses, by testing samples of ARI children out-patients from in Nanxiang Hospital in Shanghai, during the season 2006-2008.

Results:

1- Using five multiplex RT-PCR, based on previous published works [1, 2, 3, 4], we have diagnosed the presence of 17 respiratory viruses (Fig. 1 and Table 1) in nasopharyngeal samples. RNA transcripts of each of 15 RNA viruses and cloned DNA for GADPH, BOCA and ADV were used as external controls (Fig. 1).

2- A total of 490 nasopharyngeal swabs (59.2%) from 827 children experiencing ARI from October 2006 to October 2008 were positive, and co-infections were detected in 113 patients. The prevalence of known pathogens was in the order: RSV > BOCA > Rhino > INFB > INFA > PIV1 > HMPV > ADV > PIV3 > NL63 > Entero > 229E > PIV4 > HKU-1,OC43 (Fig.2). INFC and PIV2 were not detected in this study. Seasonal peaks of RSV and BOCA were observed.

3- Among 113 co-infections, 95 cases were infected by two viruses and 18 cases were infected by more than three viruses. Viruses were detected with the frequency order: BOCA(66) > RSV(48) > Rhino(26) > INFB(19) > PIV1(16) > HMPV(11) > INFA(8) > Entero, ADV, PIV3(6) > NL63(3) > 229E(20) > PIV4(1).
We found more BOCA in co-infection (66) than in single infection (54). Moreover, BOCA was usually co-infected with RSV which is the most prevalent virus in ARI.

4. The large nucleotide homology observed between viruses indicated that major subtypes were circulating, however rhinoviruses showed a large diversity of variants and new genotypes (see poster from Ting Huang et al.)

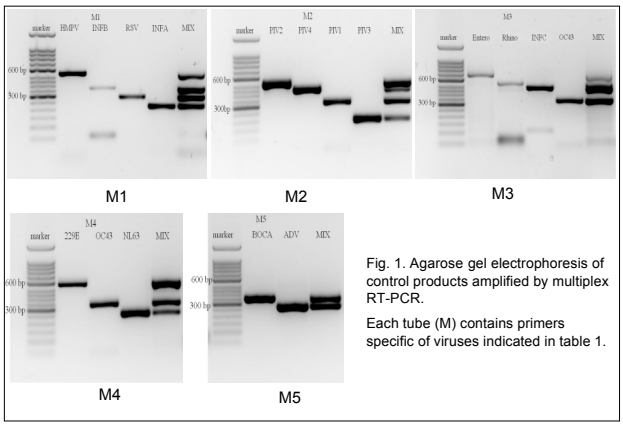
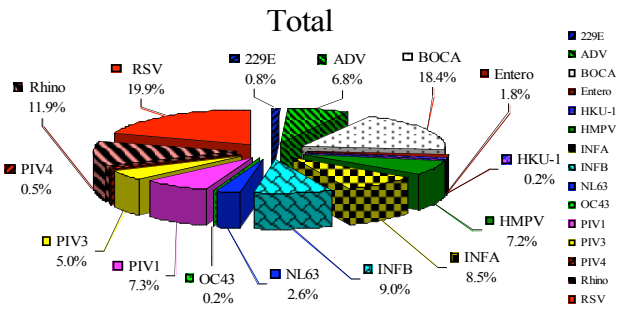


Fig. 1. Agarose gel electrophoresis of control products amplified by multiplex RT-PCR.
Each tube (M) contains primers specific of viruses indicated in table 1.

Fig.2 prevalence of 15 viruses detected in 827 samples



Conclusions:

➢ Multiplex RT-PCR successfully identified a large panel of viruses, using primers designed with consensus sequences, allowing a high throughput discriminating screening of known viruses. This technique can easily be implemented without requesting sophisticated facility.

➢Multiplex RT-PCR allowed to monitor seasonal circulation of viruses and to subsequently identify persistence or introduction of new genotypes in eastern China.

References

[1] Bellau-Pujol S, Vabret A, Legrand L, Dina J, Gouarin S, Petitjean-Lecherbonnier J, Pozzetto B, Ginevra C, Freymuth F. Development of three multiplex RT-PCR assays for the detection of 12 respiratory RNA viruses. J Virol Methods. 2005 Jun;126 (1-2):53-63

[2] Freymuth F, Vabret A, Cuvillon-Nimal D, Simon S, Dina J, Legrand L, Gouarin S, Petitjean J, Eckart P, Brouard J. Comparison of multiplex PCR assays and conventional techniques for the diagnostic of respiratory virus infections in children admitted to hospital with an acute respiratory illness. J Med Virol. 2006 Nov;78(11):1498-504.

[3] Casas I, Avellon A, Mosquera M, Jabado O, Echevarria JE, Campos RH, Rewers M, Perez-Breña P, Lipkin WI, Palacios G. Molecular identification of adenoviruses in clinical samples by analyzing a partial hexon genomic region. J Clin Microbiol. 2005 Dec;43(12):6176-82.

[4] Allander T, Tammi MT, Eriksson M, Bjerkner A, Tiveljung-Lindell A, Andersson B. Cloning of a human parvovirus by molecular screening of respiratory tract samples. Proc Natl Acad Sci U S A. 2005 Sep 6;102(36):12891-6. Epub , 2005 Aug 23.

Acknowledgments

This study is part of the RESPARI and SISEA project in the Asian-Pacific network of Pasteur Institutes and was supported by Li Kha-Shing Foundation and the Agence Française pour le Développement. Wei WANG is financed by AREVA Foundation.

中国科学院上海巴斯德研究所
INSTITUT PASTEUR OF SHANGHAI
CHINESE ACADEMY OF SCIENCES

RESPARI

SISEA
Surveillance and Investigation of Epidemic Situations in Southeast Asia