Diagnosis of dengue virus infection with IgA anti Dengue rapid tests

A. Hartono*, S.K. Sari

Clinical Pathology, School of Medicine, Surabaya, INA, Indonesia

Background: Dengue IgM, IgG Capture ELISAs and NS1 Ag ELISA have become the most widely used serological methods for dengue diagnosis until now. Previous studies reported a possible use of IgA antibodies for dengue virus as a new serologic marker to make dengue infection active. In the present study, the performance of IgA anti-dengue rapid test as a new marker of dengue infection was assessed.

Methods: In this study, sera were obtained from 70 dengue virus infection patients and 30 non dengue virus infection patients. Seventy dengue paired sera were collected twice, at the time of hospital admission (acute) and discharge (convalescent). All sera samples were characterized using dengue reference ELISAs (NS1 Ag, Dengue IgM and IgG capture ELISAs). All of the dengue and non dengue samples were evaluated by Dengue IgA Rapid Test.

Results: The results of IgA anti-dengue rapid test were compared with the corresponding dengue reference tests. The sensitivity and specificity of IgA anti-dengue rapid test respectively were 82.9% (95%CI:72.4-89.9), and 73.3%(95%CI: 55.6%-85.8%). Meanwhile, from acute sera, sensitivity of IgA anti-dengue rapid test was 83.3%(95%CI:64.5-93.7), higher than IgM (73.3%,95%CI:53.8-87.0), IgG (66.7%,95%CI:47.1-82.1) and NS1 Ag ELISAs (60%,95%CI:40.7-76.8). Based on the day of fever, sensitivity of IgA anti-dengue from day 1-2 was 66.7%, day 3-4 was 84.8%, day 5-7 was 76.2% respectively. Positive IgA anti-dengue rapid test results in acute sera was higher in secondary (84.6%) than primary infection (77.7%).

Conclusion: IgA anti-dengue rapid test can be considered as a new marker of dengue infection, because it gives a high sensitivity, especially in the acute phase and in the secondary infections as well.

http://dx.doi.org/10.1016/j.ijid.2012.05.522
this newly developed assay was validated by in vitro experiments in which ribavirin, a well-characterized flavivirus and alphavirus inhibitor, showed a dose-dependent inhibition of virus replication on cells that was assessed by viral infectivity and viral RNA production. The specificity and sensitivity of this diagnostic technology was successfully evaluated in patient samples. 

**Conclusion:** Our results demonstrate the potential of this newly developed one-step SYBR Green I-based RT-PCR assay may be a useful tool in rapid diagnosis of mosquito-borne flaviviruses and alphaviruses and monitoring the extent of viral replication possibly in patients’ samples.

http://dx.doi.org/10.1016/j.ijid.2012.05.524

**Type:** Poster Presentation

**Final Abstract Number:** 55.014  
**Session:** Diagnostics  
**Date:** Saturday, June 16, 2012  
**Time:** 12:45-14:15  
**Room:** Poster & Exhibition Area

**Presence and pattern of virulence genes in non-lactose fermenting *Escherichia coli* strains isolated from stools of children <5 years in rural and urban Bangladesh**  
A. Hossain  
ICDDR,B, Dhaka, Bangladesh

**Background:** *Escherichiae coli* (E. coli) encoding pathogenic genes are considered agent for infantile and childhood diarrhoea and determined by detecting genes from lactose fermenting (LF) colonies. Presence of virulent genes in non-lactose fermenting (NLF) *E. coli* be potential in determining those genes of ETEC, EPEC and EAEC when compared to LF *E. coli*.

**Methods:** Stools specimens from diarrhoeal children <5 years attending ICDDR, B hospital were processed for enteropathogens. The NLF *E. coli* confirmed by biochemical characterization including API 20E. Equal number of LF *E. coli* selected as controls. Strandrad Multiplex PCR was performed on both LF and NLF *E. coli* using defined primers (*bfp*a and *eae* genes for EPEC, *aat*A and *aai*C genes for EAEC and *est*A and *etB* for ETEC).

**Results:** Of 460 specimens, 74 strains (16%) were NLF of which 24 possess genes either of EPEC, EAEC or ETEC. 16 cases contain the *bfp*A and *eae* genes for EPEC, *aat*A and *aai*C genes for EAEC and *est*A and *etB* for ETEC. The presence of diarrhoeagenic genes is common in NLF *E. coli*. Specimens showing presence of NLF colonies but seronegative for *Salmonella* or *Shigella* (NLF) be further analyzed for virulence genes when no other pathogens are identified by culture, thus assist clinicians for management of childhood diarrhoea and help preventing development of EPEC and EAEC associated persistent infection being common in children. Further evaluation is suggested using a broader scale of samples.

http://dx.doi.org/10.1016/j.ijid.2012.05.525

**Type:** Poster Presentation

**Final Abstract Number:** 55.015  
**Session:** Diagnostics  
**Date:** Saturday, June 16, 2012  
**Time:** 12:45-14:15  
**Room:** Poster & Exhibition Area

**An in-house multiplex PCR for detection of *S. typhi* and *S. paratyphi* A: A comparison between detection method by agarose gel electrophoresis and lateral flow assay**  
N.A. Zulkiply, F.R. Sjafri, A. Ismail, A. Ismail  
Universiti Sains Malaysia, Kubang Kerian, Kelantan, Malaysia

**Background:** Typhoid and paratyphoid still remain as public health problem in developing and underdeveloped countries caused by Salmonella Typhi and Salmonella Paratyphi. Culture method is the gold standard to diagnose typhoid and paratyphoid fever as well as carriers. However, the method is less sensitive and produces results within 2-7 days. Thus, the study aims to develop the multiplex PCR for simultaneous detection of S.Typhi, S.Paratyphi A and Salmonella genus in presence of internal amplification control (IAC) with detection method via agarose gel electrophoresis and compare with lateral flow assay.

**Methods:** PCR was optimized with annealing temperature ranging between 55–75°C. Other parameters such as concentration of labeled primers, MgCl2, dNTPs and Taq polymerase were also optimized and the detection was performed via 2% agarose gel electrophoresis. For lateral flow assay, the nitrocellulose membrane was dotted with protein, anti-FITC, anti-Cy5, anti-Dig and anti-DNP and assembled with conjugate pad. The analytical sensitivity was carried out by agarose gel electrophoresis and lateral flow assay. Each amplicon was applied onto the dotted dipstick and the intensity of the dots was observed in 15 min. The test was validated with stool samples spiked with 25S. Typhi, 25 S. Paratyphi A and 25 otherSalmonella serovars and other bacteria. The test was also validated with nine stool samples of food handlers.

**Results:** The multiplex PCR successfully developed to detect *S. Typhi*, *S. Paratyphi A*, *Salmonella* genus and IAC without any cross-reaction. The detection limit for *S. Typhi* and *S. Paratyphi A* were 0.16 ng/ml and 0.08 ng/ml respectively using lateral flow assay compared 0.63 nl/ul for both *S. Typhi* and *S. Paratyphi A* using agarose gel electrophoresis. The test was successfully validated with spiked stool samples of 75 bacterial isolates and 9 stool samples of food handlers during typhoid outbreak with both sensitivity and specificity of 100%.

**Conclusion:** The findings suggest that the multiplex PCR-DNA dipstick is a potential assay as molecular diagnostics for detection of *S. Typhi* and *S. Paratyphi A*.

http://dx.doi.org/10.1016/j.ijid.2012.05.526