Evaluation of a New Anti-Dengue Virus IgM Particle Agglutination Kit in the Context of the Pacific Islands[¶]

A. Berlioz-Arthaud[*✉], M. Marfel[**], A.M. Durand[***] and T. Ogawa[†]*

[*]Institut Pasteur de Nouvelle-Calédonie, B.P. 61, 98845 Nouméa, New Caledonia
[**]Department of Health Services, Yap State Hospital, Yap, Federated States of Micronesia, 96943
[***]Department of Health Services, Yap, Federated States of Micronesia, 96943
[†]Pentax Corporation, 2-36-9 Maeno-cho, Itabashi-ku, Tokyo 174-8639, Japan

Abstract

The objectives of this regional study were to evaluate a new dengue IgM particle agglutination (PA) test in terms of sensitivity and specificity to compare this kit to a widely used immunochromatographic strip test and to assess its operational handling in limited settings, such as those encountered in the Pacific insular region. The sensitivity and specificity were assessed using serum-banked sera from the Pasteur Institute in New Caledonia with a commercial microplate ELISA kit taken as the reference. The prospective field part of the study was performed in the Yap State Hospital during a DENV-1 outbreak. The particle agglutination test showed a sensitivity of 76.7% and a specificity of 95.2%. For the strip assay, those characteristics were 73.3% and 83.3% respectively. The use of the particle agglutination test in Yap confirmed its easy handling and suggested a higher sensitivity. This new particle agglutination test is useful in the Pacific islands because of higher sensitivity and specificity and operational flexibility in remote locations.

Keywords: IgM particulate agglutination test, dengue, sensitivity, specificity, Pacific islands.

Introduction

Dengue in the Pacific region occurs as occasional outbreaks of a limited duration, most likely due to the importation of the virus from outside the region or from dengue-affected neighbouring islands[1]. The circulation of DENV-1 virus was first isolated and detected in the Pacific region in mid-2000, which has continued until now. Starting in Palau, the outbreaks have been identified in French Polynesia, Cook Islands, Samoa, Vanuatu, Solomon Islands, Wallis and Futuna, Fiji and New Caledonia[2]. From July 2004, foci have been reported on PacNet, the Pacific Public Health Surveillance Network (PPHSN) e-mail list, again in Palau, and in the Federated States of Micronesia, particularly on the island of Yap.

The present study aimed at the evaluation of a newly-released dengue IgM particle

[¶] This work was initiated in the framework of the Pacific Public Health Surveillance Network (PPHSN), a regional initiative from the WHO Western Pacific Regional Office (WPRO) and the Secretariat of the Pacific Community (SPC), including most of the countries and territories of the Pacific insular region. This collaborative study involved two clinical laboratories, i.e. the Pasteur Institute (New Caledonia) and the Clinical Laboratory of Yap State Hospital (Federated States of Micronesia).

✉*aberlioz@pasteur.nc;* ☎ *(687) 27-02-85, Fax: (687) 27-33-90*

agglutination (PA) test, proposed by Pentax Corporation (Tokyo, Japan) in terms of sensitivity and specificity, to assess the handling of this kit in resource-constrained situations, and to compare it with the PanBio (Brisbane, Australia) immunochromatographic strip test (ICT).

The PA test is based on sensibilised hydroxyapatite beads, a technology already successfully used by Pentax for the serological diagnosis of Japanese encephalitis.[3-5]

The Pacific Public Health Surveillance Network (PPHSN) was created in December 1996 under the joint leadership of the Secretariat of the Pacific Community (SPC), a Pacific-based international development agency comprising 27 insular states of the region and the World Health Organization (WHO). The aim of the PPHSN is to improve public health surveillance in the Pacific islands in a sustainable manner. The PPHSN comprises three operational 'arms':

- **PacNet,** an e-mail listserver of more than 500 subscribers, mainly regional health professionals, for early warning of outbreaks and as a source of technical information and support.
- **EpiNet**, the response arm of the PPHSN, which links about 100 variously-skilled national health workers.
- **LabNet,** a regional public health laboratory network. Its purpose is to develop existing laboratories in the area and to promote technical assistance between states and territories. It comprises three levels of services: first-line facilities in direct contact with patients (national level or "L1"), a first sub-regional confirmation level ("L2"), and reference laboratories ("L3") within or at the periphery of the Pacific insular zone. The role of L2 laboratories is not only to offer confirmation services to their neighbouring countries but also to propose realistic testing strategies to be implemented in L1 sites for major epidemic-prone infectious diseases.[6] The study presented here is a collaborative project between two laboratories of this network, the Pasteur Institute in New Caledonia (PINC) and the state hospital in Yap (Federated States of Micronesia). It aims to evaluate a new rapid test for dengue serology that could be regionally recommended for use in other Pacific islands in the framework of the PPHSN.

Materials and Methods

Sensitivity and specificity assessment

Stored material in the serum bank of the Pasteur Institute, New Caledonia, was utilized for the study. Two groups of samples, originally having tested positive for dengue and other diseases that could represent differential diagnoses, were simultaneously analysed by the PA kit, the ICT and a commercial microplate ELISA assay, taken as the reference. For each assessed kit, the sensitivity and specificity and the positive and negative predictive values were calculated. The data were processed anonymously using the Excel 2000® (Microsoft) software.

Biological material used

The sensitivity panel included 43 sera from dengue-confirmed patients, comprising:

- Thirty-nine DENV-1 positive samples (2004, New Caledonia), including 13 early samples, negative for IgM but

positive for viral RNA, evidenced in an in-house PCR assay, according to Lanciotti et al[7]. and 26 late samples positive for IgM (5 weak positive and 21 positive);

- Two DENV-2 IgM positive samples (1998 DENV-2 outbreak, New Caledonia);
- Two DENV-3 IgM positive samples (1995 DENV-3 outbreak, New Caledonia).

The specificity was assessed using a panel of 42 sera that tested negative for dengue IgM with the reference method but that were reactive to diseases encountered in the Pacific island region, as follows:

- Two hepatitis A IgM positive sera
- Four hepatitis B HBs antigen positive sera
- Six hepatitis C total antibody positive sera
- One rubella IgM positive sera
- Two anti-*Cytomegalovirus* (CMV) IgM positive sera
- Five anti-*Toxoplasma gondii* IgM positive sera
- Five syphilis serology positive sera (simultaneously positive for TPHA and VDRL)
- Four HIV serology positive sera
- Three complement fixation antibody positive sera (picornavirus, influenza, herpes)
- Six sera testing positive for leptospirosis serology (MAT: microagglutination test, titre over 1/800)
- Four rheumatoid factor-positive sera (simultaneously positive for Waaler-Rose and Latex tests).

Reference method

Dengue IgM capture (PanBio, Brisbane, Australia)

This kit is a widely used conventional ELISA microplate test and its performances have already been assessed and published[8,9]. It is the one currently used in the Pasteur Institute, New Caledonia, and will be considered as the reference for the evaluation of the two rapid tests.

Evaluated kits

Dengue duo IgM and IgG rapid strip test (PanBio, Brisbane, Australia)

This is a single ready-to-use immuno-chromatographic qualitative test kit. The assay procedure is very simple and requires no specific lab equipment or training. The result is available in 15 minutes for strong positives or up to 30 minutes as some very weak results may require this duration to develop. A positive result appears as a red-coloured line (of varying intensity) of the reactive zones, corresponding either to IgG or to IgM against dengue viruses. A validation control component is also included in the kit[#].

Anti-dengue virus IgM detection PA kit (Pentax Corporation, Tokyo, Japan)

The kit consists of an anti-human IgM-coated microplate and coloured hydroxyapatite beads coated with dengue virus. The assay takes less than two hours, including two incubation periods of 30 and 60 minutes, and requires only basic laboratory equipment. The final reading consists of a characteristic agglutination pattern for positive samples. The assay can be

[#]As the reference technique used in this assessment detects only the IgM, the reactivity of the IgG zone was not analysed in this study.

run in a quantitative mode using serial dilutions of the serum, and as the microplate is divided in removable strips, small batches can be processed. The kit includes both positive and negative internal controls.

In the present study, according to the manufacturer's instructions, the screening dilution for the qualitative protocol was 1/100 and positive samples were then quantified up to a 1/800 dilution, although the manual indicates a quantification up to a 1/12 800 dilution for positive samples.

Prospective field evaluation

The PA test has been used on daily requests in the clinical laboratory of Yap state hospital (Federated States of Micronesia) between July and October 2004, during a period of local circulation of DENV-1.

Prior to this study, this lab was not using any dengue test, and the aim was not only to evaluate the performances of the PA test in field conditions but also assess its handling in a peripheral site without previous experience in dengue testing.

The laboratory in Yap referred all the 53 dengue samples from this period to the Pasteur Institute in New Caledonia for confirmation, according to the recommendations made in the framework of the PPHSN. Batched samples were sent to the Pasteur Institute where those sera were tested for dengue IgM using the ELISA microplate test described above. Additionally, discrepant samples were assayed for dengue RNA by PCR.

Results

Sensitivity assessment

Among the 30 dengue IgM positive sera, 23 were correctly identified by the PA kit and 22 by using the ICT. Five samples were not identified by both rapid tests (Table 1). The false negatives were seen mostly in early samples (i.e. drawn 3–6 days after symptom onset) exhibiting a low reactivity: sample/cut off (S/Co) ratio with the reference microplate ELISA lower than 2, except for one that tested negative only with the ICT (S/Co=3.6, PA titre=1/800). Nineteen of the 23 sera that tested positive for the PA test exhibited a titre equal or higher than 1/800, whereas one of them was positive only at the screening dilution (1/100). The PA titre was usually consistent with the S/Co: for example all the samples with titres higher than 1/800 had a ratio of over 2.8 (range: 2.88–7.56) (Table 1).

Finally, none of the 13 DENV-1 PCR positive sera but negative for IgM with the study reference test gave a positive result, neither with the PA kit nor with the ICT.

Therefore, the calculated sensitivities are 76.7% for the PA test and 73.3% for the ICT when compared to the reference microplate IgM-ELISA.

Specificity assessment

Among the samples positive for diseases other than dengue, two false positive reactivities were identified with the PA kit and seven with the ICT; therefore, the respective specificities were 95.2% and 83.3% (Table 1).

The two false positive samples with the PA test gave a weak result, as only the screening dilution (1/100) was found to be positive in one HIV and one CMV-IgM-positive serum. The ICT was reactive with two among four HBs antigen positive sera, with one positive for syphilis serology and the four samples containing a rheumatoid factor (Table 2).

Table 1. *Specificity, sensitivity and positive and negative predictive values of the tests assessed for dengue fever IgM, in comparison to the New Caledonia Pasteur Institute reference technique (conventional microplate immunocapture – ELISA dengue IgM assay)*

Panel	Kit assessed			Reference method (Dengue IgM capture, PanBio)		Performances of evaluated kits			
	Name	Kit result[a]	Number	Positive	Negative	Sensitivity	PPV[b]	Specificity	NPV[c]
Sensitivity	Anti-dengue virus IgM detection PA kit, Pentax	Positive (≥1/100)	23	23	0	76.67%	100%	–	–
		Negative	20	7	13				
	Dengue duo IgM and IgG rapid strip test, PanBio	Positive (trace or +)	22	22	0	73.33%	100%	–	–
		Negative	21	8	13				
Specificity	Anti-dengue virus IgM detection PA kit, Pentax	Positive (≥1/100)	2	0	2	–	–	95.24%	100%
		Negative	40	0	40				
	Dengue duo IgM and IgG rapid strip test, PanBio	Positive (trace or +)	7	0	7	–	–	83.33%	100%
		Negative	35	0	35				
Total of the two groups	Anti-dengue virus IgM detection PA kit, Pentax	Positive (≥1/100)	25	23	2	76.67%	92.00%	96.36%	88.33%
		Negative	60	7	53				
	Dengue duo IgM and IgG rapid strip test, PanBio	Positive (trace or +)	29	22	7	73.33%	75.86%	87.27%	85.71%
		Negative	56	8	48				

[a] interpretation according to the manufacturer's instructions
[b] positive predictive value
[c] negative predictive value

Positive and negative predictive values

Among the total 85 samples tested, representing a population with an average dengue IgM prevalence of 35%, both rapid tests had close negative predictive values (superior to 85%), but the positive predictive value was found to be significantly better with the PA kit (92%) whereas it was only 75.9% with the ICT (Table 1).

Prospective study

The following results were obtained from the 53 samples included in the study and referred to the Pasteur Institute in New Caledonia for confirmation using the ELISA microplate test (EIA) described above and by PCR for some of them:

- 34 sera gave concordant results with both methods: 16 negatives and 18 positives.
- 19 discrepancies that were also tested by PCR in order to have more argument for discriminating between false and true positives. Those 19 samples split as follows:
  - Positive for PA/negative for EIA/DENV-1 PCR-positive: 6 (considered as PA true positives/EIA false negatives);

Table 2. *Specificity of the tests assessed for dengue fever serodiagnosis, in comparison to the New Caledonia Pasteur Institute reference technique, with regard to samples which tested positive for other diseases*

Composition of the group			Reference method		Kits evaluated			
			Dengue IgM capture, PanBio		Anti-dengue virus IgM detection PA kit, Pentax		Dengue duo IgM and IgG rapid strip test, PanBio	
Pathology	Parameter	Number	Positive	Negative	Positive	Specificity	Positive	Specificity
Hepatitis A	IgM	2	0	2	0	100%	0	100%
Hepatitis B	HBs Ag	4	0	4	0	100%	2	50%
Hepatitis C	Total Ab	6	0	6	0	100%	0	100%
Rubella	IgG+IgM	1	0	1	0	100%	0	100%
CMV	IgM	2	0	2	1	50%	0	100%
Toxoplasmosis	IgM	5	0	5	0	100%	0	100%
Syphilis	TPHA and VDRL	5	0	5	0	100%	1	80%
HIV	Total Ab	4	0	4	1	75%	0	100%
Influenza/Herpes/Pico[a]	CFR Ab[a]	3	0	3	0	100%	0	100%
Auto-immune disease	Rheumatoid factor	4	0	4	0	100%	4	0%
Leptospirosis	Total Ab	6	0	6	0	100%	0	100%
Total non-dengue pathologies		42	0	42	2	95.24%	7	83.33%

[a] antibodies to various viruses detected by complement fixation reaction

- Negative for PA/positive for EIA/DENV-1 PCR-positive: 5 (considered as PA false negatives/EIA true positives)
- DENV-1 PCR negative: 8, classified inconclusive as the absence of detection of viral RNA may be due to the delay of sampling after onset (four of them where drawn from day 5 or later) or to a possible degradation having occurred during storage or the overseas transfer.

The statistical interpretation of those results is, therefore, biased and not adequate for an absolute sensitivity and specificity estimation in field conditions. However, among the 11 PCR positive samples, considered as real dengue cases, six true results were obtained for the PA versus five for the PanBio EIA, which appears to be equivalent and indicates close performances of both IgM tests.

Discussion

Sensitivity assessment

The two field tests evaluated in the retrospective part of this study were found to have quite similar sensitivities – 76.7% (PA) and 73.3% (ICT). For the ICT, this finding is lower than that reported in recent evaluations, where the sensitivity is ranging from 79% to 100%[9,10,11]. None of the two tests was able to detect IgM earlier than the reference test as evidenced on the panel of PCR DENV-1-positive sera. But, in the prospective study, performed in Yap, it has been possible to detect samples simultaneously positive by PCR and with only one of the two IgM tests kits used: the PA and the ELISA microplate assay. Therefore, it is suggested that the two reagents have comparable sensitivities but may not exactly react in the same way on early samples. This is possibly due to different antigen

preparation used in the two kits. Those findings need to be confirmed in another prospective study of a larger scale and performed on a unique site in order to minimize the bias described above.

Finally, the four sera from DENV-2 and DENV-3 outbreaks were correctly identified, but were not numerous enough to ensure a fully satisfactory detection of those serotypes. Unfortunately, no serum from DENV-4-infected patient was available as this virus has not been circulating in the Pacific region for a long time.

Specificity assessment

We report a specificity of 83.3% for the ICT, comparable to the findings of recent studies, ranging from 81% to 95%[9,10,11,12,13]. The PA kit was found to have a significantly better specificity (95.2%). Additionally, it can be mentioned that with a cut-off dilution of 1/200 instead of 1/100, the specificity of the PA kit is 100%. This modification in the protocol results only in a limited drop of sensitivity, from 76.7% to 73.3%, equivalent to the ICT.

As already described by others[14], a major interference when using the ICT was seen with specimens containing the rheumatoid factor, whereas no cross-reactivity was detectable with the PA test. Poor results were also found with HBs antigen-positive samples with the ICT (50% specificity among the four sera included in the panel). With regard to the high prevalence of chronic hepatitis B in most parts of the Pacific region, this may be a frequent cause of false positives in field conditions when using this reagent for dengue testing. Therefore, further evaluation of this interference is needed.

The cross-reactivity of antibodies to non-dengue arboviruses was not assessed here and is usually reported as being important[15], but this may not be a major problem in the Pacific region as many of these arboviruses have not been described regionally (except a few and limited Ross river virus and Japanese encephalitis outbreaks)[16,17].

Positive and negative predictive values

In remote places where, for technical and economical reasons, only rapid tests can be run, those characteristics must be clearly known as overseas confirmation is usually not available in a timely manner with regard to the emergency public health response needed. In this study, we report for both field tests an acceptable negative predictive value (superior to 85%). This means that with a limited number of consecutive negative results in ill patients (i.e. 20 to 30, which is a realistic figure in islands with small populations) it is probably enough to rule out dengue as the causative agent of an emerging outbreak of acute febrile syndromes.

Conversely, the positive predictive value of the PA kit (92%) is, in our evaluation, significantly higher than for the ICT (75.9%) and could reach 100% for a cut-off value of 1/200. Therefore, the value of a single positive result, in the Pacific island field conditions (i.e. situation of the first locally imported case on a dengue-free island in the context of a regional outbreak) is better if obtained with the PA kit, especially if the quantitative assay shows an antibody titre of 1/200 or higher. Although the confirmatory testing for samples reacting with any rapid test is always recommended, it is especially needed when using the ICT as initial screening test.

Finally, because of its good performance, the Pentax dengue IgM PA kit evaluated in this

study is recommended for use in initial dengue case screening.

The accurate confirmation of the first dengue cases of an outbreak is particularly important in the Pacific insular context where resources are often limited and the herd immunity of the population is low. The very satisfactory positive predictive value of the PA test assessed in this study makes it a reliable tool for decision-makers in charge of the public health response, usually based on expensive vector control measures.

As shown by the prospective section of this study, performed in Yap, this test can be easily performed without special equipment and by staff that have neither an academic laboratory technician training nor previous experience in dengue testing. Those conditions are frequently seen in remote places such as in most of the Pacific islands, where lab technicians are usually trained "on the bench" and where dengue is not endemic but epidemic, thus making any locally sustained dengue testing activity difficult.

Acknowledgements

The rapid tests used in this study were donated by the manufacturers: Pentax Corporation (Japan) and PanBio (Australia). Thanks are due to Mr Haemesdael, Mrs Triballi and Mrs Rolly of the Pasteur Institute Virology Laboratory, for their excellent technical work.

References

[1] Kiedrzynski T, Souares Y and Stewart T. Dengue in the Pacific: an updated story. *Pacific Health Dialog*. 1998; 5: 129-136.

[2] Lepers C. *Dengue update* – 17 January 2003. Inform Action (PPHSN quarterly bulletin). 2003; 13: 12-13.

[3] Pandey B, Yamamoto A, Morita K, Kurosawa Y, Rai S, Adhikari S, Kandel P and Kurane I. Serodiagnosis of Japanese encephalitis among Nepalese patients by the particle agglutination assay. *Epidemiol Infect*. 2003; 131(2): 881-885.

[4] Yamamoto A, Nakayama M, Tashiro M, Ogawa T and Kurane I. Hydroxyapatite-coated nylon beads as a new reagent to develop a particle agglutination assay system for detecting Japanese encephalitis virus-specific human antibodies. *J Clin Virol*. 2000; 19(3): 195-204.

[5] Yamamoto A, Nakayama M, Kurosawa Y, Sugo K, Karasawa H, Ogawa T, Takasaki T and Kurane I. Development of a particle agglutination assay system for detecting Japanese encephalitis virus-specific human IgM, using hydroxyapatite-coated nylon beads. *J Virol Methods*. 2002; 104(2): 195-201.

[6] Vilayleck M. PPHSN launches its regional network of public health laboratories. *Inform'Action: PPHSN quarterly bulletin*. 2000; 7: 26-28.

[7] Lanciotti RS, Calisher CH, Gubler DJ, Chang GJ and Vorndam AV. Rapid detection and typing of dengue viruses from clinical samples by using reverse transcriptase-polymerase chain reaction. *J Clin Microbiol*. 1992; 30(3): 545-551.

[8] Sang T, Cuzzubbo A and Devine P. Evaluation of a commercial capture enzyme-linked immunosorbent assay for detection of immunoglobulin M and G antibodies produced during dengue infection. *Clin Diagn Lab Immunol*. 1998; 5(1): 7-10.

[9] Groen J, Koraka P, Velzing J, Copra C and Osterhaus A. Evaluation of six immunoassays for detection of dengue virus-specific immunoglobulin M and G Antibodies. *Clin Diagn Lab Immunol*. 2000; 7(6): 867-871.

[10] Kittigul L and Suankeow K. Use of a rapid immunochromatographic test for early diagnosis of dengue virus infection. *Eur J Clin Microbiol Infect Dis*. 2002; 21(3): 224-226.

[11] Vajpayee M, Singh UB, Seth P and Broor S. Comparative evaluation of various commercial assays for diagnosis of dengue fever. *Southeast Asian J Trop Med Public Health*. 2001; 32(3): 472-475.

[12] Porter KR, Widjaja S, Lohita HD, Hadiwijaya SH, Maroef CN, Suharyono W and Tan R. Evaluation of a commercially available immunoglobulin M capture enzyme-linked immunosorbent assay kit for diagnosing acute dengue infection. *Clin Diagn Lab Immunol*. 1999; 6(5): 741-744.

[13] Wu S, Paxton H, Hanson B, Kung C, Chen T, Rossi C, Vaughn D, Murphy G and Hayes C. Comparison of two rapid diagnostic assays for detection of immunoglobulin M antibodies to dengue virus. *Clin Diagn Lab Immunol*. 2000; 7(1): 106-110.

[14] Jelinek T, Wastlhuber J, Proll S, Schattenkirchner M and Loscher T. Influence of rheumatoid factor on the specificity of a rapid immunochromatographic test for diagnosing dengue infection. *Eur J Clin Microbiol Infect Dis*. 2000; 19(7): 555-556.

[15] Lam SK and Devine PL. Evaluation of capture ELISA and rapid immunochromatographic test for the determination of IgM and IgG antibodies produced during dengue infection. *Clinical and Diagnostic Virology*. 1998; 10(1): 75-81.

[16] Paul WS, Moore PS, Karabatsos N, Flood SP, Yamada S, Jackson T and Tsai TF. Outbreak of Japanese encephalitis on the island of Saipan, 1990. *J Infect Dis*. 1993; 167(5): 1053-1058.

[17] Sammels LM, Coelen RJ, Lindsay MD and Mackenzie JS. Geographic distribution and evolution of Ross River virus in Australia and the Pacific Islands. *Virology*. 1995; 212(1): 20-29.