Evaluation of reagents for the serological diagnosis of Dengue EPINET II Workshop (Noumea, March, 2002)

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1. INTRODUCTION

During the EPINET II Workshop in Noumea (March 2002), it was agreed that the most peripheral laboratories ('Level 1') of the Pacific Public Health Surveillance Network (PPHSN) should all have access to rapid Dengue diagnosis kits. Given the sub-optimal performances of these reagents, all positive test results, or even negative ones in suspicious epidemiological or clinical contexts, should subsequently be systematically confirmed by a Level 2 Laboratory, using a most reliable operating protocol (such as microplate ELISA) or reference techniques.

In order to set up a rapid and reliable supply system for Pacific Island states and territories, it seemed logical to use products made by the PanBio firm (Brisbane, Australia). Some of these have already undergone an international assessment process (Dengue Duo Rapid Strip [1]) and are already being distributed to some Pacific Island countries.

The following kits could be used for Dengue testing:

- Rapid single test for Level 1 laboratories:
  - Dengue Duo IgM and IgG Rapid Cassette Test, currently in its pre-release phase, PanBio reference DEN-25S (25 tests);
- Confirmation test for Level 2 laboratories (Guam, Nouméa, Papeete and Suva):
  - ELISA (Enzyme Linked Immuno Sorbent Assay) Dengue IgM Capture kit, PanBio reference DEN-200 (96 tests).

At the present stage of implementation of the EPINET II Workshop final technical recommendations, it was necessary for a Level 2 laboratory with enough experience in Dengue bioassays to use these reagents under real conditions for a limited period. Indeed, without attempting another complete assessment, it was important for a regional Level 2 laboratory to:

- Master handling of these products, in order to provide technical support, if necessary, to the other laboratories of the network;
- verify the stated performance in terms of sensitivity and specificity;

This task, restricted in scope, was performed at the New Caledonia Pasteur Institute (IPNC) in August 2002.

2. MATERIAL AND METHODS

2.1 Assessment principle

Using material stored in the IPNC serum bank, two groups of samples, originally having tested positive for Dengue and other diseases that could represent differential diagnoses, were simultaneously analysed by the rapid test and the PanBio microplate ELISA method, then again with the IPNC usual technique, i.e. the 'in-house' IgM test protocol using the MAC-ELISA immunocapture technique, according to Bundo [2] and Chungue [3], taken in reference in this study.
Each result obtained by an assessed kit was identified as a true positive (TP) or true negative (TN) if it was identical to that obtained using the reference technique; otherwise, it was considered as a false positive (FP) or a false negative (FN).

The total number (Σ) of each type of result obtained (ΣTP, ΣTN, ΣFP and ΣFN) made it possible to work out the following characteristics [4]):

– sensitivity (%):ΣTP x 100/ ΣTP + ΣFN
– specificity (%):Σ TN x 100/Σ TN +Σ FP
– positive predictive value (%):Σ TP x 100/Σ TP + ΣFP
– negative predictive value (%):ΣTN x 100/Σ TN +Σ FN

NB: Given the small number of samples used in the study panels, the statistical values of the above parameters were not calculated.

2.2 Biological material used

2.2.1 Sensitivity panel

17 positive sera, comprising:
- 10 serotype 1 positive samples (from 2002, including 8 samples from New Caledonia (with 3 sequential samples from the same patient), one sample from Vanuatu and one from Solomon Islands);
- 3 serotype 2 positive samples (1998-99, New Caledonia);
- 4 serotype 3 positive samples (1989, New Caledonia).

8 negative sera, comprising:
- 5 also negative for leptospirosis;
- 3 from clinically suspect patients from Vanuatu.

2.2.2 Specificity panel

This group comprised 25 sera that were reactive to diseases frequently encountered in the Pacific Islands region, as follows:
– 4 Hepatitis A IgM positive sera
– 2 Hepatitis B (anti-HBc) IgM positive sera
– 2 anti CMV IgM positive sera,
– 2 anti *Toxoplasma gondii* IgM positive sera
– 2 syphilis serology positive sera (simultaneously positive for TPHA and VDRL),
– 2 HIV serology positive sera (2 mixed ELISA HIV1/2),
– 2 influenza serology positive sera (with titre 1/40 or higher for Complement Fixation antibodies),
– 5 sera testing positive for leptospirosis serology (MAT: microagglutination test);
– 4 rheumatoid factor positive sera (simultaneously positive for Waaler-Rose and Latex tests).

2.3 Evaluated Products

- *Dengue Duo IgM and IgG Rapid Strip Test, PanBio reference DEN-25S*: single ready-to-use immunochromatographic test kit: results in 10 minutes. A positive result appears a coloured spot (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.
  NB: as the reference technique used in this assessment detects only the IgM, the reactivity of the IgG zone was not analysed in this study.
- *Dengue IgM Capture (PanBio reference DEN-200):* ELISA microplate test, ready to use, and taking approx. 2.5 hours.

3. RESULTS AND ANALYSIS

The general characteristics of the evaluated products are assembled in Table 1 and the quantitative analysis of the cross-reactions are shown in Table 1.

3.1 Dengue Duo Rapid Cassette test

Extremely simple to use and requiring no apparatus, the use of this reagent is possible in the most remote and under-equipped locations. It should, however, be mentioned that interpretation of the result is not always easy, in particular in case of low reactivity 10 minutes after starting the reaction. This fact prompted a computation of the assessment parameters (specificity, sensitivity, negative and positive predictive values) in two modes: by considering the low results (traces) either as positive or negative.

When low results were considered as positives, as recommended by the manufacturer, sensitivity was excellent (100% agreement with the reference technique), but the specificity was not acceptable: only 40% with the non-Dengue disease group (comparable to a population with a zero % prevalence for Dengue) and 62.5% with the 'sensitivity' group, comparable to a high prevalence population (overall specificity on both groups together: 45.5%). This way of reading the result, however, did lead to a maximum negative predictive value (NPV) of 100%, which is good for a screening reagent, the main purpose of which is to identify with certainty patients who are not ill and who will not undergo further testing.

The alternative interpretation, in which low results were considered as negatives, lead to better specificity (76%), to the detriment of the sensitivity and the NPV, which declined to 52.9% and 75.8% respectively.

The diseases giving the most frequent cross-reactions, not found with the reference technique, are auto-immune illnesses with presence of a rheumatoid factor (4/4), toxoplasmosis (2/2), syphilis (2/2) and leptospirosis (3/5).

3.2 Dengue IgM Capture microplate test

This reagent is easy to use since its components are delivered ready for use, except the antigen mixture, which comes in freeze-dried form (6 flasks per kit, which makes it possible to perform tests on small batches of 11 samples). The technique does, however, require some specific equipment: incubator, microplate washer and reader, precision pipettes: for that reason, it can only be used in an experienced laboratory by adequately trained staff.

The assay duration is optimised by an original protocol comprising the simultaneous parallel incubation of samples on the anti human IgM sensitised microplate and of conjugate with the antigen mixture. This operating procedure makes it possible to reduce the number of steps and, therefore, the time needed to obtain results. [5]

The validation and interpretation of the test results use positive, negative and cut-off controls. With the PanBio test it can be noted that there is more scope for differentiating between results than with the technique used as reference here. This is objectified by comparison of the means of the sample/threshold optical density ratios obtained with the 'sensitivity' group (25 samples): for 18 positives the mean ratio was 1.9 with the commercial kit and 1.3 with the 'in-house' reference technique, for the 7 negatives, these ratios were respectively 0.35 and 0.55.

All the documented positive samples were detected by the PanBio kit, whose sensitivity was therefore 100% for this group.

The only non-specific reactivity revealed concerned a sample that was highly positive for rheumatoid factor. In particular, no cross-reactivity was recorded with leptospirosis, a frequent differential diagnosis for Dengue in the Pacific, as confirmed by other studies [6,7,8]. In view of the lack of endemic agents in the Pacific Islands, the interference of other arboviruses was not studied in this project; however, the absence of cross-reactions with yellow fever vaccinations or Japanese encephalitis has already been published [8,9]. In complement, interference due to Ross River infection (a potentially epidemic arbovirus in the Western Pacific region) and malaria should be assessed.

The specificity was, therefore, assessed at 96% on the non-Dengue group and 97% for all the samples tested. The positive predictive value (PPV) of a result was 94.4%, so the use of this product for confirmation of the previous rapid test would appear to be recommended.

4. CONCLUSION

Because of its high sensitivity, comparable to that of the reference method used in this study, the Dengue Rapid Cassette Duo reagent is suitable for initial Dengue case screening. It can be implemented without special equipment and by staff who do not have any specialised laboratory technician training. It should be pointed out, however, that the result interpretation procedure must be strictly adhered to, both in terms of timing (10 minutes) and interpretation (any trace of colour must be considered as a positive result). If this is done, all those patients who are not infected with Dengue will be identified, but, because of the time taken for seroconversion, a negative result in a clinico-biological context suggestive of Dengue should lead to the examination of a later sample. However, the lack of specificity of the positive results (approx. 45%) makes confirmation by another method absolutely necessary and could currently slow the release process of this kit.

The Dengue IgM Capture microplate method offers the required characteristics for this role. In the assessment presented here, its performances emerged as identical to those given by the reference technique, but a higher power of discrimination between positive and negative samples was noted. In addition, it is simple to perform and within the scope of any adequately equipped laboratory.

Both products can be recommended to Level 1 and 2 laboratories in the Pacific Public Health Surveillance Network for the serological diagnosis of Dengue (Dengue Duo Rapid Cassette test is not yet available on the market at this stage).

5. REFERENCES

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Panel	Kit assessed			Reference method IgM Dengue MAC (a) ELISA		Performances of evaluated kits			
	Name	Kit Result	Number	Positive	Negative	Sensitivity	PPV (b)	Specificity	NVP (c)
Specificity	Dengue Duo Rapid Cassette (PanBio)	Positive (trace or +)	15	0	15	-	-	40%	-
		Negative	10	0	10				
	Dengue Duo Rapid Cassette (PanBio)	Positive (+)	6	0	6	-	-	76.0%	-
		Negative or trace	19	0	19				
	Dengue IgM Capture (PanBio)	Positive	1	0	1	-	-	96%	-
		Negative	24	0	24				
Sensitivity	Dengue Duo Rapid Cassette (PanBio)	Positive (trace or +)	20	17	3	100%	-	62.5%	-
		Negative	5	0	5				
	Dengue Duo Rapid Cassette (PanBio)	Positive (+)	11	9	2	52.9%	-	75%	-
		Negative or trace	14	8	6				
	Dengue IgM Capture (PanBio)	Positive	17	17	0	100%	-	100%	-
		Negative	8	0	8				
Total of the two groups	Dengue Duo Rapid Cassette (PanBio)	Positive (trace or +)	35	17	18	100.0%	48.6%	45.5%	100,0%
		Negative	15	0	15				
	Dengue Duo Rapid Cassette (PanBio)	Positive (+)	17	9	8	52.9%	52.9%	75.8%	75.8%
		Negative or trace	33	8	25				
	Dengue IgM Capture (PanBio)	Positive	18	17	1	100.0%	94.4%	97.0%	100.0%
		Negative	32	0	32				

(a) : M Antibody Capture, (b) : positive predictive value, (c) : negative predictive value

Table I: Specificity, sensitivity, positive and negative predictive values of the tests assessed for dengue fever serodiagnosis, in comparison to the New Caledonia Pasteur Institute reference technique (IgM Dengue MAC ELISA).

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Composition of the group :			Reference method (IgM MAC ELISA)		PanBio Kits evaluated					
					Dengue Duo Rapid Cassette				Dengue IgM Capture	
Pathology	Parameter	Number	Positive	Negative	Positive	Specificity	+ or trace	Specificity	Positive	Specificity
Hepatitis A	IgM	4	0	4	0	100.0%	1	75.0%	0	100.0%
Hepatitis B	HBc IgM	2	0	2	0	100.0%	1	50.0%	0	100.0%
CMV	IgM	2	0	2	0	100.0%	0	100.0%	0	100.0%
Toxoplasmosis	IgM	2	0	2	1	50.0%	2	0.0%	0	100.0%
Syphilis	TPHA & VDRL	2	0	2	0	100.0%	2	0.0%	0	100.0%
HIV	Total Ab	2	0	2	0	100.0%	1	50.0%	0	100.0%
Influenza	Total Ab	2	0	2	1	50.0%	1	50.0%	0	100.0%
Auto-immune disease	*Rheumatoid Factor	4	0	4	3	25.0%	4	0.0%	1	75.0%
Leptospirosis	Total Ab	5	0	5	1	80.0%	3	40.0%	0	100.0%
Total non-dengue pathologies		25	0	25	6	**76.0%**	15	**40.0%**	1	**96.0%**

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

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