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## Assessment of NS1 antigen detection tests during DEN-4 epidemic in French Polynesia

## Introduction



Rapid and specific dengue diagnosis is essential for both the clinical management of infected patients and the surveillance and containment of the disease.

During the disease's febrile phase, such confirmation is usually obtained through either viral isolation or reverse transcriptase polymerase chain reaction (or RT-PCR). However, these methods, which are costly and difficult to set up technically, are generally reserved for specialised laboratories. Since 2002, detection of the NS1 protein, a non-structural protein secreted by the dengue virus, has offered a new approach for diagnosis during the disease's acute phase (mainly the first five days) [1]. Several commercial NS1 antigen (NS1 Ag) detection kits using immunoenzyme, ELISA or immunochromatographic techniques (i.e. rapid tests) are now available, and most clinical laboratories can easily perform them.

These tests offer excellent specificity but their sensitivity is

thought to vary depending on the viral serotype [2-4] and the type of infection (primary vs secondary) [5, 6].

In French Polynesia, contrary to other transmission zones, dengue is spread in an endemo-

epidemic mode. The epidemics associated with the introduction of a new serotype are followed by a period of low viral circulation, with no sustained co-circulation of several serotypes. In this situation, tests with a dual diagnostic and surveillance function should be at once specific and sensitive, while remaining affordable and easy to use on a large scale.

The dengue 4 (DEN-4) epidemic that affected French Polynesia in 2009 gave us an opportunity to assess the performance of three commercial kits for this serotype (Biorad Platelia<sup>™</sup>, Dengue NS1 Ag ELISA, Panbio® Dengue Early ELISA – second generation and Standard Diagnostics Dengue NS1 Ag ELISA), using RT-PCR as our reference technique.

#### The study

Between February and April 2009, 181 consecutive patients presenting with acute-phase dengue-like syndrome, from the islands of Taha'a and Bora Bora

# Case definition of dengue-like syndrome

The clinical definition of a suspected case (dengue-like syndrome) requires at least the following simultaneous symptoms:

- High fever (≥ 38.5°C), of a sudden onset, for less than one week;
- Algic syndrome: headaches (retroorbital pain in particular), arthralgia or myalgia; and
- Absence of any symptoms suggestive of another infection (particularly respiratory or ear, nose, throat).

Source: French Polynesia Health Department's Health Watch Office.







(French Polynesia), were included in the study. Serum specimens were taken before day 6<sup>1</sup> of the disease.

NS1 antigen detection was done with the following commercial kits: Biorad Platelia<sup>™</sup> Dengue NS1 Ag ELISA 'NS1 Biorad', Panbio® Dengue Early ELISA (second generation 'NS1 Panbio') and Standard Diagnostics Dengue NS1 Ag ELISA 'NS1 SD', in compliance with the manufacturers' instructions. The viral RNA and viral serotype identification were performed on all the sera of the study by multiplex RT-PCR [7]. Lastly, IgM and IgG type antibody testing was done in every case (Panbio® Dengue Immocapture IgM and Panbio® Dengue Indirect IgG ELISAs). In our study, IgG type antibodies made it possible to gauge patients' immunological status regarding dengue (primary or secondary).

The average age of patients was 20 years (standard deviation (SD)  $\pm$  16) and the median age was 14 years (0–73 years). On average, the sample was taken at day 3 (SD  $\pm$  1). In total, 139 patients (76.8%) had anti-dengue IgG at the time the specimen was taken; 102 samples (56.4%) were positive for a DEN-4 virus by RT-PCR. No other type of dengue virus was identified, confirming the absence of viral co-circulation during the study period.

#### Results

The performances of the three kits, in terms of sensitivity and specificity for the detection of the NS1 antigen as regards DEN-4, are summarised in Table 1.

# Table 1. Sensitivity and specificity of NS1 antigen detection tests, based on results of RT-PCR (N=181).

	No. of positive tests	Sensitivity (%) [95% Cl*]	Specificity (%) [95% CI]
Platelia™ Dengue NS1 Ag ELISA	62	59.8	98.7
Biorad Laboratories		[52.7–67.0]	[97.1–100.0]
Panbio® Dengue Early ELISA (second generation) Inverness Medical Innovations	69	66.7 [59.8–73.5]	98.7 [97.1–100.0]
Dengue NS1 Ag ELISA	81	76.5	96.2
Standard Diagnostics		[70.3–83.7]	[93.4–99.0]

\*CI=confidence interval.

The specificity of the kits assessed in this study is high, between 96.2% and 98.7%, with no significant differences between the three kits (p = 0.44).

Five 'false positives' (i.e. RT-PCR negative / NS1 Ag positive) were found: three with the SD NS1 kit (sera taken at day 2, day 3 and day 5), one with the Biorad kit (day 2) and one with Panbio (day 3).

The sensitivity of the tests seems more variable with 59.8%, 66.7% and 76.5% for the NS1 Biorad, Panbio and SD kits, respectively. A significant difference is found between the Biorad and SD tests (p = 0.01).

<sup>&</sup>lt;sup>1</sup> 6<sup>th</sup> day following the onset of clinical signs of the disease, knowing that the onset of signs designates day 1 of the disease.







In general, while the sensitivity of the three kits seems lower in patients having anti-dengue IgG (57.3%, 65.2% and 73.3%, respectively) compared with patients in which these were not detected (75.0%, 75.0% and 84.6%, respectively) (Table 2), this difference is not significant in our study.

Table 2. Sensitivity of NS1 detection tests as related to IgG status	•
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	Sensitivity IgG + (n=138) [95% CI*]	Sensitivity IgG - (n=42) [95% Cl]
Platelia™ Dengue NS1 Ag ELISA	57.3	75.0
Biorad Laboratories	[49.0–65.6]	[64.2–89.7]
Panbio® Dengue Early ELISA	65.2	75.0
(second generation)	[57.2–73.1]	[64.2–89.7]
Inverness Medical Innovations		
Dengue NS1 Ag ELISA	73.3	84.6
Standard Diagnostics	[68.1–82.5]	[73.7–95.5]

\*CI=confidence interval.

## Conclusion

We have assessed the performance of three NS1 antigen diagnostic detection tests during the DEN-4 epidemic that affected French Polynesia in 2009.

Alongside specialised direct diagnosis methods such as viral isolation and RT-PCR, the detection of NS1 Ag has already shown its value in early dengue diagnosis. Despite an increasing number of publications, few data exist on the performance of NS1 Ag detection tests in the case of DEN-4 virus infections.

Performed on a sample of more than 100 confirmed DEN-4 virus infections, our study showed that, in the first five days of the disease, the ELISA kits currently on the market have comparable performances. We did, however, record a significant difference in sensitivity between the NS1 Biorad kit and the NS1 Standard Diagnostics kit. The latter seemed to yield better results than Biorad when compared with the reference technique (RT-PCR).

The single specimen taken from each patient did not make it possible to take a clear position on the cases of 'false positives' found with the three NS1 antigen diagnostic detection tests. These results reduce test specificity, although these could in fact be real cases of dengue not detected by RT-PCR. Some teams have, in fact, shown that this technique can lose sensitivity from day 4 and that NS1 Ag provided confirmation of positive patients for whom RT-PCR gave a false negative result [4, 8]. These 'false positives' could also be associated with flavivirus infections other than dengue.

Observation of diminished sensitivity in the presence of anti-dengue IgG is consistent with previous studies [4, 5]. We were unable to make any further progress with the classification of patients on the basis of infection status (primary versus secondary) but the presence of anti-dengue IgG, and in all probability of anti-NS1 IgG, produced following prior heterotypical infections, does seem to reduce the analytical performances of the kits. This being so, it is essential to gauge the immunisation rate of the population to be assessed in order to make an accurate interpretation of the results of NS1Ag testing.







If NS1 Ag detection used as the first-line method is negative, these results would seem to weigh in favour of continuing with dengue diagnosis, either by RNA testing in the early days of the disease, or by anti-dengue antibody testing, after day 5.

Our study shows in particular that the detection of the NS1 antigen, through these affordable and easily implemented ELISA tests, makes specific dengue diagnosis accessible to patients presenting early (before day 5) to first-line laboratories, without referring to a specialised laboratory (except for serotyping). Its exact place in the confirmation strategy will need to be specified in order to maximise diagnosis performance.

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