Implications of variable dengue diagnostic test performance

Importance of diagnostic tests in dengue control

Diagnostic testing in public health or reference laboratories is generally conducted to either aid in the clinical management of a patient or guide a population health response in a bid to contain a community-wide disease outbreak. In most cases, dengue diagnostic testing is conducted to aid in the clinical management of the dengue-infected patient. However, depending on whether the patient is located in a known dengue endemic or non-endemic area, the results of such testing may also significantly influence the public health response and vector control activities. Indeed, the public health importance of a positive dengue result in dengue non-endemic areas varies, depending on whether vector density (*Aedes aegypti*, *Aedes albopictus, Aedes polynesiensis*) is high enough to support sustained virus transmission and whether the geographical area favours dengue outbreaks. Sensitive yet reliable test results are particularly important in non-endemic dengue areas that are susceptible to dengue transmission and establishment. In these areas, positive results are likely to trigger a rapid population health response including contact tracing and deployment of vector control teams. The occurrence of repeated false-positive test notifications can result in unnecessary diversion of population health and environmental health staff from real public health issues, needless expenditure of scarce public health funds, frustration for the laboratory and a loss of enthusiasm for a rapid and vigorous dengue outbreak response – 'the boy who cried wolf' syndrome.

Diagnostic tests have to walk a very fine line, particularly those that are used to satisfy both clinical and public health needs. Ideally, tests should be sensitive enough to pick up every real disease case, while being specific enough not to produce false-positive results – something that is rarely if ever achievable in reality. Clearly, in the case of dengue, a diagnostic test that misses even a few dengue cases could result in a large-scale dengue outbreak, while a test that produces too many false-positive cases could result in unnecessary expenditure and a blunting of future population health responses.

It is important that no matter which test is used to support a diagnostic or public health response, laboratory, clinical and population health staff are familiar with the performance characteristics of the test. A test that fails to identify a number of true-positive cases needs to be 'accommodated' by supplemental 'fever surveillance' and population health approaches to ensure that alternative follow-up measures are in place (repeated sampling of epidemiologically and clinically suspect cases) to identify false-negative cases. Similarly, alternative strategies need to be implemented where a test produces a number of false-positive results. Strategies could include referral to a reference laboratory, collection of follow-up serum samples to monitor a rise or fall in antibody levels, or the use of a second test from a different supplier to test and confirm reactive samples.

Variable performance

Following a number of apparent false-positive dengue samples that were referred to us from Fiji and Samoa, we were approached by the WHO Regional Office in Suva, Fiji, to contribute to a joint posting on PacNet briefly detailing our experiences in Queensland, Australia, with the PanBio IgM dengue test that is used extensively in Australia and the Western Pacific.

Queensland situation

We carefully reviewed our data relating to PanBio tests conducted by QHPS (Queensland Health Pathology Services) laboratories and they are presented below. We receive a large number of samples from both public and private laboratories (the majority from private laboratories) for dengue confirmation, but we do not know which particular test is being performed by the private pathology laboratories nor their results. We presume they have all been reactive. Thus, results relating to samples submitted by private laboratories are not discussed further. However, since all Queensland Health public pathology laboratories are linked to us by a common laboratory information management system, we know what tests have been used and the results produced by those tests. All QHPS laboratories performing this testing (Cairns, Townsville and Royal Brisbane Hospital) are ISO certified and NATA accredited and we are confident that they are performing the

test according to the manufacturer's recommendations, although clearly we cannot guarantee this. Those data are presented below and refer specifically to samples tested on the PanBio IgM ELISA test.

Samples tested on the PanBio Igm ELISA test from 2002 to 2007

	2002	2003	2004	2005	2006	2007#
Submitted QHPS	1728	2401	2826	2878	2555	1537
Non-reactive QHPS	1674	2192	2523	2751	2367	1365
Reactive QHPS	38	153	172	95	119	130
Equivocal QHP	16	56	131	32	69	42
% Positive PanBio	2.2%	6.4%	6.1%	3.3%	4.7%	8.4%
Confirmed positive by QHSS	33	113	86	47	31	32
% Confirmed*	86.8%	73.9%	50%	49.5%	26.1%	24.6%
Dengue confirmed outbreaks / cases Qld	25	>300†	>550†	74	36	46

Year to July
*Percentage of PanBio reactive samples confirmed by QHSS
† Outbreaks in Cairns, Townsville and the Torres Strait continued from 2003 into 2004, with a total of 902 cases during this period

Our figures indicate that the number of false positives on the dengue PanBio test have been increasing over time and in our original PacNet posting we suggested that this apparent rise could be due to changes in manufacturing procedures.

There is a possibility that our in-house reference tests have lost sensitivity over time rather than that there has been a loss of specificity by the PanBio IgM, but this seems unlikely. All dengue-reactive results are notifiable in Queensland and all prompt a population health follow-up. If there is any suggestion that these patients represent a true dengue case (recent travel history, consistent clinical presentation) then they are retested (including molecular testing). When necessary, a follow-up sample may be requested.

It has been rightly suggested that the figures we originally presented in the PacNet posting might have been biased by the number of true dengue cases occurring in earlier years (higher predictive power during outbreaks than during inter-epidemic periods). We alluded to this possibility in our original posting. While it is true that the total number (in real terms) of PanBio reactive samples that were confirmed by QHSS in 2003 and 2004 was higher than in either 2002 or 2005, the percentage of confirmed positives has fallen over time. In 2002, a particularly quiet year for dengue in Queensland, there were only 25 confirmed cases yet that was the year that QHSS and PanBio had the greatest agreement, with 86.8% of PanBio IgM reactive samples confirmed as positive by our tests. In contrast, the percentage of confirmed PanBio IgM- reactive samples dropped to 50% in 2004 when we had over 500 confirmed dengue cases in Queensland.

Conclusion

Clearly, the apparent drop in the specificity or positive predictive value of the PanBio IgM ELISA that we have observed over the past six years cannot be explained simply in terms of a variation of dengue activity in Queensland during this period. The purpose of this paper is simply to alert readers of Inform'ACTION to our experiences with samples that have been referred to us for confirmation that initially tested positive in the PanBio IgM ELISA by the referring laboratory. This is not a laboratory comparison and we are unable to say whether the commercial test was performed according to the manufacturer's instructions. Nevertheless, our results and those of other reference laboratories in the region indicate that appropriate laboratory and public health approaches need to include strategies to accommodate the current performance of the PanBio IgM ELISA.

Greg Smith & Carmel Taylor
Public Health Virology Laboratory
Queensland Health Scientific Services

Word layout – Inform'ACTION is the bilingual bulletin of the PPHSN. http://www.spc.int/phs/PPHSN/
produced by the Secretariat of the Pacific Community (SPC), © 2007