



The need for regulation and standardisation of *in vitro* diagnostic (IVD) assays for the diagnosis of acute tropical infections: dengue as a case study

Introduction

A recent review of diagnostics for the developing world¹ stated that the characteristics of the ideal diagnostic test were:

- Affordable by those at risk of infection.
- Sensitive (few false-negatives).
- Specific (few false-positives).
- User-friendly (simple to perform and requiring minimal training).
- Rapid (to enable treatment at first visit) and robust (does not require refrigerated storage).
- Equipment-free.
- Delivered to those who need it.

The need for such tests has lead to the development of rapid *in vitro* diagnostic (IVD) assays for tropical infections such as dengue, tuberculosis, leptospirosis, melioidosis and malaria, with many based on immunochromatographic test (ICT) principles.

The rapid IVD market is largely unregulated, with the exception of the USA where IVDs require approval by the Food and Drug Administration (FDA); however, most assays can be ordered via the internet with little or no independent verification of the manufacturers' performance claims. Furthermore, there is a disturbing lack of standardisation in the way rapid IVDs are assessed, even in peer-reviewed studies. There is an urgent need for regulation of such assays by national testing authorities to verify their

**Stuart D Blacksell &
Nicholas PJ Day**

Centre for Clinical Vaccinology
and Tropical Medicine
Nuffield Department of Clinical Medicine
Churchill Hospital, University of Oxford
Oxford, UK
and
Wellcome Trust – Mahidol University
Oxford Tropical Medicine Programme
Faculty of Tropical Medicine
Mahidol University, 420/6 Rajvithi Road
Bangkok 10400 Thailand

claimed diagnostic performance, which, if lacking, could lead to widespread misdiagnosis of serious disease. To highlight this need, we have used the results of recent studies of rapid dengue IVD ICT tests as an example.

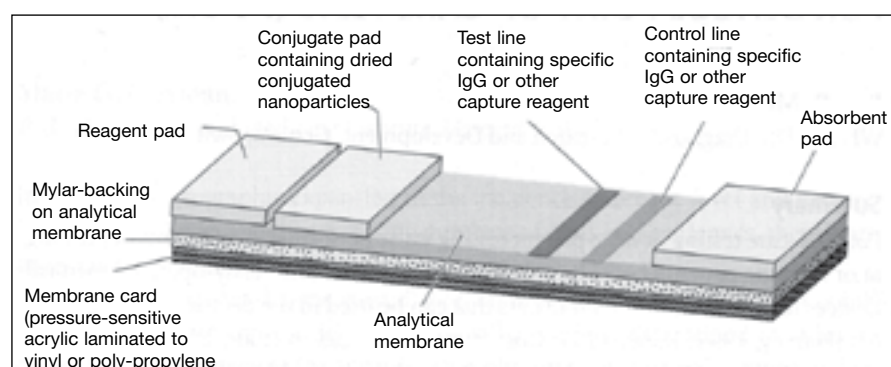
Dengue virus ICTs: the principle behind the technology

Most manufacturers of dengue virus rapid tests use ICTs either as a lateral flow (flat) or wick-style (upright in tube) format. Dengue virus antigen and colloidal gold

labelled anti-dengue virus monoclonal antibody are dried on a pad at the head of a nitrocellulose strip which is impregnated with both anti-human IgM and IgG antibody lines (Figure 1). Test sample (serum or plasma) and running buffer are added to the pad which activates the reagents and facilitates the migration of the reagents and sample by capillary action along the nitrocellulose strip towards the anti-human IgM and IgG antibody lines. The presence of dengue virus IgM and IgG is signified by the development of maroon lines in the location of the anti-human IgM and IgG antibody lines.

The dengue virus ICT assay has the advantage that it can be performed in approximately 10-30 minutes and requires no specialised equipment or training, making it ideal for low technology environments.

Figure 1. Lateral flow test strip construct (World Health Organization: reproduced with permission).





Methods of assessing diagnostic accuracy

Diagnostic assays are usually evaluated in terms of sensitivity and specificity calculated using a 2x2 cross-tabulation (Figure 2) where a 'gold standard' result (the peer-acknowledged, most accurate test) or reference standard result (normally, the test most widely used) is compared with the rapid test to determine diagnostic accuracy. A test that is 100% sensitive and specific is a perfect test.

Many peer-reviewed studies have attempted to determine dengue ICT diagnostic accuracy. However, variation in testing methodologies (i.e. use of different gold standard comparators and timing of patient samples) resulted in a wide range of accuracy estimates. A meta-analysis of previously published studies evaluated 11 ICT assays² and found that sensitivity ranged from 45-100% and specificity from 57-100%. This level of heterogeneity was significant and made it difficult to compare the accuracy estimates.

To improve the standardisation of diagnostic accuracy testing of IVDs, guidelines have been created by the Standards for Reporting of Diagnostic Accuracy (STARD) working group³ as well as listing the information that should be reported from diagnostic accuracy studies.

Figure 2. A 2x2 table used to derive True positive (Tp), True negative (Tn), False positive (Fp) and False negative (Fn) values to calculate IVD sensitivity ($Tp/(Tp+Fn)$) and Specificity ($Tn/(Fp+Tn)$).

		Reference assay result		
		Positive	Negative	
Rapid Test	Positive	Tp	Fp	(Tp + Fp)
	Negative	Fn	Tn	(Fn + Tn)
		(Tp + Fn)	(Fp + Tn)	(Tp + Fp + Fn + Tn)

Variation in diagnostic accuracy

Using standardised assessment parameters, a recent comparison of eight commercially available dengue ICT assays⁴ commissioned by the World Health Organization demonstrated generally poor diagnostic accuracy with sensitivities that ranged from 6-65% and specificities ranged from 69-100%. Of the eight tests, only two had sensitivities greater than 50%, considered to be clinically useful, and, of these, one had relatively low specificity (69%). A key component of this study was the use of a final dengue diagnosis using a combination of clinical, PCR and IgM/IgG ELISA results, accounting for the lower, but 'real life', sensitivity scores.

The results from this study differed markedly from the manufacturers' claimed performance characteristics and strongly suggested that most of the dengue IVDs assessed were unsuitable for the diagnosis of acute dengue virus infection.

Conclusions – Caveat emptor, free market and government regulation

The reality remains that *caveat emptor* must be exercised when selecting IVD for disease diagnosis. Selection criteria must be based on the results of independent diagnostic accuracy assessments rather than relying solely of the performance

characteristics provided by the manufacturer. As such, purchasing IVDs via the internet from storefronts with professional looking websites is fraught with potential problems (i.e. unknown IVD quality, repackaging of inferior products and temperature fluctuations during transport) and should be avoided where possible.

Regulations such as those in the USA require IVD assays to be approved by the FDA prior to legal sale⁵. A similar form of regulation of IVD assays in Australia is to be implemented by the Therapeutic Goods Administration (TGA) and will come into effect during 2006⁶.

Acknowledgements

Stuart Blacksell and Nick Day are funded by the Wellcome Trust of Great Britain.

References

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MICRO-FACT

Mosquitos are the world's most deadly insect. They transmit the microbe *Plasmodium falciparum* which causes malaria, a disease that kills one child every 30 seconds. They also transmit Dengue virus.