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Evaluation of Abbott Architect high-sensitivity troponin I assay for haemolysis interference

Sir,

Assays for cardiac troponin (cTn), the preferred cardiac biomarker,[1] have undergone improvements in analytical performance, resulting in the development of 'high-sensitivity' assays, able to measure cTn in a majority of healthy individuals.[2] The measurement of these low cTnI concentrations, however, may be more vulnerable to confounding by various interfering factors.[2] Sample haemolysis is one such factor, that has previously been reported to interfere with both cTnI and T assays, giving positive or negative bias depending on the assay platform (the level of haemolysis at which this bias occurs also varies between assays).[3–5]

One previous study, evaluating haemolysis interference on a new Abbott high-sensitivity cTnI assay, found minimal interference at free haemoglobin (Hb) levels up to 3.00 g/L, but only included samples with cTnI concentrations $\leq$72.9 ng/L.[5] As a changing cTn concentration is an important part of the diagnostic criteria for acute myocardial infarction (AMI),[1] we aimed to further evaluate the effect of haemolysis on this assay, including a broader cTnI concentration range.

Non-haemolysed plasma pools of varying cTnI concentrations were spiked with haemolysate as previously described.[6] The spiked samples were then assayed in triplicate using an Abbott STAT High Sensitive Troponin I assay (Abbott Architect ci8200 analyser; Abbott Diagnostics, USA). The laboratory decision levels for this assay are $\leq$16 ng/L in females and $\leq$34 ng/L in males and the limit of detection 1.9 ng/L. In-house QC data (using pooled plasma samples and Liquichek Cardiac Markers Plus Control LT material; Bio-Rad Laboratories, USA) showed assay CV of 9.9% (at 4.2 ng/L), 5.5% (10.8 ng/L), 4.7% (12.7 ng/L), 5.2% (21.3 ng/L), 3.3% (23.9 ng/L), 2.7% (74.0 ng/L), 2.0% (193.9 ng/L), 1.8% (14,991.6 ng/L). The free Hb concentration of the haemolysate was measured using a Sysmex XE-2100 analyser (Sysmex, Japan).

The sample groups (of varying free Hb concentrations) were assessed for overall significant difference using the Friedman (non-parametric) test and for difference from the unspiked group using multiple Wilcoxon signed rank tests (with Bonferroni correction; p value 0.0083 for significance). Individual results were also assessed for significant change, defined as exceeding a critical difference [CD; $2.77 \times \mathrm{SD}_{\mathrm{Analytical}}$ ($p = 0.05$); with SD derived from linear regression of QC data, excluding the highest QC concentration][5,7] and results were compared to proposed serial cTnI change criteria (>20% or $\geq$28 ng/L).[2,8] cTnI replicate outliers were excluded from the data analysis, and were defined as exceeding CD [$3.65 \times$ CV ($p = 0.01$); using CV at similar and lower, where available, QC concentration] from both other replicates. Statistical analysis was performed using software from SigmaPlot (Systat Software, USA), Excel (Microsoft, USA) and SAS 9.3 (SAS, USA).

The pooled plasma samples (cTnI range 2.3–1126.0 ng/L), after spiking with haemolysate (to free Hb 6.14 g/L) had cTnI recovery of 91.7–107.4% (Fig. 1).

Statistically significant difference was found between the sample groups overall ($p < 0.0001$) and for each of the spiked sample groups compared to the unspiked group (except the group with the lowest spiked free Hb and the free Hb 2.53 g/L group) (Table 1). Nine spiked samples had cTnI results exceeding CD (all giving lower values), six of which occurred in the two groups with the highest spiked free Hb concentrations (Table 1). None of the spiked samples had a >20% change but three samples had changes $\geq$28 ng/L (at cTnI concentrations 467.7 ng/L and 1126.0 ng/L) (Table 1).

Only one of the cTnI replicates was excluded as an outlier, occurring in the lowest cTnI pool at free Hb concentration 2.53 g/L (replicate results were 2.4 ng/L, 2.4 ng/L, 3.3 ng/L).

Our study found the Abbott high-sensitivity cTnI assay had minimal interference from haemolysis. This was supported by our data showing spiked samples (free Hb up to 6.14 g/L; cTnI concentration 2.3–1126.0 ng/L) giving a cTnI recovery of 91.7–107.4%, with cTnI decreases only exceeding CD in grossly haemolysed samples with free Hb $\geq$4.96 g/L (and in three samples at free Hb $\geq$1.28 g/L but with substantially elevated cTnI of 304.3 ng/L).

These findings are consistent with a previous report evaluating a high-sensitivity cTnI assay from the same manufacturer, at cTnI concentrations $\leq$72.9 ng/L, that found cTnI recovery of 91–108% at free Hb <3.0 g/L but in grossly haemolysed samples (free Hb 5.50 g/L), greater negative bias ($\leq$14% at cTnI $\leq$3.8 ng/L) and cTnI reductions exceeding CD ($2.77 \times$ SD) occurred.[5] Another study evaluating the same assay, reported minimal haemolysis interference (free Hb

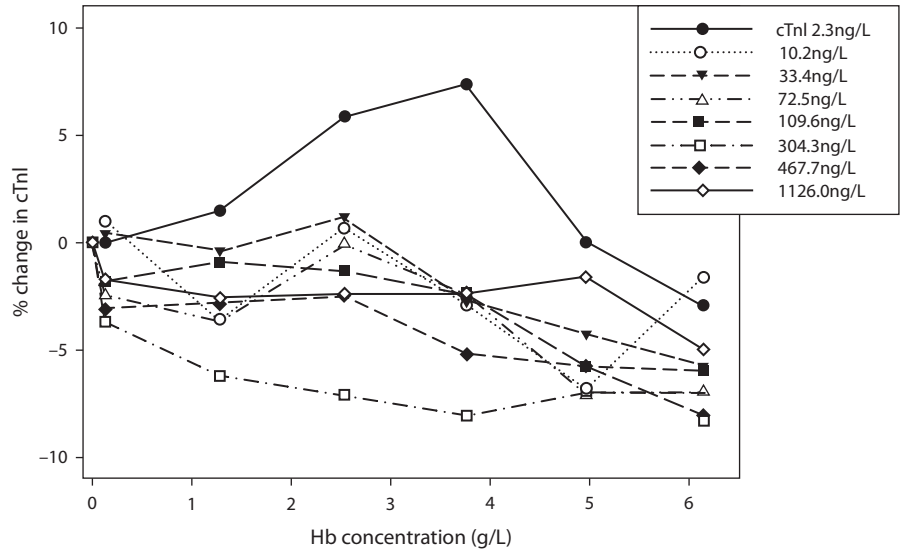

Fig. 1 % change from baseline in mean cTnI results (assayed in triplicate) of six non-haemolysed plasma pools (cTnI 2.3–1126.0 ng/L) that were spiked with haemolysate (up to free Hb concentration 6.14 g/L). cTnI, cardiac troponin I; Hb, haemoglobin.

Table 1 Mean cTnI results (assayed in triplicate) of six non-haemolysed plasma pools (cTnI 2.3–1126.0 ng/L) spiked with haemolysate (up to calculated free Hb concentration of 6.14 g/L)

Free Hb, g/L	Mean (SD) cTnI, ng/L							
0.0	2.3 (0.1)	10.2 (0.1)	33.4 (0.8)	72.5 (2.1)	109.6 (2.4)	304.3 (4.0)	467.7 (4.2)	1126.0 (34.7)
0.13	2.3 (0.2)	10.3 (0.6)	33.6 (1.7)	70.7 (3.4)	107.6 (5.9)	293.0 (6.1)	453.0 (2.0)	1106.3 (44.8)
1.28[a]	2.3 (0.1)	9.9 (0.6)	33.3 (1.3)	69.9 (0.5)	108.6 (2.6)	**285.3** (5.5)	454.3 (0.6)	1096.7[b] (4.2)
2.53	2.4 (0.0)	10.3 (0.5)	33.8 (1.7)	72.5 (1.8)	108.1 (3.1)	**282.7** (3.1)	456.0 (16.4)	1098.7 (23.5)
3.76[a]	2.4 (0.2)	9.9 (0.3)	32.5 (0.8)	70.8 (3.1)	107.0 (6.9)	**279.7** (6.1)	443.3 (4.0)	1099.0 (27.1)
4.96[a]	2.3 (0.4)	9.5 (0.3)	32.0 (0.3)	**67.4** (1.1)	103.3 (1.4)	**283.3** (12.7)	**440.7** (8.6)	1107.7 (51.6)
6.14[a]	2.2 (0.2)	10.1 (0.5)	31.5 (1.2)	**67.5** (1.8)	103.0 (6.6)	**279.0** (6.1)	**430.0**[b] (11.5)	1069.7[b] (46.3)

cTnI, cardiac troponin I; Hb, haemoglobin; SD, standard deviation.

Samples exceeding a critical difference ($2.77 \times SD_{Analytical;}\ p = 0.05$) are shown in bold font.

[a] Groups with statistically significant change in cTnI concentrations ($p \leq 0.0083$) from unspiked samples group.

[b] Spiked samples with ≥28 ng/L difference from unspiked concentration.

<8.60 g/L; with under −3% mean change in results), although their actual data were not published.[9]

Other cTn assays have previously been shown to have haemolysis interference, including a contemporary cTnI assay from Ortho Clinical Diagnostics that had >10% positive bias (at free Hb ≥0.43 g/L) and a contemporary and high-sensitivity cTnT assay from Roche, that both had >10% negative bias (at free Hb ≥1.32 g/L).[3]

According to the universal definition of acute myocardial infarction (AMI), an elevation of cTn to above the 99th percentile upper reference limit with a changing pattern on serial measurements, supports a diagnosis of AMI.[1] However, as the degree of haemolysis may differ between samples in serial measurements, assessing for cTn change may be less reliable when one of the samples is haemolysed (if using an assay subject to this interference) and the other is not.[3]

We also applied previously proposed serial cTnI change criteria (supporting AMI diagnosis) to our spiked samples[2,8] and found no cTnI results changed by >20%. Using another suggested (high-sensitivity) cTnI change criterion from a study of patients with recent onset chest pain ($n = 130$; ST-elevation AMI excluded; outcome measure acute coronary syndrome hospitalisation; Abbott Architect assay),[8] we found only three results changed by ≥28 ng/L but these all occurred at high cTnI concentrations ≥467.7 ng/L, where diagnostic uncertainty is unlikely.

Sample haemolysis is an important source of pre-analytical error and is common, particularly in the Emergency Department, where cTn testing is frequently performed (with reported haemolysis rates of 6.5–9.2% at free Hb ≥0.50 g/L).[10,11] Laboratories should routinely monitor samples for haemolysis and be aware of the potential effect of this interferent on their cTn assay.

In conclusion, the new Abbott high-sensitivity cTnI assay shows minimal interference due to haemolysis and this is not a confounding factor for clinical interpretation in most situations.

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Evaluation of the Dual Path Platform syphilis point of care test in North Queensland

Sir,

The rate of diagnosis of syphilis in North Queensland Aboriginal and Torres Straight Islander populations has continued to rise over the past 6 years, from 16.1% in 2009 to 36.4% in 2013.[1,2] In particular, the regions surrounding Mount Isa have been marked by ongoing syphilis outbreaks, with a total of 284 cases from January 2010 to March 2015. Of these cases, 99% occurred in patients of Indigenous origin, and 95% aged less than 35 years.[1] Resurgence of disease in rural and remote communities has been attributed to geographical and sociocultural constraints delaying disease presentation.[3]

The current algorithm for diagnosis of infectious syphilis in Pathology Queensland (Fig. 1) uses two treponemal tests [enyme immunoassay IgG (EIA IgG; Abbott, USA) and the *Treponema pallidum* particle agglutination test (TPPA; Fujirebio, Japan)], and one non-treponemal antibody test [rapid plasma reagin (RPR; BD, USA)]. Non-treponemal tests, while non-specific, become elevated with acute infection and subsequently increase the sensitivity of diagnosis. This allows for the detection of active disease. Treponemal tests are utilised in a confirmatory capacity.[4] Treponemal antibody tests remain elevated for life, and therefore do not always reflect active infection.[4,5] A positive TPPA test in conjunction with a positive RPR test is indicative of current infection with *T. pallidum*, while a positive TPPA test with a negative RPR test is indicative of inactive infection.[3–5]

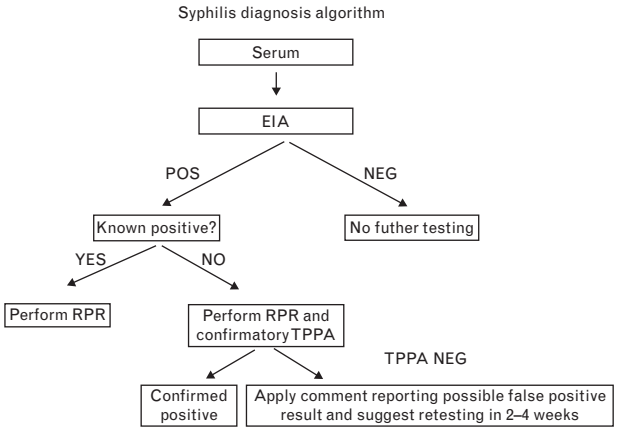

Fig. 1 Townsville laboratory syphilis diagnosis algorithm. EIA, enzyme immunoassay IgG (Abbott, USA); RPR, rapid plasma reagin (BD, USA); TPPA, *Treponema pallidum* particle agglutination test (Fujirebio, Japan).

A new point of care test (PoCT), the Dual Path Platform (DPP) Syphilis Screen and Confirm Assay (Chembio, USA) is based on the simultaneous detection of non-treponemal and treponemal antibodies. It improves on previous syphilis PoCT assays, with the additional ability to detect non-treponemal antibodies. This consequently allows for a differentiation between active infection and inactive disease, with the non-treponemal antibody decreasing or becoming absent in treated disease.[4]

Ethics approval was granted and a total of 449 stored frozen sera obtained during sexual health clinic screens over the last 12 months in North Queensland were tested with the DPP Syphilis PoCT. The DPP Syphilis PoCT consists of both a treponemal marker and non-treponemal marker (Fig. 2), in addition to a procedural control marker. The treponemal marker (line 1) detects IgG to *T. pallidum*, whilst the non-treponemal marker (line 2) detects IgM and IgG antibodies to lipoidal material and cardiolipin antibodies.[6,7] If the antibodies to syphilis (treponemal and non-treponemal) are present, then two pink/purple lines are produced, in addition to the control line, and signify active infection. The absence of treponemal and/or non-treponemal antibodies results in no pink/purple lines in the test area.[6] The test was performed according to instructions provided by the manufacturers, and interpreted by medical staff in the Townsville laboratory.

Comparison was made with the gold standard tests EIA, TPPA, and RPR. Known positive and negative results were de-identified to ensure interpretation was non-biased, and a spectrum of serum titres ranging from 2 to 256 were tested. Discrepant results were retested with the DPP Syphilis PoCT, but the result of the gold standard was accepted over the result of the DPP Syphilis PoCT. Results were then entered into a central database. Each specimen had been previously tested according to the standard testing regimen at the Townsville laboratory to provide a comparison of PoCT to gold standard.

Of the 449 specimens, 217 tested positive by the DPP Syphilis PoCT (non-treponemal line) compared to 227 by the comparator RPR test (Table 1). Analysis of these results