Supplementary material MidsMTvsDBS 01OCT20 v1.6

SUPPLEMENTARY MATERIAL

Appendix 1: Laboratory Methodology

All postal samples received in the laboratory were unpackaged, date-stamped, barcoded and booked onto the laboratory information management system (LIMS). At this stage any samples failing to meet the minimum testing requirements (e.g.: insufficient sample [<500µl for mini-tubes (MT), or fewer than 4 spots for dried blood spot (DBS)], no sample, no patient details, excessively haemolysed or leaking MT samples), were rejected and reported as such with relevant details. Prior to testing, MT samples were microcentrifuged for 10 minutes at 13,000 rpm and the supernatant transferred to a fresh barcoded 2ml tube. For DBS testing four 10mm discs, each containing at least 50µl of dried blood were required from each patient. The four discs were submerged in 1.5ml elution buffer (0.05% Tween®20 made up in phosphate-buffered saline) in a 25ml universal container and left overnight on a shaker at room temperature. The eluate was then centrifuged for 10 minutes at 10,000 rpm and the supernatant was transferred to a barcoded 2ml tube.

All samples were tested on an Abbott ARCHITECT serological analyser using the ARCHITECT HIV antigen/antibody (Ag/Ab) Combo assay for HIV and the ARCHITECHT Syphilis IgG/IgM assay for syphilis. The 'off-label' use of the ARCHITECT HIV Ag/Ab combo assay and the ARCHITECT Syphilis IgM/IgG assay for DBS samples was validated locally and the process has been accredited for routine use following a successful United Kingdom Accreditation Service [UKAS] inspection). Both DBS filter paper and MT were CE marked.

HIV:

The ARCHITECT HIV (Ag/Ab) Combo assay is a chemiluminescent microparticle immunoassay (CMIA) that allows the simultaneous qualitative detection of HIV p24 antigen and antibodies to HIV type 1 and/or type 2. Abbott claim an HIV-1 p24 antigen sensitivity of 0.87 IU/ml. This was confirmed during local verification of the assay using the WHO first international reference reagent (NIBSC code 90/636), where an average sensitivity of 0.78 IU/ml was achieved in serum samples. Due to the dilution of samples during DBS processing, the observed sensitivity for blood spots was reduced approximately 8-fold to 6.24 IU/ml. This reduction in sensitivity is unlikely to be of clinical significance for a screening assay in which HIV antibody levels would be expected to be high in sera and/or plasma. The validation process demonstrated quite a significant gap between the Architect values generated by clear positives and clear negative DBS samples. This implies that the apparent loss of DBS sensitivity demonstrated with the WHO controls does not necessarily have the same impact when looking at real populations of clinical samples.

Syphilis:

The ARCHITECT Syphilis (IgM/IgG) assay is a chemiluminescent microparticle immunoassay (CMIA) that allows for the qualitative detection of syphilis IgM and IgG antibodies to syphilis. During local verification of the assay, an average sensitivity of 0.025 IU/ml was achieved in serum samples (WHO first international reference reagent (NIBSC code 05/132)). Due to the dilution of samples during DBS processing, the observed sensitivity for blood spots was reduced approximately 8-fold to 0.2IU/ml. During the validation process, it was noted that at the lower end of the dilution series, yielded values were still distinguishable from negative results, despite being below the standard assay cut off (OD of 1 for Architect assays).

Sensitivity and specificity studies were conducted prior to this study to validate both DBS and MT assays, which were directly compared with known HIV-positive and syphilis antibody positive whole blood samples.

Authors: Page, Atabani, Arumainayagam, Wilson, Hartland, Taylor

Supplementary material MidsMTvsDBS 01OCT20 v1.6

All results were automatically downloaded into the LIMS and released to the Saving Lives server following relevant technical and clinical checks. Reactive postal HIV results were checked by repeat HIV testing at the local genitourinary clinic using a 4th generation (Ag/Ab) venous HIV blood test (Roche Cobas 6000 platform). Reactive postal syphilis results were checked by repeat syphilis testing at the local genitourinary clinic using venous syphilis blood tests (Abbott platform). In addition to IgM/IgG being tested, subsequent rapid plasma reagin (RPR) and Treponema pallidum particle agglutination assay (TPPA) tests were also performed to verify infection and to quantify disease activity (essential for differentiating between previously treated syphilis and re-infection).

Appendix 2: Information Governance

The Saving Lives STI self-sampling postal kit request system consists of a database housed on a secure N3-compatible server. A request module is called into a client clinic's website via a simple iFrame. Patients complete a demographic questionnaire which enables them to select an appropriate kit, and the database system generates unique identifiers which monitor both patient and sample through each stage of the system: request, kit despatch, sample receipt, laboratory analysis and resulting. Each stage of this process is information governance (IG) compatible, and is fully compliant with all current and pending requirements of the IG toolkit programme. All data is recorded in real time and stored within an N3-compliant environment, and can be anonymised for all necessary applications.

Appendix 3: Independently successfully processed samples for HIV and syphilis tests for mini-tube and dried blood spot

***MT:* Total HIV results 561, Total STS results 544.**
***DBS:* Total HIV results 2583, Total STS results 2579.**

Blood collection system	Successful HIV only sample processing & analysis/ blood sample return, n(%)	Successful STS only sample processing & analysis/ blood sample return, n(%)	Overall HIV only result obtained/ STI kits requested, n(%)	Overall STS only result obtained/ STI kits requested, n(%)	HIV RRR, n	STS RRR, n
MT	561/945 *(59.4)*	544/945 *(57.6)*	561/1515 *(37.0)*	544/1515 *(35.9)*	2.7	2.8
DBS	2583/2727 *(94.7)*	2579/2727 *(94.6)*	2583/4155 *(62.1)*	2579/4155 *(62.1)*	1.6	1.6
***p* value**	***<0.001***	***<0.001***	***<0.001***	***<0.001***	--	--
% difference[95%CI]	***35.3[32.1, 38.6]***	***37.0[33.7, 40.3]***	***25.0[21.6, 28.7]***	***26.2[22.6, 29.7]***		

p values in bold and italic typeface denote statistical significance
For proportion percentage differences with 95%CI; negative values favour MT, positive values favour DBS
DBS, dried blood spot; MT, mini-tube; RRR, request-to-result ratio; STI, sexually transmitted infection; STS, serological test for syphilis

Authors: Page, Atabani, Arumainayagam, Wilson, Hartland, Taylor

Supplementary material MidsMTvsDBS 01OCT20 v1.6

Appendix 4: Reasons why returned samples did not lead to a meaningful test result

Returned unprocessed samples: ***MT = 431, DBS = 149.***

Blood collection system	No request form, n(%)	No PID label on sample, n(%)	Insuff. sample, n(%)	Haemolysed sample, n(%)
MT	9/945 (1.0)	0/945 (0.0)	305/945 (32.3)	68/945 (7.2)
DBS	42/2727 (1.5)	43/2727 (1.6)	55/2727 (2.0)	0/2727 (0.0)
***p* value**	0.183	***<0.001***	***<0.001***	***<0.001***
% difference[95%CI]	*0.6[-0.2, 1.4]*	***1.6[n/a]***	***-30.3[-27.2, -33.3]***	***-7.2[n/a]***

p values in bold and italic typeface denote statistical significance
For proportion percentage differences with 95%CI; negative values favour MT, positive values favour DBS
DBS, dried blood spot; MT, mini-tube; PID, patient information details

Blood collection system	Equivocal (HIV), n(%)	Unknown reason, n(%)	Other reason, n (%)
MT	0/945 (0.0)	49/945 (5.2)	0/945 (0.0)
DBS	1/2727 (0.0)	6/2727 (0.2)	2/2727 (0.1)
***p* value**	1.000	***<0.001***	1.000
% difference[95%CI]	*0.04[n/a]*	***-5.0[3.5, 6.4]***	*0.07[n/a]*

p values in bold and italic typeface denote statistical significance
For proportion percentage differences with 95%CI; negative values favour MT, positive values favour DBS
DBS, dried blood spot; MT, mini-tube

Appendix 5a, 5b & 5c: Comparisons with similar study in North-West of England[10]

5a: Snapshot baseline characteristics (most populous only) - comparison between Midlands and North-West participants

Location	Sex n(%)	Median age, yrs (IQR)	Ethnicity n(%)	Sexuality n(%)
Midlands	*Female*		*White British*	*Heterosexual*
	3839/5670	*26*	*4191/5670*	*4959/5670*
	(67.7)	*(22-31)*	*(74.0)*	*(87.5)*
North-West	*Female*		*White British*	*Heterosexual*
	347/550	*26*	*495/550*	*471/550*
	(63.1)	*(22-31)*	*(90.0)*	*(85.6)*

IQR, interquartile range

5b: System process successes - cross comparison between Midlands and North-West Data (HIV data only)

Location	Blood collection system	STI kit req/ret, n(%)	Blood test ret/kit req, n(%)	HIV res/ bld ret, n(%)	HIV res/ STI req, n(%)	RRR
Midlands	***MT***	1072/1515 (70.8)	945/1072 (88.2)	561/945 (59.4)	561/1515 (37.0)	2.7
	DBS	3133/4155 (75.4)	2727/3133 (87.0)	2583/2727 (94.7)	2583/4155 (62.1)	1.6
North-West	***MT***	189/275 (68.7)	167/189 (88.4)	93/167 (55.7)	93/275 (33.8)	3.0
	DBS	183/275 (66.5)	164/183 (89.6)	162/164 (98.8)	162/275 (58.9)	1.7

DBS, dried blood spot; MT, mini-tube

Authors: Page, Atabani, Arumainayagam, Wilson, Hartland, Taylor

Supplementary material MidsMTvsDBS 01OCT20 v1.6

5c: False Positive results -cross-comparison between Midlands and North-West Data (HIV data only)

Location	Blood collection system	HIV reactive, n (%)	Confirmed reactive, n (%)	False Positive, n (%)
Midlands	***MT***	35/561 (6.1)	1/30* (3.3)	29/556 (5.2)
	DBS	13/2583 (0.5)	1/11* (9.1)	10/2582* (0.4)
North-West	***MT***	5/93 (5.4)	0/5 (0.0)	5/93 (5.4)
	DBS	0/162 (0.0)	0/0 (0.0)	0/0 (0.0)

DBS, dried blood spot; MT, mini-tube

*reductions due to no evidence of confirmatory test by service provider or GP

Authors: Page, Atabani, Arumainayagam, Wilson, Hartland, Taylor