

Revised HIV testing validation protocol

Background

Following the Pago Pago meeting on expanding HIV testing in the Pacific in 2008 (SPC, 2008), it was agreed to contract a laboratory to validate a rapid diagnostic test (RDT) based HIV testing algorithm which included confirmatory testing and which could be implemented in-country. The evaluation protocol was developed by the HIV Testing Task Force (HTTF; 2009) which is comprised of members of the regional development partners.

Phase I (completed): laboratory based assessment of potentially suitable tests and recommendation of an algorithm.

Phase II (not yet begun): small scale field pilot of suitable algorithm(s) identified in Phase I.

Phase III: broader implementation and ongoing monitoring.

A number of factors have contributed to significant delays in the implementation of phase II and there are a number of documented cases in which the absence of in-country confirmatory testing has directly contributed to a variety of poor health outcomes, including vertical transmission of HIV from mother to child. These issues are summarised in greater detail in the accompanying document “Fast tracking HIV RDT evaluation.pdf”.

To improve health outcomes in PICTs in-country confirmatory testing should be implemented as a matter of urgency. Accordingly, a revised protocol which combines phase II and phase III has been developed.

Phase 1 results

Results from the phase I validation work indicate that a combination of two tests, UniGold (Trinity Biotech) and Insti (Biolytical), run in parallel in the second test position of the algorithm will yield the highest sensitivity, specificity, positive predictive value and negative predictive value of the overall HIV testing algorithm. The recommended algorithm is screening with Determine, with all reactive specimens then tested with UniGold and Insti in parallel. The algorithm is shown in figure 1 below and described in greater detail in table 1.

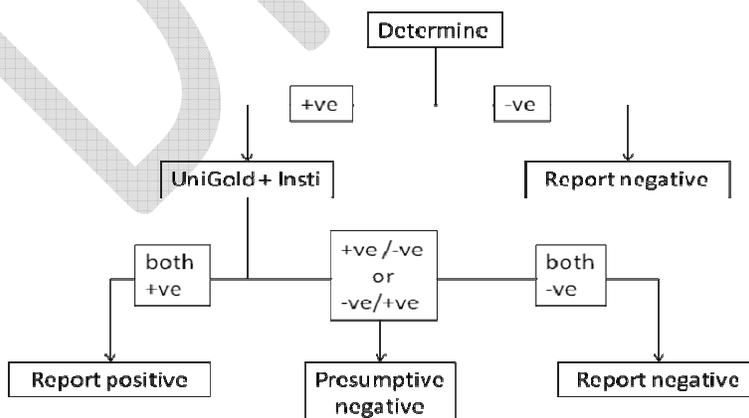


Figure 1: The proposed in-country HIV testing algorithm. Note that tests will be performed in the order indicated - Determine screening must always happen before any further testing with UniGold and Insti in parallel.

Table 1: Proposed in-country HIV testing algorithm including follow up actions depending on test outcomes at each stage of the algorithm.

TEST RESULTS			REPORT	COMMENT
DETERMINE	UniGold	Insti		
-	TNR*	TNR	Anti-HIV Negative	HIV antibody not detected
+	-	-	Anti-HIV Negative	HIV antibody not present, false positive screen. Refer to NRL for quality assurance
+	+	-	Presumptive Anti-HIV negative	Most likely HIV negative. There is a small possibility that these may indicate early HIV infection. Refer to NRL for quality assurance and confirmatory testing. It is strongly recommended that another sample be drawn in 4-6 weeks for testing to confirm these results.
+	-	+		
+	+	+	Anti-HIV Positive	Reactivity in HIV tests indicative of HIV infection. Refer to NRL for quality assurance purposes. Should the NRL result differ a second sample should be drawn as soon as possible for further testing at NRL.

* Testing not required

More detailed results and analysis from phase I are provided in the document “HIV Validation Phase 1 Report”.

Revised Phase II : Combined Phase II & III to Fast Track Confirmatory Testing

The main revision to the evaluation protocol does not require any deviation from the original protocol in terms of technical detail. Rather, the revision is specifically to roll out quality assured, in-country confirmatory testing in more countries more quickly. To achieve this a combined Phase II/ Phase III approach will be taken. The NRL will take the lead in training in-country staff on the appropriate use of the proposed algorithm. NRL staff will be partnered by staff with the relevant technical expertise from other partner agencies.

It is intended that from late June 2010 one to two PICTs per month will receive appropriate training and commodities to implement the RDT-based testing algorithm which will include delivering the final test result as indicated in table 1. Simultaneously, for each sample tested the laboratory will collect an additional serum or plasma specimen and dried serum or plasma spots for referral to NRL for quality assurance and continued validation of the algorithm until such time as the Task Force deems repeat testing of all specimens is no longer necessary.

Training

Training will be comprised of two components. Theory and practical training in the use, interpretation and quality assurance of HIV RDTs and the HIV testing algorithm will be provided for in-country laboratory technicians and managers. It is critically important that hospital and Ministry of Health administrators who are involved in HIV testing policy and/or commodity purchasing understand that all HIV RDTs have different properties and that once an algorithm is defined it must not be changed in any way without appropriate technical evaluation. Accordingly, basic training in the theory of HIV testing algorithms will be

provided to these staff. Further, it is critical that all staff at participating clinics understand the algorithm and the absolute necessity to provide adequate sample volume for the entire algorithm and adequate information about the individuals being tested. Therefore, training of these individuals will be undertaken to ensure these requirements are discussed and understood.

Samples

Samples from all individuals presenting at relevant clinics for HIV testing will be tested in the recommended algorithm. For this purpose, an additional tube of blood will be collected and serum or plasma prepared to ensure adequate volume for testing in the confirmatory rapid test algorithm and quality assurance by NRL as necessary. Ideally this second specimen will be collected in a serum separation tube (SST) or plasma preparation tubes (PPT). In addition, dried serum or plasma spots (DSS/DPS) will be prepared from the serum/plasma to investigate the possibility of using DSS/DPS as a retesting quality assurance method for future work. DSS and DPS are not considered a hazardous substance which greatly simplifies international shipping.

Testing

All testing will be conducted at the in-country reference laboratory (ICRL).

- Samples received at the ICRL will be centrifuged. The entire volume of serum must be retained.
- Serum samples will be tested first at the ICRL's using Determine;
- No further testing will be performed on samples negative on Determine;;
- All Determine reactive samples will be tested in each of the rapid tests in the recommended algorithm

The testing in the rapid tests may be performed in batches.

- No more than 10 samples should be tested in one rapid test run.
- Tests will always be used within their expiry dates.
- Positive and negative control samples will be purchased and provided by NRL. The controls will be at regular intervals, and with each new operator, each new shipment and each change of reagent lot number.
- Testing will be performed as specified in the manufacturers' package inserts.
- If possible, all test results will be read independently by two readers, who will resolve any differences in consultation with each other.
- Test runs will be validated according to the manufacturer's criteria and runs that do not meet the validation criteria will be repeated.

Records

NRL will provide a simple form for recording samples' demographic information and worksheet templates that can be copied to guide test runs..

For each run of each test, a worksheet will be completed. The worksheet will record:

- the identifiers of the samples tested and the test results along with the names of each assay used and the sequence in which they were used
- test kit name, batch number and expiry date
- run date

- operator
- rapid test results

Following completion of the test run the worksheet will be used to record:

- results for the samples and controls (where applicable)
- incubation start time and read time;
- the number of instances where differences between readers occurred.

Copies of all the worksheets will be sent to the NRL for collation and analysis of results from all the ICRLs. NRL will prepare a report for the Taskforce.

Reporting of Test Results

Results will be reported to individuals based on the results of the rapid test algorithm. Samples negative on Determine will be reported negative; samples reactive on Determine and subjected to supplemental testing using the rapid tests in the algorithm will be reported and commented upon according to the protocol in Table 1.

Competency and Quality Assurance

Prior to the commencement of testing, an appropriate member of NRL, PPTC or SPC will travel to the ICRLs to train the staff in performance of the rapid tests, completing the worksheets, and reporting the results according to the agreed protocol. At the end of this training ICRL staff will complete a QA exercise. The NRL will provide a panel of 10-20 coded samples to each site that a technician at the ICRL will test and return the results to NRL. Additional panels and/or photographic EQAS may be provided during the validation period as necessary.

Samples found reactive on Determine at the ICRL will be sent to NRL for further validation of the rapid test algorithm. For this purpose, the entire remaining volume of serum/plasma should be either retained in the SST/PPT or removed into a single, sterile tube; and stored at 2-8oC for up to 6 days prior to shipment to NRL for testing. Samples that will be stored for longer periods should be frozen. Samples will be shipped to NRL from the ICRL regularly in batches, along with the demographic information and copies of relevant worksheets. On arrival at NRL serum/plasma will be tested and results communicated to the relevant ICRL.

Determine reactive samples will continue to be sent to NRL until such time as the Taskforce deems the algorithm to be adequately validated and the laboratories to be delivering accurate results.