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☐ Single site proposal
☒ "Core" proposal (for multicentre study)
☐ Centre-specific proposal under multicentre study
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Form II

HRP Research Proposal: Project details

Laboratory-based Evaluation of Point-Of-Care Tests for Trichomoniasis

1. Project summary

a. Background information and rationale

WHO estimates that approximately 357 million people aged 15 to 49 are infected each year with four curable sexually transmitted infections (STIs), chlamydia, gonorrhea, syphilis, and trichomoniasis, of which trichomoniasis is the most frequently reported. Studies have shown that trichomoniasis, caused by the protozoan, *Trichomonas vaginalis (Tv)*, is associated with adverse outcomes of pregnancy and facilitates HIV transmission and acquisition. Early detection and treatment of trichomoniasis in pregnant women, HIV infected women and women at high risk of infection are critical in preventing adverse outcomes.

According to the 2013 WHO manual on the Laboratory Diagnosis of Sexually Transmitted Infections, there are four methods for the laboratory diagnosis of trichomoniasis: wet preparation microscopy, antigen detection, culture, and nucleic acid amplification tests (NAATs). NAATs offer the most accurate results but are costly and require sophisticated laboratory equipment. There is currently one commercially available qualitative NAAT for the detection of Tv ribosomal RNA using vaginal swabs.

Since all these diagnostic methods are laboratory-based, in countries where laboratory infrastructure is limited, patients often do not have access to the laboratory diagnosis of trichomonaisis. In recent years, rapid tests that can be used at the point-of-care (hereafter referred to as POCTs) have become commercially available. These tests detect Tv antigen from vaginal swabs, are easy to use and can give a result in 15 minutes. A new generation of NAATs that can be used as near POCTs have also become commercially available. These tests are performed on automated sample-in, answer-out platforms, requiring minimal training and are highly accurate. They are more expensive than POC antigen detection tests and results are available in 1.5 hours.

b. Study hypothesis and objectives

The overall objective of laboratory-based evaluations is to provide evidence on the analytical performance and operational characteristics of commercially available Tv POC or near POC tests. The specific objectives of this evaluation are to determine the analytical performance of Tv POC and near POC tests compared to that of reference standard assays, to assess the operational characteristics of Tv POC and near POC tests, including the ease of use, technical complexity, reproducibility and repeatability and to provide evidence for triaging Tv POC and near POC tests into clinic-based evaluations.

c. Study methods

Simulated specimens should be used. If this is not possible, laboratory-based evaluations are to be conducted with leftover human samples from routine care or a research project, respecting relevant national laws and regulations. In general, samples collected as part of routine clinical standard of care or a research project can be used for the evaluation if the following criteria are met: Sample is characterized for Tv; Sample is de-identified from any personal identifier; Sample cannot be directly or indirectly traced to patients; No promise or implied promise was made to patients/clients that the samples would be destroyed; A research ethics committee approves the use of these samples for research.

Sample size calculation depends on the expected performance of the test compared to the reference technology. If the POCT is expected to have at least 90% sensitivity and 98% specificity compared to the reference test, then a sample size of 150 positive and 100 negative samples would be adequate to give a precision of ±5% around the point estimates of sensitivity and specificity respectively. If the expected sensitivity is higher, the sample size can be decreased accordingly.

Staff at the study site should be trained on the study protocol, including test performance procedures, and interpretation and recording of testing results. Data from the evaluation will be entered into a standardised data recording form. The sensitivity, specificity, positive and negative predictive values for each POCT will be calculated by comparing the POCT results to the validated reference test results. In addition to quantitative evaluation of test performance, qualitative assessment of the suitability for use of the POC or near POC test in the laboratory will be assessed by the technician.

2. Detailed description of the project

2.1 Background information and rationale

WHO estimates that approximately 357 million people aged 15 to 49 are infected each year with four curable sexually transmitted infections (STIs), chlamydia, gonorrhea, syphilis, and trichomoniasis, of which trichomoniasis is the most frequently reported. Studies have shown that trichomoniasis, caused by the protozoan, *Trichomonas vaginalis (Tv)*, is associated with adverse outcomes of pregnancy and facilitates HIV transmission and acquisition. Early detection and

treatment of trichomoniasis in pregnant women, HIV infected women and women at high risk of infection are critical in preventing adverse outcomes.

According to the 2013 WHO manual on the Laboratory Diagnosis of Sexually Transmitted Infections, there are four methods for the laboratory diagnosis of trichomoniasis: wet preparation microscopy, antigen detection, culture, and nucleic acid amplification tests (NAATs). NAATs offer the most accurate results but are costly and require sophisticated laboratory equipment. There is currently one commercially available qualitative NAAT for the detection of Tv ribosomal RNA using vaginal swabs.

Since all these diagnostic methods are laboratory-based, in countries where laboratory infrastructure is limited, patients often do not have access to the laboratory diagnosis of trichomonaisis. In recent years, rapid tests that can be used at the point-of-care (hereafter referred to as POCTs) have become commercially available. These tests detect Tv antigen from vaginal swabs, are easy to use and can give a result in 15 minutes. A new generation of NAATs that can be used as near POCTs have also become commercially available. These tests are performed on automated sample-in, answer-out platforms, requiring minimal training and are highly accurate. They are more expensive than POC antigen detection tests and results are available in 1.5 hours.

Laboratory-based evaluation of the performance of these POC and near POC tests is an important priority for STI control programmes and for improving the specificity of syndromic management of vaginal discharge. Laboratory-based evaluations will provide data on the analytical performance of these tests, which can be used to guide WHO and national health authorities on whether to conduct further evaluation to determine the clinical performance of these tests in settings of intended use, i.e. at POC settings, and to inform adoption and test introduction.

2.2 Study hypothesis and objectives

The overall objective of laboratory-based evaluations is to provide evidence on the analytical performance and operational characteristics of commercially available Tv POC or near POC tests. The specific objectives of this evaluation are:

1) To determine the analytical performance of Tv POC and near POC tests compared to that of reference standard assays
2) To assess the operational characteristics of Tv POC and near POC tests, including the ease of use, technical complexity, reproducibility and repeatability
3) To provide evidence for triaging Tv POC and near POC tests into clinic-based evaluations

2.3 Study conceptual framework

Question: Are POCTs for the diagnosis of trichomoniasis as performant as the gold standard tests in a laboratory environment?

P (participants): NA

I (intervention): perform the Tv POCT in accordance with the manufacturers' directions

C (control): perform the Tv gold standard diagnostic tests on the same sample at the same time

O (outcome): sensitivity, specificity, predictive values (if possible) and operational characteristics

T (timeframe): until the required sample size is reached, maximum 8 months from inception to dissemination of the results.

2.4 Study design

The evaluation will be conducted according to the following guiding principles:

1) A diagnostic test should be evaluated for a clearly defined indication.

2) A diagnostic test should be evaluated using methods and equipment fit for the purpose.

3) Laboratory staff performing the reference tests should be qualified and competent in proficiency to perform the tests under evaluation and the reference standard tests.

4) There should be a regular independent assessment of the laboratory quality assurance/quality control procedures.

5) The evaluations should be conducted in compliance with Good Laboratory Practice.

2.5 Procedures

2.5.1 Study site(s)

These evaluations will be conducted in laboratories at a WHO Collaborating Centre for STIs, these laboratories should be able to demonstrate:

- proficiency at performing the reference test (≥90% score on a minimum of 2 testing events over the last year)
- experience and expertise in conducting diagnostic evaluations
- access to well characterised evaluation panels that allow quantitative assessment of test sensitivity, specificity, reproducibility and repeatability, as well as qualitative assessment of ease of use, technical complexity and ease of interpretation

2.5.2 Study participants

NA

2.5.3 Participant recruitment

NA

2.5.4 Sampling and allocation

NA

2.5.5 Sampling size calculation

Sample size calculation depends on the expected performance of the test compared to the reference technology. If the POCT is expected to have at least 90% sensitivity and 98% specificity compared to the reference test, then a sample size of 150 positive and 100 negative samples would be adequate to give a precision of ±5% around the point estimates of sensitivity and specificity respectively. If the expected sensitivity is higher, the sample size can be decreased accordingly.

2.5.6 Description of the intervention

2.5.6.1 Drugs and devices

POCTs for STIs are described in a landscape report commissioned by WHO RHR. All test kits to be considered for this study must be tests that can be used at POC or near POC. WHO will contact the manufacturers of the candidate test kits to inform them of the laboratory-based evaluation. If the company is interested, they will be asked to donate test kits in accordance with the terms specified under a WHO Confidentiality and Material Transfer Agreement.

2.5.6.2 Innovation in service delivery

To increase access to screening and diagnostic testing, it is important that the tests to be included in this evaluation should have characteristics that are consistent with those set out in the Target Product Profiles (TPPs) developed by consensus at the first WHO RHR Technical Consultation on POCTs for STIs in 2014. These include the following operational characteristics:

1. Rapid -- test result is available within the duration of the clinic visit.
2. Simple -- test can be performed in 2-3 steps, requiring minimal training and no equipment
3. Easy to interpret -- card or strip format with visual readout or using a small reader

2.5.7 Admission procedure

NA

2.5.8 Follow-up procedures

NA

2.5.9 Criteria for discontinuation of a participant

NA

2.5.10 Criteria for discontinuation of the study

NA

2.5.11 Laboratory and other investigations

The PI should review the core protocol with the study team from each site and determine if any changes are necessary to adapt to local conditions. This will minimize procedural differences amongst sites, which may account for the difference in study outcomes from site to site. Each POC or near POC test should be performed in accordance with the procedure described in the instructions for use in the package insert and should not have site to site differences.

The general guidelines are summarized as the following:

1) Note lot number and expiry date: a kit should not be used beyond the expiry date.
2) Ensure correct storage conditions: do not use the kit if the desiccant has changed colour.
3) If test kits are stored in the refrigerator, they should be brought to room temperature (at least 30 minutes) before use.

4) Damaged kits should be discarded.
5) Use test kits immediately after opening.
6) Reagents from one kit should not be used with those of another kit.
7) Test should be performed exactly as described in the manufacturer's instructions for use.

Each specimen will be given a study number which is a combination of the laboratory abbreviation and a consecutive 3-digit number. This study number can only be used for linking all study results. If the test under evaluation requires subjective reading, each test result will be read independently by two technicians to determine variability in the interpretation of test results. Technician 1 reads the (near) POCT result within the reading window of time as recommended by the instructions. Technician 2 reads the (near) POCT result within the reading window of time as recommended by the instructions and one hour later.

The reference test for Tv should be a laboratory-based NAAT approved by a stringent regulatory authority, such as US FDA, and agreed on by WHO and the PI(s). The reference assay should be performed by a reference laboratory technician on an evaluation panel of simulated swab samples in accordance with the manufacturers' instructions for use. If the study is multi-centred, the same Tv reference NAAT should be used across all the sites. Both POC or near POC test results and reference test results should be recorded in the Data Recording Form (appendix 1).

The composition of the evaluation panel (n=100) will be as follows:

- To challenge sensitivity: 60 Tv positive samples with 10 samples at each concentration ranging from 10^2-10^7 Tv genome copies based on quantitative PCR

- To challenge specificity: 10 samples with high concentration of closely related Trichomonas species; 20 samples of other causes of vaginal discharge (5 each of swab samples positive for *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, bacterial vaginosis, Candida species), and 10 negative samples consisting of buffer only

These simulated swab samples will be made by dilutions of a highly concentrated Tv stock preparation (from a reference strain) into NAAT buffer. This stock preparation should be quantified using quantitative PCR, and appropriate dilutions of this stock preparation will be made in NAAT buffer to make up the evaluation panel as above. Each member of the evaluation panel must be validated using the reference test before being used to evaluate the POC or near POC tests under evaluation.

2.6 Study instruments

Appendix 1. Data Recording Form

Appendix 2. Operational characteristics of POC or near POC tests

2.7 Project management

The study team in each laboratory will consist of at a minimum:

- Principal investigator (PI)
- Technical supervisor
- Technical staff for reference testing

- 2 laboratory technicians

1) PI
- If necessary, obtain ethical committee approval for the evaluation study prior to the conduct of activities
- Coordinate activities within the laboratory to ensure the evaluation is conducted according to the core protocol as approved.
- Collect data and collate in a central database for analysis
- Participate in the overall review and analyses of evaluation results.

2) Technical supervisor
- Supervise the day-to-day activities associated with the evaluation, including the validation of the evaluation panel, training technicians on study procedures including test interpretation, reviewing and signing off on test results daily during the evaluation, and trouble-shooting.
- Ensure that the technicians performing the tests under evaluation are blinded to the results of the evaluation panel
- Ensure that if the test under evaluation requires subjective reading, the results should be read independently by 2 technicians to assess inter-reader variability in test interpretation.
- Collate the results from the laboratory-based evaluation and enter them into the study database.
- Send the final database to the PI, and WHO for analysis

3) Technical staff for reference testing:
- Assemble the evaluation panel
- Perform the reference test on the evaluation panel, in accordance with the manufacturer's instruction for use

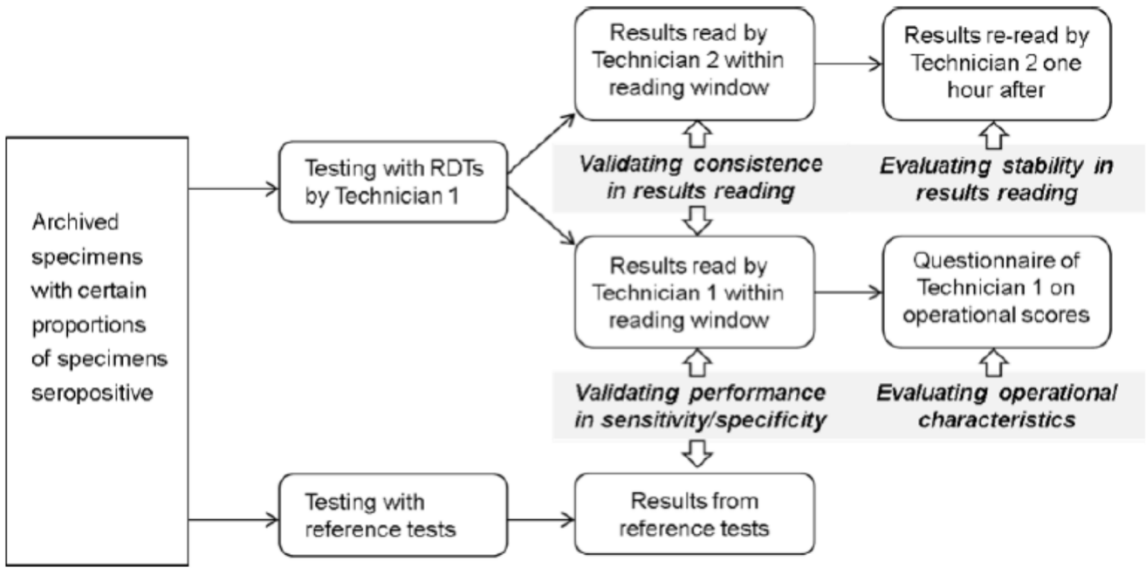
4) Technician 1:
- Perform and read POC or near POC test under evaluation in accordance with the study protocol
- Record results in the Data Recording Form.
- if the test under evaluation requires subjective reading (i.e. reading by eye), prepare completed tests for Technician 2 to read and record independently

5) Technician 2:
- If the POC or near POC test requires subjective reading, read the result of the test under evaluation at two separate times: once within the time stated in the instructions for use, and another one hour after the test was performed
- Record results in a separate Data Recording Form

Staff at the study site should be trained on the study protocol, including test performance procedures, and interpretation and recording of testing results. Standard operating procedures (SOPs) should be developed to describe all the procedures used in the evaluation. The technicians who perform the tests under evaluation and/or read the testing results will first practice the testing procedure with positive and negative control specimens provided by the staff performing the reference testing, under the supervision of the technical supervisor. If the results are read by eye, the test results should be read independently by both technicians to ensure consistency of test

results. The study should only proceed when the technical supervisor is confident of their ability to conduct the study, and all the materials required for the study are in place.

The study procedures are summarized in the following diagram:



All indeterminate results in POC or near POC testing should be recorded as indeterminate and excluded from sensitivity and specificity analysis. The number of indeterminate results should, however, be presented in the overall evaluation report. In addition to quantitative evaluation of test performance, qualitative assessment of the suitability for use of the POC or near POC test in the laboratory will be assessed by the technician. At the end of the study, the technician who performs the POC or near POC tests will answer a short questionnaire, using a scoring evaluation, on clarity of kit instructions (only for those who are familiar with the language in which the instructions are written), ease of use (technical complexity), ease of interpretation of results, time to complete the test, training time, and hands on time (appendix 2).

2.8 Data quality assurance

The national reference laboratory should support the study sites to ensure quality assurance principles are adhered to such as strict observation of SOPs for each step of the evaluation process. Daily quality control specimens (positive and negative controls) should be run by laboratory staff members.

The reproducibility of the test is a measurement of precision of the closeness of agreement between test results when the conditions for testing or measurement changes. Reproducibility can be measured between operators (Technician 1 and 2), between different test sites, and using different kit lots. To measure reproducibility, Technician 2 will independently perform 30 tests under evaluation and compare the results to those of Technician 1.

The repeatability of the test refers to measuring the closeness of test results when no conditions of measurement changes. Repeatability can be measured by Technician 1 repeating 30 specimens (20 positive and 10 negative specimens) from the evaluation panel and comparing the results.

2.9 Data management

Depending on the site. Will be specified in the site consensus protocols. Laboratory evaluation of diagnostic tests does not affect subjects in any way and is therefore exempted from having a DSMB.

2.10 Data analysis plan

Data from the evaluation will be entered by the technical supervisor into the Excel spreadsheet at the evaluation laboratories. The technical supervisor will send the Excel spreadsheet to the PI.

The results from the POC or near POC tests under evaluation will be compared to the NAAT reference testing results. In each case, the sensitivity, and specificity for the test under evaluation will be calculated in comparison with the reference test results using the following formulas.

Table 1. Evaluation data summarization format

Result for Test under evaluation	Reference test results		
	+	–	Total
+	a	b	a+b
–	c	d	c+d
Total	a+c	b+d	a+b+c+d

POC or near POC test sensitivity = a/(a+c)

POC or near POC test specificity = d/(b+d)

If leftover clinical specimens from a defined population cohort are used, then positive and negative predictive values can be calculated as follows:

- Positive predictive value = a/(a+b)
- Negative predictive value = d/(c+d)

Reproducibility and repeatability will be calculated as the % of test results that are concordant.

For the operational characteristics of the test under evaluation, a composite score will be calculated by adding the scores from each of the criteria assessed, with a high score being very suitable and a low score being unsuitable.

2.11 Study timeline

	Timeline							
	Jan	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**
Finalise study protocol by adapting the core protocol								
Submit to WHO ethics committee								
Submit to local ethics committee, when necessary								
Obtain ethical approvals								
Validate proficiency at performing the reference test								
Train staff on the study protocol								
Prepare supplies and reference testing kits								
Prepare and validate evaluation panel								
Receive testing kits under evaluation								
Pilot SOPs on control specimens								
Conduct evaluation								
Analyse the study data								
Send testing results to WHO								
Provide results for selection in clinic-based evaluation								
Prepare the evaluation report								
Disseminate the study results								

2.12 Main problems anticipated and proposed solutions

Will be included in the consensus protocols as they are site-specific.

2.13 Applicability of results

Laboratory-based evaluations of Tv POCTs will be the source of the Tv POCTs used for the clinic-based evaluations.

2.14 Links with other projects

Laboratory-based evaluations of Ng and Ct POCTs will be performed. Clinic-based evaluations of POCTs for Ct, Ng and Tv will be done in case management of women complaining of vaginal discharge and in screening of women with high risk of these infections, and Ct and Ng in screening of MSM.

2.15 References

3. Gender considerations

3.1 Describe how women and men are affected by the public health need that the study addresses, and whether this is a need expressed or felt by women and/or men

NA

3.2 Explain how the research contributes to identifying and/or reducing inequities between women and men in sexual and reproductive health and health care

NA

3.3 Describe measures taken to facilitate the individual participation of women or men in the research process in light of their different life situations.

NA

3.4 Describe measures taken to ensure that community involvement is inclusive

NA

3.5 Describe the sex composition of the research team, and their duties and responsibilities in the proposed research

NA, will be added in site specific consensual protocols

4. Ethical issues

4.1 Ethical considerations:

The study proposal should be reviewed by the local Ethics Committee and any other National review body, as necessary.

The use of simulated specimens in the evaluation panel does not require the approval of Research Ethics Committee that oversees research involving human subjects.

If simulated specimens are not possible and laboratory based evaluations are to be conducted with leftover human samples from routine care or a research project, then the study protocol must be consistent with relevant national laws and regulations. In general, samples collected as part of routine clinical standard of care or a research project can be used for the evaluation if the following criteria are met:

- Sample is characterized for Tv

- Sample is de-identified from any personal identifier[1]
- Sample cannot be directly or indirectly traced to patients
- No promise or implied promise was made to patients/clients that the samples would be destroyed
- A research ethics committee approves the use of these samples for research.

4.1.1 Study population, recruitment strategy and informed consent process

NA

4.1.2 Perceived risks and benefits of the study, both at the individual and community levels

NA

4.1.3 Safeguards to protect any recognized vulnerability of the study participants

NA

4.1.4 Reimbursement or compensation to study participants

NA

4.1.5 Access to treatment or counselling for conditions either identified during screening of potential participants or resulting from the study intervention

NA

4.1.6 Responsiveness of the project to community needs and priorities

NA

4.1.7 Deception

NA

4.2 Forms required (include or attach as a scanned copy, as appropriate)

4.2.1 Information sheet for participants and/or responsible persons

NA

4.2.2 Informed consent forms for participants and if appropriate, responsible persons

[1] In general leftover samples are sent to reference labs already de-identified and receive a new lab-code upon arrival in the lab. To ascertain supplementary de-identification for this evaluation, the samples with the required characteristics will be designated and a technician with no connection to the previous research including these samples and who is not a member of the technical team of the evaluation, will be appointed to assign consecutives numbers/codes to the designated samples without linkage to nor the lab-code, nor the preceding research.

NA

4.2.3 Local (institutional, community and/or national) ethics approval

NA

5. Environmental impact of the project

Biosafety guidelines for laboratory staff

- Treat all specimens as potentially infectious
- Wear protective gloves and laboratory gown while handling specimens
- Do not eat, drink or smoke in the laboratory
- Do not wear open toe footwear in the laboratory
- Clean up spills with appropriate disinfectants e.g. 1% bleach
- Decontaminate all materials with an appropriate disinfectant
- Dispose of all waste, including test kits, in a biohazard container

6. Plans for dissemination and use of project results

These data should be published in national or international peer-reviewed journals.

7. Other support for the proposed research project

7.1 Project support by other institution(s)

Proficiency of the participating laboratories will be assessed at the initiation of the study through Tv proficiency programs sponsored by the WHO and US CDC or other licensed proficiency panel providers. Other possible project support will be site specific.

7.2 Consideration of the proposal by other institution(s)

NA

8. Other current research projects of the principal investigator.

NA

Please list all other research projects currently in progress.

Title of project	Source of support	Duration of project (dates)	Per cent of time spent on project by principal investigator

9. Curricula vitae of the principal investigator and co-investigator(s)

NA

10. Additional Information

Appendix 1. Data Recording Form

Name of test under evaluation: _____
Manufacturer: _____
Lot number: _____
Expiry date (day/month/year): _____/_____/_____
Evaluation site: _____
Results read by: ☐ Technician 1 ☐ Technician 2

(Technician 1 reads and records the results "Within the time stated in the instruction for use"; and Technician 2 reads and records the results "Within the time stated in the instruction for use" AND "60 min. later".)

Study number	**Reference test results**	**Result for Test under Evaluation (Positive/Negative/Indeterminate)**	
		Within the time stated in the instruction for use	**60 min. after the time stated in the instruction for use**
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Appendix 2 Operational Characteristics of POC or near POC Tests

Name of test under evaluation: _____
Manufacturer: _____
Survey date (day/month/year): _____/_____/_____
Name of staff: _____

	Score for test under evaluation	Scoring guide
1. Clarity of kit instructions		
• Difficult to follow		0
• Fairly clear		1
• Very clear		2
• Excellent		3
2. Ease of use		
• Complicated		0
• Fairly easy		1
• Very easy		2
• Excellent		3
3. Ease of interpretation of results		
• Difficult		0
• Fairly easy		1
• Very easy		2
• Unambiguous		3
4. Rapidity of test results		
• >30 minutes		0
• 20-30 minutes		1
• <20 minutes		2
5. Hands-on time		
• >10 minutes		0
• 5-10 minutes		1
• <5 minutes		2
6. Training time required		
• >2 hour		0
• 1-2 hours		1
• 30-59 minutes		2
• <30 minutes		3

Appendix 3 Budget template for laboratory-based evaluation of POC or near POC Tests

Item	Cost (USD)	Subtotal/Total (USD)
Personnel: 1. Study coordinator (0.5 FTE) 2. Technician 3. Clinic health worker **4.** data manager (0.5 FTE)	**15,000**	**15,000**
Supplies (consumables: gloves, pipettes, etc)	**1,000**	
Reference testing	**3,000**	**4,000**
Publishing	**6,000**	**6,000**
Other (please specify and justify below)		
TOTAL BUDGET		**25,000**

The study budget does not include costs of POCTs as these will be provided from the companies.

International travel for research staff will be budgeted separately.