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Integrated Laboratory Protocol for Diagnosis of Sexually Transmitted Infections (STIs): ELISA, Rapid Tests, Hemagglutination, and Confirmatory Algorithms

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Abstract

This protocol presents a fully integrated and sequential laboratory workflow for the serological diagnosis of sexually transmitted infections. It describes a combined approach that incorporates rapid immunochromatographic screening for HIV and syphilis, non-treponemal testing using the Rapid Plasma Reagin assay, treponemal confirmation through hemagglutination (TPHA), and enzyme-linked immunosorbent assays (ELISA) for the detection of HIV-1/2, HSV-2, HCV IgG, Chlamydia trachomatis IgG, and HBsAg. When available, automated confirmation using the Abbott Architect/MEIA platform is included to strengthen diagnostic validity. Designed for laboratories operating in low- and middle-income settings, the protocol follows ISO 15189 and CLSI recommendations and outlines all pre-analytical, analytical, and post-analytical requirements necessary to ensure reliable reproducibility in clinical and research environments.

Materials

5.1. Consumables
BD Vacutainer® sterile tubes (dry), Micropipettes (calibrated) and tips, 96-well ELISA plates (kit-specific), RPR carbon antigen cards (BD Macro-Vue®), TPHA test plates (Rapid Labs)

5.2. Reagents

Pathogen	Kit	Type	Manufacturer	Notes
HIV-1/2	ELISA	IgG/IgM	Bio-Rad / ZEUS	Confirmation required
HBsAg	ELISA	Antigen	Bio-Rad	Neutralization needed
HCV	ELISA	IgG	CTK Biotech	Confirmation: RNA
HSV-2	ELISA	IgG gG2	Zeus	Type-specific
C. trachomatis	ELISA	IgG	Bio-Rad	Past exposure marker
Syphilis confirmation	TPHA + RPR	Antibody	Rapid Labs / BD	Screening +

5.3. Equipment
Refrigerated centrifuge, Microplate reader (450 nm ± reference filter 620–630 nm)
Plate washer, Dry block incubator, Abbott Architect i1000 or MEIA system (optional confirmation)

Safety warnings

4.2. Safety Notes
All serum handling must be performed in a Class II biological safety cabinet. Human specimens are considered potentially infectious and must be processed using universal safety precautions. Laboratory activities must comply with ISO 15190 standards for biosafety and facility protection.

4.3. Pre-analytical Specifications
Venous blood is collected at a standardized volume of 5 mL into a dry serum tube without anticoagulant. Centrifugation is performed within a maximum of six hours after collection. Samples are transported to the laboratory in a cooler box containing refrigerated packs to maintain a stable temperature during transit. Serum intended for delayed testing is stored at −80 °C for long-term preservation, while short-term storage at +4 °C is acceptable for periods not exceeding seventy-two hours. When stored at −80 °C, serum samples maintain analytical integrity for several weeks before ELISA testing is performed.

Ethics statement

All procedures were reviewed and approved by the Biomedical Ethics Committee of the University of Antananarivo. Written informed consent was obtained from adult participants, and assent with parental consent was required for minors prior to sample collection.

Before start

4.1. Ethics Approval

All procedures were reviewed and approved by the Biomedical Ethics Committee of the University of Antananarivo. Written informed consent was obtained from adult participants, and assent with parental consent was required for minors prior to sample collection.

Before You Begin

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5 Refrigerated centrifuge, Microplate reader (450 nm ± reference filter 620–630 nm), Plate washer, Dry block incubator, Abbott Architect i1000 or MEIA system (optional confirmation)

Procedure Overview

6 Workflow Diagram (Text Format)
Sample Reception
→ Verification 26 Labeling
→ Serum Preparation
→ Step 1: HIV/Syphilis Combo Rapid Test

→ Step 2: If HIV reactive → ELISA confirmation
→ Step 3: RPR Screening
→ Step 4: TPHA Confirmation
→ Step 5: ELISA Panel (HBsAg, HCV IgG, HSV-2 IgG, CT IgG)
→ Step 6: Confirm reactive samples (HBsAg neutralization, HCV RNA, etc.)
→ Quality Control 26 LIMS Entry
→ Biological Validation
→ Final Report

Step-by-Step Method

7 Allow tubes to clot 20–30 minutes.
Centrifuge at 2500 g for 10 minutes.
Transfer serum to sterile cryovials.
Perform testing or store at −80 °C.

8 Follow IFU of CTK Biotech.
Add 10 µL serum + 2 drops buffer
Read at 15 minutes
If HIV reactive → proceed to Step 4 (HIV ELISA)
If syphilis reactive → proceed to Step 3 (RPR)

9 Pipette 50 µL serum into card circle
Add 1 drop of RPR antigen
Rotate for 8 minutes
Read macroscopically
Reactive → Confirm with TPHA
Non-reactive → Report as screening negative

10 Dilute serum 1:40
Add 25 µL to test well
Add sensitized RBCs
Incubate 45 minutes at room temperature
Agglutination = Treponemal infection confirmed

11 Dilute samples 1:21 unless otherwise specified
Add 100 µL to wells
Incubate 25 min at 37 °C
Wash ×5
Add 100 µL conjugate
Incubate 25 min
Wash ×5
Add 100 µL TMB
Incubate 10–15 min
Add 50 µL stop solution

Read at 450 nm

12 The interpretation of ELISA results follows the manufacturer's standardized optical density thresholds. Values equal to or below 0.90 are considered negative and indicate the absence of detectable antibodies or antigen. Results falling between 0.91 and 1.09 are classified as equivocal and require repeat testing on a freshly prepared aliquot to exclude technical variability. Values equal to or greater than 1.10 are interpreted as positive and indicate immunological reactivity consistent with prior exposure or current infection, depending on the pathogen under investigation.
Negative ≤ 0.90
Equivocal 0.91–1.09
Positive ≥ 1.10

Confirmatory Testing

13 All reactive results obtained through screening or ELISA procedures must undergo confirmatory evaluation using pathogen-specific reference methods. HIV reactivity is confirmed through immunoblot assays or nucleic acid testing. Detection of HBsAg requires confirmation through a neutralization assay to verify antigen specificity. HCV serological reactivity is followed by RNA PCR to determine active infection. Chlamydia trachomatis seropositivity should be complemented by NAAT, which represents the diagnostic gold standard for active infection. HSV-2 serology generally does not require confirmatory testing unless clinical uncertainty exists or lesion-based sampling is indicated.
HIV Immunoblot or NAT
HBsAg Neutralization assay
HCV RNA PCR
CT NAAT recommended
HSV-2 No confirmatory test required unless clinical doubt

Quality Control

14 Quality assurance is maintained through the systematic inclusion of negative controls, positive controls, and calibrators in duplicate on every plate. The assay run is accepted only when all controls fall within the expected ranges and when the coefficient of variation between duplicates remains below 15 percent. Laboratories implementing this protocol are encouraged to participate regularly in external quality assessment schemes to ensure continued analytical performance and comparability with international standards.
Run NC, PC, Calibrators in duplicate each plate
Acceptable CV 3c 15%
Participate in external QA programs

Troubleshooting Guide

15 High background | Inadequate washing | Increase cycles, verify washer
Low PC signal | Degraded reagents | Check expiry and storage
High CV | Pipetting error | Recalibrate pipettes
Weak sample signal | Specimen degradation | Re-test using fresh aliquot

Data Management

16 All laboratory results are recorded in the Laboratory Information Management System (LIMS), which ensures complete traceability in accordance with ISO 15189 requirements. Raw data, including plate readings, internal quality control logs, and validation sheets, are archived securely and preserved for a minimum period of two years or longer if required by institutional policy. Access to the database is restricted to authorized personnel, and all records are maintained under strict confidentiality.

Limitations

17 This protocol presents inherent limitations related to the biological nature of serological testing. Early window-period infections may not be detected, particularly for HIV, HBV, or HCV, when antibody concentrations remain below assay thresholds. Detection of *Chlamydia trachomatis* IgG by ELISA reflects past immunological exposure rather than an active infection and therefore cannot be interpreted as evidence of current disease. HSV serology does not distinguish between primary and past infections, which limits its clinical specificity. The RPR assay may produce false-positive reactions in various conditions, including pregnancy, autoimmune disorders, and certain viral infections. For these reasons, all reactive results must be interpreted within a broader clinical and epidemiological context, and confirmatory testing is recommended whenever applicable.

References

18 1. CTK Biotech Inc. RecombLISA HCV IgG ELISA [package insert]. Poway (CA): CTK Biotech; n.d.
2. Bio-Rad Laboratories. Mouse anti Chlamydia trachomatis LPS, clone 1312/236 [datasheet 1990-2809].
3. Bio-Rad Laboratories. Mouse anti Hepatitis B Surface Antigen (HBsAg), clone 1834 (MCA4658) [datasheet].
4. CTK Biotech Inc. OnSite HIV/Syphilis Ab Combo Rapid Test: Instructions for Use. Poway (CA): CTK Biotech; n.d.
5. Bio-Rad Laboratories. Mouse anti HIV-1 p24 monoclonal antibody, clone 28E0 [datasheet 4999-8607].
6. Becton, Dickinson and Company. Macro-Vue RPR Card Tests 18 mm Circle Card Test [package insert].

7. Becton, Dickinson and Company. Macro-Vue RPR Card Tests: composition, précautions et performances.
8. Rapid Labs Ltd. TPHA 100/200/500 Tests — Instructions for Use (Rev. 9; 08/03/2023). Colchester (UK): Rapid Labs
9. ZEUS Scientific. ZEUS ELISA HSV gG-2 IgG Test System Instructions for Use (Rev. 12/19/2017).
10. ZEUS Scientific. ZEUS ELISA HSV gG-2 IgG Test System Performances (études multi-sites et panel CDC).

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Acknowledgements

7. **Step-by-Step Method**

1. **Sample Preparation**
   1. Allow tubes to clot 20–30 minutes.
   2. Centrifuge at 2500 g for 10 minutes.
   3. Transfer serum to sterile cryovials.
   4. Perform testing or store at −80 °C.

2. **HIV/Syphilis Combo Rapid Test**
   - Follow IFU of CTK Biotech.
   - Add 10 µL serum + 2 drops buffer
   - Read at 15 minutes
   - If HIV reactive → proceed to Step 4 (HIV ELISA)
   - If syphilis reactive → proceed to Step 3 (RPR)

3. **RPR Test (Non-Treponemal Screening)**
   1. Pipette 50 µL serum into card circle
   2. Add 1 drop of RPR antigen
   3. Rotate for 8 minutes
   4. Read macroscopically
   - Reactive → Confirm with TPHA
   - Non-reactive → Report as screening negative

4. **TPHA Confirmation**
   1. Dilute serum 1:40
   2. Add 25 µL to test well
   3. Add sensitized RBCs
   4. Incubate 45 minutes at room temperature
   5. Agglutination = Treponemal infection confirmed

5. **ELISA Panel Procedure**
   (Applicable to HIV ELISA confirmation, HBV, HCV, CT, HSV-2)

5.1 **General ELISA Steps (Unified Format)**
   1. Dilute samples 1:21 unless otherwise specified
   2. Add 100 µL to wells
   3. Incubate 25 min at 37 °C
   4. Wash ×5
   5. Add 100 µL conjugate
   6. Incubate 25 min
   7. Wash ×5

8. Add 100 µL TMB
9. Incubate 10–15 min
10. Add 50 µL stop solution
11. Read at 450 nm

5.2 **Interpretation (Standard)**

- The interpretation of ELISA results follows the manufacturer's standardized optical density thresholds. Values equal to or below 0.90 are considered negative and indicate the absence of detectable antibodies or antigen. Results falling between 0.91 and 1.09 are classified as equivocal and require repeat testing on a freshly prepared aliquot to exclude technical variability. Values equal to or greater than 1.10 are interpreted as positive and indicate immunological reactivity consistent with prior exposure or current infection, depending on the pathogen under investigation.

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9. **Quality Control**

Quality assurance is maintained through the systematic inclusion of negative controls, positive controls, and calibrators in duplicate on every plate. The assay run is accepted only when all controls fall within the expected ranges and when the coefficient of variation between duplicates remains below 15 percent. Laboratories implementing this protocol are encouraged to participate regularly in external quality assessment schemes to ensure continued analytical performance and comparability with international standards.

Run NC, PC, Calibrators in duplicate each plate

Acceptable CV 3c 15%

Participate in external QA programs

10. **Troubleshooting Guide**

Problem	Possible Cause	Solution
High background	Inadequate washing	Increase cycles, verify washer
Low PC signal	Degraded reagents	Check expiry and storage
High CV	Pipetting error	Recalibrate pipettes
Weak sample signal	Specimen degradation	Re-test using fresh aliquot

11. **Data Management**

All laboratory results are recorded in the Laboratory Information Management System (LIMS), which ensures complete traceability in accordance with ISO 15189 requirements. Raw data, including plate readings, internal quality control logs, and validation sheets, are archived securely and preserved for a minimum period of two years or longer if required by institutional policy. Access to the database is restricted to authorized personnel, and all records are maintained under strict confidentiality.

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