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Integrated Diagnostic Protocol for Syphilis Using Immunochromatographic HIV/Syphilis Combo, RPR, and TPHA Tests Detection of Antibodies anti Treponema pallidum V.1

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Faramalala Ramamonjiarisoa, MD[1,2,3], HDR RAZAFIARIMANGA Zara Nomentsoa[4,5], Dr. Ramaroson Roseline PhD[6]

[1]Doctoral Candidate, Doctoral School of Life and Environmental Sciences (SVE);
[2]Research Team: EAD 7 – Immunology, Immunopathology, Immunodiagnostics;
[3]Medical Biologist, Research and Training Laboratory in Medical Biology (LBM), Faculty of Medicine, University of Antananarivo, Madagascar;
[4]Associate Professor – Director of Documentation, International Publications, and Editing, University of Antananarivo;
[5]President of the Faculty College of Teachers, Faculty of Sciences, University of Antananarivo;
[6]Specialization in Fundamental and Applied Biochemistry, Faculty of Sciences, University of Antananarivo, Madagascar

framamonjiarisoa

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We use this protocol and it's working

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Abstract

This protocol presents a standardized diagnostic workflow for syphilis using three complementary laboratory methods: (1) Immunochromatographic HIV/Syphilis Combo test for initial screening, (2) Rapid Plasma Reagin (RPR) test for detecting non-treponemal antibodies, and (3) Treponema pallidum Hemagglutination Assay (TPHA) for confirming treponemal infection. The combined approach improves diagnostic accuracy and facilitates simultaneous HIV screening. Immunochromatographic HIV/Syphilis Combo test is designed to simultaneously detect antibodies for both HIV and syphilis, providing a rapid and efficient initial screening method. RPR detects reagin, an antibody-like substance found in syphilitic individuals and sometimes in those with other conditions. The reagin binds to the cardiolipin-coated cholesterol particles in the test, causing flocculation. TPHA uses avian erythrocytes coated with T.pallidum antigens. Specific antibodies in the plasma or serum bind to these antigens, causing erythrocytes to agglutinate, forming a characteristic pattern in the test well. Non-specific reactions are prevented by absorbents. The integration of these methods enhances the overall diagnostic process, ensuring both accurate syphilis and HIV diagnosis.

Guidelines

This protocol complies with IFU, ISO 15189 (Medical Laboratories – Requirements for Quality and Competence), CLSI EP05/EP09/EP12 guidelines, and WHO recommendations for sexually transmitted infection testing and quality assurance in medical laboratories.

Materials

- OnSite® HIV/Syphilis Ab Combo Rapid Test (CTK Biotech)
- BD Macro-Vue™ RPR Card Test kit
- Rapid Labs TPHA kit
- Micropipettes and sterile tips
- Clean glass slides or test cards
- Timer, centrifuge, and mixing rotator
- Personal protective equipment (gloves, lab coat, goggles)
- Sample types: serum, plasma, or whole blood

Safety warnings

Perform procedures under Biosafety Level 2 (BSL-2) conditions. Wear gloves, laboratory coat, and protective eyewear. Dispose of waste according to local biosafety and environmental regulations. Treat all biological specimens as potentially infectious. Dispose of used materials in accordance with institutional biosafety and national biomedical waste guidelines. Sample Handling: Acceptable specimens: Serum or plasma (per kit IFU). Store samples at 2–8 °C up to 7 days or ≤ −20 °C for long-term storage. Avoid repeated freeze-thaw cycles.

Ethics statement

The study protocol was reviewed and approved by the Ethics Biomedical Committee of the University of Antananarivo. All procedures were conducted in accordance with national research ethics guidelines. Written informed consent was obtained from all participants. Calibrate pipettes and microplate readers. Store reagents at 2–8 °C and allow to reach room temperature before use. Verify internal quality controls (positive/negative sera) before performing

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Before Start

1 Calibrate pipettes and microplate readers. Store reagents at 2–8 °C and allow to reach room temperature before use. Verify internal quality controls (positive/negative sera) before performing.

Safety Warnings

2 Perform procedures under Biosafety Level 2 (BSL-2) conditions. Wear gloves, laboratory coat, and protective eyewear. Dispose of waste according to local biosafety and environmental regulations.
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Laboratory procedure

3 Allow reagents and samples to reach room temperature (20–25°C).

4 Add 50 µL of specimen into the test well.

5 Add two drops (≈80 µL) of sample diluent.

6 Read results at 15 minutes.

6.1 - One control line: Negative.

6.2 - Control + test line: Positive.

6.3 - No control line: Invalid.

7 Equilibrate reagents and samples to room temperature.

8 Place 50 µL of serum on a test circle.

9 Add 18 µL of antigen suspension.

10 Rotate the card for 8 minutes at 100 ± 2 rpm.

11 Examine for macroscopic flocculation.

12 For quantitative determination, perform serial twofold dilutions.

13 Dilute serum 1:20 with sample diluent.

14 Add 25 µL of diluted sample to each well.

15 Add 75 µL of Test Cells to test wells and Control Cells to control wells.

16 Mix gently and incubate for 45–60 minutes at 15–30°C.

17 Interpret results:

17.1 - Reactive: diffuse mat formation.

17.2 - Non-reactive: compact button formation.

Interpretation of Results

18 Step 1: Perform HIV/Syphilis Combo rapid test.
- If reactive positive or no → confirm with RPR.
Step 2: If RPR positive or no → perform TPHA.
Final interpretation:
- RPR (+) / TPHA (+): Active syphilis.
- RPR (–) / TPHA (+): Past/treated infection.
- RPR (+) / TPHA (–): Biological false positive.

Quality Control

19 Include positive and negative controls in each run. Do not use expired reagents. Repeat the test if control results are invalid. Participate in external quality assurance programs periodically.

Data Storage and Confidentiality

20 LIMS Database: All raw data and test results are stored securely in the Laboratory Information Management System (LIMS), ensuring traceability and compliance with ISO 15189 standards. Confidentiality: Patient data are handled confidentially, and all personal information is stored securely in compliance with ethical guidelines and privacy laws.

Limitations

21 Early primary infection may yield false negatives.
Biological false positives may occur in pregnancy, autoimmune diseases, or viral infections.
Cross-reactivity with yaws and other treponematoses possible.
Qualitative tests require clinical correlation.

Workflow Algorithm

22 Sample Reception → Verification of identification → Serum/Plasma preparation →
Initial Rapid Screening (CTK Biotech Syphilis HIV Ab PLUS Combo)
RPR BD Macro-Vue® Screening → If Reactive or no
TPHA Rapid Labs Confirmation
Consolidate all results → Quality Control verification → Biological validation
Final report issue

Protocol references

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