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Enzyme-Linked Immunosorbent Assays (ELISA) Protocol Detection of Antibodies (_Chlamydia trachomatis_, HIV-1/2, HSV-2, HCV) and Antigen (HBsAg, HBV) V.2

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We use this protocol and it's working

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Abstract

This protocol describes a comprehensive multisero logical algorithm for the detection of major sexually transmitted infections (STIs), including hepatitis B virus (HBV), hepatitis C virus (HCV), herpes simplex virus type 2 (HSV -2), and Chlamydia trachomatis assays and automated confirmations using the Abbott Diagnostic.

Enzyme-linked immunosorbent assays (ELISAs) are the most common immunoassay type and used to detect the presence and measure the amount/concentration of specific antibodies or antigens in a sample. In diagnostics, ELISAs are frequently used to determine whether and how many antibodies have been produced in response to pathogen exposure or vaccination.

This protocol outlines a standardized ELISA procedure suitable for the qualitative detection of: - HIV-1/2, HSV-2 (type-specific gG-2) IgG, HCV, Chlamydia trachomatis antibodies and HBsAg (Hepatitis B surface antigen).

The procedure follows kit-specific IFUs, ISO 15189 and CLSI standards and provides a reproducible and traceable workflow for diagnostic and research laboratories.

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

Materials Provided: Reagents (typical kit components)

Each Test System contains the following components in sufficient quantities to perform the number of tests indicated on the packaging label.
1. Plate: 96 wells configured in twelve, 1×8-well, strips coated with affinity antibody or purified antigen. The strips are packaged in a strip holder and sealed in an envelope with desiccant.
2. Conjugate: Conjugated HRP (horseradish peroxidase) goat anti-human IgG 15mL
3. Positive Control : 0.35mL
4. Calibrator : 0.5mL
5. Negative Control : 0.35mL
6. SAVe Diluent®: 30mL bottle containing Tween-20, bovine serum albumin and phosphate-buffered-saline, (pH 7.2 ± 0.2).
7. TMB: 15mL, amber-capped, amber bottle containing 3, 3′, 5, 5′ tetramethylbenzidine (TMB).
8. Stop Solution: 15ml, bottle containing 1M H_2SO_4, 0.7M HCl.
9. Wash Buffer Concentrate (10X):

Equipment required but not provided
1. ELISA microwell reader capable of reading at a wavelength of 450nm.
2. Pipettes capable of accurately delivering 10 - 200µL.
3. Multichannel pipette capable of accurately delivering 50 - 200µL.
4. Reagent reservoirs for multichannel pipettes.
5. Manual Wash bottle or microwell washing system.
6. Distilled or deionized water.
7. One liter graduated cylinder.
8. Serological pipettes.
9. Disposable pipette tips.
10. Paper towels.
11. Laboratory timer to monitor incubation steps.
12. Disposal basin and disinfectant (i.e.: 10% household bleach - 0.5% Sodium Hypochlorite).
13. Incubator or controlled room temperature bench.

Reagents for Specific Procedure of Detection of Ag HBs: for Sandwich ELISA
1. Coating Buffer
   - Anhydrous Na_2CO_3, 1.5 g
   - Anhydrous $NaHCO_3$, 2.93 g
   - Distilled water, 1 liter, pH to 9.6
   - For an alternative coating buffer use ELISA coating buffer
2. Blocking buffer
   - Phosphate Buffered Saline (PBS) containing 1% w/v BSA
   - For an alternative blocking buffer
3. Wash buffer

- Phosphate Buffered Saline (PBS) containing 0.05% v/v Tween®-20
- For an alternative wash buffer use ELISA wash buffer

4. Recommended Substrates and Stop Solutions
- TMB Core+, for use with HRP-conjugated antibodies. Stop with 0.2M H2SO4.pNPP, for use with alkaline phosphatase-conjugated antibodies. Stop with 1M NaOH.

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. Handle all specimens as potentially infectious; Stop Solution: Corrosive and toxic. Avoid contact and inhalation. TMB and Wash Buffer: Irritating to skin and eyes. Sodium Azide (present in some reagents) can form explosive residues in metal pipes—flush drains with plenty of water. Avoid light exposure to reagents, especially TMB. Dispose of waste using approved biohazard procedures. 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.

Before start

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

Preparation:
- Bring all reagents to 20–25 °C before use.
- Mix gently by inversion; avoid foaming.
- Prepare 1:21 sample dilution (10 µL serum + 200 µL diluent), unless otherwise specified.
- Protect TMB from light.

Method

1 Coat microtiter plate wells with 100 µl of the appropriate coating antibody, at a concentration between 1-10 µg/ml in coating buffer. Cover the plate and incubate overnight at 4°C. Wash the plate 5 times in PBS.
Add 200 µl of blocking buffer to each well. Incubate for 60 min at RT. Wash 5 times in wash buffer.
Dilute samples (typically 1:21).
Add 100 µL sample , incubate 25 min.Wash x5.
Add 100 µL Conjugate, incubate 25 min.Wash x5.
Add 100 µL TMB, incubate 10–15 min.
Add 50 µL Stop Solution , mix.
Read at 450 nm ≤ 30 min.

Quality Assurance

2 Define run acceptance criteria (Required each run: RB, NC, CAL x3, PC) in the laboratory SOP. Compute mean OD of CAL triplicate (discard any ▯15% from mean). Accept run if QC criteria (kit-specific) are met. Maintain equipment calibration logs and temperature records. Participate in external quality assessment (EQA) when available.
Typical acceptance (unless otherwise stated): NC ≤ 0.250 - CAL ≥ 0.300 - PC ≥ 0.500 - NC/CAL ≤ 0.90 - PC/CAL ≥ 1.25 If any QC fails, invalidate and repeat run.

Calculation of Cutoff and Results

3 Correction Factor (CF): per lot, see label.
Cutoff OD = CF × Mean CAL OD.
Index (OD Ratio) = Sample OD ÷ Cutoff OD.
Index Interpretation Action ≤ 0.90 Negative Report as non-reactive 0.91–1.09 Equivocal Retest in duplicate; confirm if still equivocal ≥ 1.10 Positive Report as reactive; confirmatory testing required

Confirmatory 26 Test-Specific Notes

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Test	Target	Follow-up confirmation
HIV-1/2 Ab	Antibody	Perform **HIV-1/2 differentiation immunoassay** and/or **NAT** per national algorithm.
HSV-2 gG-2 IgG	Type-specific antibody	**Serology is qualitative** and indicates exposure; does **not** distinguish active vs. past infection. For active lesions, collect a **NAAT/PCR from lesion swab**. Equivocal results: **retest/alternate method** or **repeat specimen in 1–3 weeks**.
HCV Ab	Antibody	Confirm with **HCV RNA (NAT)** to confirm active infection.
Chlamydia trachomatis	Antigen or antibody	Confirm with **NAAT** (swab/urine).
HBsAg	Antigen	Confirm with **neutralization** or alternate method; interpret with full HBV serologic profile.

Storage and Stability

5 Store kits and reagents as specified by the manufacturer (commonly 2–8 °C). Protect TMB from light. Store processed data and plate maps per institutional policy (≥2 years or as required).

Data Storage and Confidentiality

6 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

7 Early infection may yield false negatives (window period). Cross-reactivity possible; follow kit-specific validation data. Hemolytic, lipemic, or icteric samples may interfere. ELISA is qualitative, not quantitative. Confirm all reactive results with an appropriate secondary test.

Troubleshooting Summary

8 Problem: High background (NC OD above limit) Possible Cause: Insufficient washing; reagent contamination; plate not dried Solution: Increase wash cycles; verify washer; tap plate dry; use fresh reagents
Problem: Low PC / calibrator signal Possible Cause: Expired/degraded reagents; improper temperatures; short incubation Solution: Check lot/expiry; adhere to times/temps; verify reader wavelength
Problem: High duplicate CV Possible Cause: Pipetting error; bubbles; edge effects Solution: Use calibrated pipettes; remove bubbles; avoid using edge wells if

recommended
Problem: Weak sample signals Possible Cause: Specimen degradation; incorrect dilutions Solution: Confirm storage history; re-test with fresh aliquot; verify dilution scheme
Problem: All wells low (incl. PC) Possible Cause: Conjugate or substrate omitted; HRP inactivated Solution: Audit steps; prepare fresh conjugate/substrate; confirm HRP activity.

Quick Bench Card

9 ELISA Summary (All kits) - Dilute sample → Add 100 µL → Incubate 25 min → Wash ×5 - Add Conjugate 100 µL → Incubate 25 min → Wash ×5 - Add TMB 100 µL → Incubate 10–15 min → Add Stop 50 µL → Read 450 nm ≤30 min - Required QC: RB, NC, CAL×3, PC - Interpretation: Neg ≤0.90 | Eq 0.91–1.09 | Pos ≥1.10 - Confirmatory tests: HIV → Immunoblot/NAT; HCV → RNA; CT → NAAT; HBsAg → Neutralization/Panel.

Workflow Algorithm (Text-Based Flow)

10 Sample Reception → Verification of identification → Serum/Plasma preparation → Initial Rapid Screening (CTK Biotech Syphilis HIV Ab PLUS Combo) ↓ If HIV Reactive → Confirm by Third-Generation ELISA (Biorad 26 Zeus Scientific) ↓ If Confirmed HIV Positive → Report and record in LIMS ↓ Perform ELISA Panel for HBsAg, HCV IgG, HSV-2 IgG, and Chlamydia trachomatis ↓ If HBsAg or HCV Reactive → Confirm using Abbott Diagnostic MEIA Consolidate all results → Quality Control verification → Biological validation → Final report issue

Protocol references

1) WHO and national guidelines for HIV, HBV, and HCV testing algorithms.
2) CDC. Sexually Transmitted Infections and Viral Hepatitis testing recommendations.
3) Manufacturer Instructions for Use (IFU) for each specific ELISA kit.
4) CLSI documents (e.g., EP05, EP12) for precision and qualitative test evaluation.