SPECIMENS FOR HIV TESTING

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Disclosures

- The speaker has nothing to disclose
Objectives

- Describe the various sample matrices used for HIV testing, including serum, plasma, whole blood, oral fluids and dried blood spots
- Summarize the points to consider for specimen collection, handling and storage
- Discuss the selection of laboratory tests for HIV diagnosis and management
Spectrum of HIV Tests

- Laboratory-Based Immunoassays (IAs)
- Rapid Tests (Point-of-Care or Near Patient)
- Western blot (WB), Immunofluorescence (IFA)
- NAAT for Proviral DNA or RNA
- Molecular Viral Load Assays
- Antiretroviral Drug Susceptibility Tests
  - Genotype
  - Phenotype
  - Tropotype
Selection of Tests to Perform

- Depends on the potential population
- The clinical situation
- Intended use of the assay
# Test Selection

<table>
<thead>
<tr>
<th>CLINICAL SITUATION</th>
<th>RECOMMENDED TESTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood donor screening</td>
<td>Antibody screening IAs; Ag/Ab combo IAs; WB; RNA-specific NAAT</td>
</tr>
<tr>
<td>Routine screening/diagnosis of HIV infection (excluding infants and acute infection)</td>
<td>Ab-only (lab-based and rapid POC) or Ag/Ab combo screening IAs; WB</td>
</tr>
<tr>
<td>Acute HIV infection</td>
<td>Ag/Ab combo screening IAs; RNA or proviral DNA NAAT</td>
</tr>
<tr>
<td>Infant (≤18 months of age) born to an HIV-infected mother</td>
<td>RNA or proviral DNA NAAT</td>
</tr>
<tr>
<td>Indeterminate or negative HIV-1 Western blot</td>
<td>RNA or proviral DNA NAAT; repeat screening IAs and WB in ~2-3 weeks; perform HIV-2-specific Ab screening IAs and WB</td>
</tr>
<tr>
<td>Prognosis</td>
<td>RNA-specific molecular quantification</td>
</tr>
<tr>
<td>Response to therapy</td>
<td>RNA-specific molecular quantification</td>
</tr>
<tr>
<td>Antiretroviral drug resistance</td>
<td>Genotypic, phenotypic, tropotypic drug susceptibility assays</td>
</tr>
</tbody>
</table>
Specimen Matrices

- Serum or plasma are the most widely used.
- Tests that use other fluids have distinct uses and advantages.
- Other specimen matrices:
  - Whole blood (both fingerstick and venipuncture)
  - Oral fluids
  - Urine
  - Cerebrospinal fluid
  - Cadaveric blood
  - Dried blood spots (DBS)
Collection of Serum or Plasma

- Serum has been the industry standard for years
- Plasma can be used as an acceptable alternative
- For obtaining serum, collection tubes should not contain preservatives or anticoagulants

- Typical anticoagulants for plasma collection
  - Potassium EDTA
  - Sodium & Lithium heparin
  - Sodium citrate
  - Acid-citrate-dextrose

- Invert tubes to mix
Blood Specimen Processing

- Serum or plasma should be removed from clotted blood or anticoagulated red cells ASAP to avoid hemolysis.
- Blood collection tubes with gel barriers can be used for rapid and efficient separation of serum or plasma from other blood components.
Blood Specimen Concerns

- Best results generally observed for serum or plasma that are clear and nonhemolyzed
- Lipemic, icteric, or hemolyzed specimens should be avoided when possible
- Specimens containing unremoved clots, red blood cells, or particulate matter may give inconsistent results
  - Clarify by centrifugation before testing
- Specimens with obvious microbial contamination should not be used
Blood Specimen Storage

- **UNPROCESSED BLOOD**
  - Maintain at 2-8°C shortly after collection and during transport to laboratory
  - If kept at RT, do not hold for extended time

- **PROCESSED SERUM OR PLASMA**
  - May be stored at 2-8°C pending test completion (usually no longer than 48-72 h, but can be up to 7 days); long-term storage at -20°C or colder
  - If an extended delay in transport or testing is anticipated, freeze sample at -20°C or colder
  - Avoid repeat freezing and thawing
  - Do not store samples in frost-free freezers
  - Heat inactivation of specimen is not recommended unless otherwise specified
Blood Specimen Shipping

- Preferable that blood samples be processed before shipping
- Understand not always possible
- Can ship whole blood at 2-8°C or RT
- Serum or plasma can be shipped cold or frozen at -20°C or colder (dry ice)
- Ship in compliance with applicable state, federal, international regulations
Specimens Compatible with ARCHITECT HIV Ag/Ab Combo Assay

<table>
<thead>
<tr>
<th>Glass Tubes</th>
<th>Plastic Tubes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>Serum</td>
</tr>
<tr>
<td>K$_3$EDTA</td>
<td>Serum Separator (SST)</td>
</tr>
<tr>
<td>Na$_2$EDTA</td>
<td>Lithium heparin with gel</td>
</tr>
<tr>
<td>Sodium heparin</td>
<td></td>
</tr>
<tr>
<td>K$_2$EDTA</td>
<td>K$_2$EDTA with gel (PPT)</td>
</tr>
</tbody>
</table>

We prefer plasma as our specimen of choice and prefer that blood be collected in plasma preparation tubes (PPT) with K$_2$EDTA with gel.

We always recommend that collection tubes be filled to top with blood to assure enough processed plasma to complete all HIV-related testing.

Can have up to 5 freeze-thaw cycles.
Matching Specimens and Tests

<table>
<thead>
<tr>
<th>Assay</th>
<th>Serum</th>
<th>Plasma in:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>K$_2$EDTA</td>
</tr>
<tr>
<td>HIV Ag/Ab Combo</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(w/ or w/o separator)</td>
</tr>
<tr>
<td>HAVAB (anti-HAV IgG)</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HAVAB-M (anti-HAV IgM)</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HBsAg (including confirmation assay)</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td>CORE (anti-HBc total)</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CORE-M (anti-HBc IgM)</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUSAB (anti-HBs)</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCV (anti-HCV)</td>
<td>YES</td>
<td>YES</td>
</tr>
</tbody>
</table>

Available commercial HIV Western blot assays can be done on serum or plasma as well.
Rapid HIV Antibody Assays

- Used in acute care settings, EDs, STD clinics, medical field setting, and developing countries
- Currently, 7 approved by FDA; only 4 are CLIA-waived
- Multiple specimen types - oral fluids, whole blood, serum, plasma
- Oral fluids a reliable specimen for detecting antibodies to HIV-1
  - Noninvasive specimen collection procedure
  - Greater safety, increased patient compliance, an alternative to phlebotomy
  - POC and In-Home HIV tests (OraSure OraQuick)
<table>
<thead>
<tr>
<th>Assay</th>
<th>Specimen Type</th>
<th>CLIA Category</th>
<th>Virus Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>OraQuick ADVANCE</td>
<td>Oral Fluid</td>
<td>W</td>
<td>HIV-1/2</td>
</tr>
<tr>
<td></td>
<td>Whole Blood</td>
<td>W</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Plasma</td>
<td>MC</td>
<td></td>
</tr>
<tr>
<td>Uni-Gold Recombigen</td>
<td>Whole Blood</td>
<td>W</td>
<td>HIV-1</td>
</tr>
<tr>
<td></td>
<td>Serum/Plasma</td>
<td>MC</td>
<td></td>
</tr>
<tr>
<td>Reveal G-3</td>
<td>Serum</td>
<td>MC</td>
<td>HIV-1</td>
</tr>
<tr>
<td></td>
<td>Plasma</td>
<td>MC</td>
<td></td>
</tr>
<tr>
<td>Multispot</td>
<td>Serum</td>
<td>MC</td>
<td>HIV-1/2 Differentiates</td>
</tr>
<tr>
<td></td>
<td>Plasma</td>
<td>MC</td>
<td></td>
</tr>
<tr>
<td>Clearview STAT-PAK</td>
<td>Whole Blood</td>
<td>W</td>
<td>HIV-1/2</td>
</tr>
<tr>
<td></td>
<td>Serum/Plasma</td>
<td>MC</td>
<td></td>
</tr>
<tr>
<td>Clearview COMPLETE</td>
<td>Whole Blood</td>
<td>W</td>
<td>HIV-1/2</td>
</tr>
<tr>
<td></td>
<td>Serum/Plasma</td>
<td>MC</td>
<td></td>
</tr>
<tr>
<td>INSTI HIV-1</td>
<td>Whole Blood</td>
<td>MC</td>
<td>HIV-1</td>
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<td></td>
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We prefer plasma as our specimen of choice and prefer that blood be collected in plasma preparation tubes (PPT) with $K_2$EDTA with gel.

Concerns for RNA degradation; avoid extreme environmental conditions and nucleases (RNases).
Specimen Storage:

- Whole blood, plasma, or serum may be stored for up to 72 hours from time of draw at ≤25°C (RT).
- Specimens should not be stored at temperatures between 25-30°C for more than 24 h.
- Processed specimens may be stored an additional five days at 2-8°C.
- Can have up to 3 freeze-thaw cycles.
- Long-term storage at -70°C.
Specimen Considerations for HIV-1 RNA Quantitative Assays

- Viral load assays require plasma collected in K$_2$EDTA.

- Plasma-Preparation Tubes (PPT) are most helpful:
  - Provides means for collection, processing and transport of neat plasma in a closed evacuated system.

- Other acceptable EDTA-containing tubes can be used, but then changes the processing requirements.

- Specimens need to be centrifuged to separate cells from plasma within specified time limits.

- Processing times may vary depending on assay used.

- Volume of specimen required generally higher than for other HIV tests.
Plasma Storage in PPT and Viral Load

- Specimens should be processed without significant delay
- Freshly collected whole blood specimens
  - Can be held at 15-30°C (RT) for up to 6 h or at 2-8°C for up to 24 h prior to processing
- Plasma removed from blood
  - May be stored at 15-30°C for up to 24 h or at 2-8°C for up to 5 days without significant loss of viral RNA
- No more than 3 freeze/thaw cycles
- Once thawed, plasma can be stored at 2-8°C for up to 6 h before testing
- Long-term storage of plasma at -70°C or colder
Plasma Storage in PPT and Viral Load

- Previous studies: Freezing of plasma in situ in PPT results in elevated HIV-1 viral loads when quantified in Roche COBAS Amplicor HIV-1 Monitor assay.
- For current Abbott RealTime HIV-1 RNA Assay, plasma separated in PPTs can be stored frozen in situ until time of testing with no effect on viral load results.
Dried Blood Spots (DBS)

- Collect and store whole blood on filter paper
- Appears to be suitable for:
  - Qualitative DNA or RNA analysis for diagnosis of infections in infants, others
  - Quantitative viral load measurements
  - Gene sequencing for detection of drug resistance
- Easy way to collect and ship specimens for testing; particularly important in resource-limited settings
- Precludes need for phlebotomist and maintenance of cold chain transport and storage
- Facilitates testing of specimens with limited collection volume
Whatman 903 Cards

- Filter paper is relatively inexpensive and readily obtained
- DBS card samples can be prepared from a few drops of blood; typically 15-200 μl; 50-100 norm
- Make 6 or more spots/patient
- Blood can be obtained by finger stick
- Viral RNA and DNA, antibodies or other proteins, and antiviral drugs or their metabolites remain stable on DBS cards for relatively long times when stored with dessicant in closed bags at RT or below
- Cards are small and light and safe and easy to transport
- Punched out disks (3-6 mm) from card are used
Whatman 903 Cards

NAME  CTC  904

DATE   28/3-08
New Proposed HIV Testing Algorithm

A1: 4th generation HIV-1/2 immunoassay

- A1+
  - Negative for HIV-1 and HIV-2 antibodies and p24 Ag
- A1(-)

A2: HIV-1/HIV-2 differentiation immunoassay

- HIV-1 +
  - HIV-1 antibodies detected
  - Initiate care (and viral load)
- HIV-2 +
  - HIV-2 antibodies detected
  - Initiate care
- HIV-1&2 (-)
  - NAAT
    - NAAT
      - Acute HIV-1 infection
      - Initiate care
    - NAAT
      - Negative for HIV-1

Conclusions

- Proper specimen collection and processing is vital to downstream performance of HIV tests.
- There must be constant communication between laboratory and providers and continuous education on appropriate procedures.
- Depending on the testing performed, judicious selection and standardized handling of specimens can have a positive impact on the overall process.