Epidemiologists and Laboratory Science

Najib Aziz, M.D.
Adjunct Associate Professor
Department of Epidemiology
UCLA, Fielding School of Public Health
January 2014
Introduction:

• Advance in the laboratory science technologies enable us to detect trace amount of material of interest and determine from what organism it came from.

• Epidemiology describes the distribution of health and disease in a population and determinant of that distribution.

• The laboratory science tools enable the epidemiologist to moves the epidemiologic studies beyond the detection of risk factors and probable transmission mode, to identify mechanisms of disease pathogenesis and describe transmission systems.

• Every laboratory measurement is subject to error from variety source and the goal should be to reduce the error source so the true biological variation can be observed.

• Laboratory test is only useful if it is reliable, valid and interpreted appropriately. The validity and reliability of the test assured by quality control and quality assurance programs.
Planning
- Define the possible causes of the outbreak
- Decide which clinical specimens are required to confirm the cause of the outbreak.

Selection of the laboratory for testing
- Decide who will collect, process and transport the specimens
- Define the procedures necessary for specimen management
Quality Assurance Cycle of Laboratory Work

1. Pre-Analytic:
   a) Patient or subject Preparation
   b) Sample Collection
   c) Personnel Competency Test Evaluations
   d) Sample Receipt and Accessioning
   e) Sample Transport

1. Analytic:
   a) Quality Control
   b) Testing

2. Post-Analytic:
   a) Reporting
   b) Record Keeping
The Quality Assurance Cycle

- Data and Lab Management
- Safety
- Customer Service
- Patient/Client Prep
- Sample Collection
- Sample Receipt and Accessioning
- Sample Transport
- Quality Control
- Record Keeping
- Personnel Competency Test Evaluations
- Reporting

CDC
Optimal laboratory work flow for an investigational or outbreak project

Minimizing of the potential error
Specimens collection
Transportation
Processing
Storage
Data analyze
1. Specimen collection

- Type of specimen
- Time of collection (effect of circadian rhythms)
- Who collect the specimen (Subject, Research associate, investigator)
  
  trained and retrained periodically to ensure protocols are followed

- How collect the specimen (e.g. clean-catch midstream of urine)
- Quality of specimen collected
- Specimen labeling
Specimen Type:

- Blood for smears culture
- Blood for Serum plasma
- Cerebrospinal Fluid (CSF)
- Stool samples
- Nasopharyngeal swabs
- Urine
- Water sample
- Blood for
- Blood for
- Blood for
- Blood for
- Sputum
- Throat swabs
- Rectal swabs
- Saliva
- Food sample
Vacutainer Venous Blood collection tubes

Gold  Red  Gray
Light green  Tiger green  Red top  Orange  Royal Blue  Green
Gray  Tin  Lavender  White  Yellow  Pink  Light Blue
Urine collection Device

Saliva collection Device

Swabs collection Device
Whole blood:

- Cellular elements (red blood cells or RBC, white blood cells or WBC, and platelets or PLT)
- Liquid component, which is either serum or plasma.
- In general, adult blood has about 40% cellular elements and 60% serum or plasma

*Serum* is the liquid expressed from clotted blood does not contain fibrinogen or other coagulation factors, preferred specimen for most chemistry, blood bank and serology tests.

*Plasma* is the liquid portion of the blood present in anticoagulated specimens. Plasma contains all the coagulation factors, including fibrinogen.
Common errors affecting all types of specimens

- Failure to label a specimen correctly and to provide all pertinent information
- Failure to use the correct container/tube for appropriate specimen preservation
- Insufficient quantity of specimen to run test or QNS (quantity not sufficient).
- Inaccurate and incomplete subject instructions prior to collection.
- Failure to tighten specimen container lids, resulting in leakage and/or contamination of specimens
SERUM PREPARATION ERRORS

- Failure to separate serum from red cells within 60 minutes of venipuncture
- Failure to allow the specimens to clot before centrifugation.
- Hemolysis: red blood cells break down and components spill into serum
- Lipemia: cloudy or milky serum sometimes due to the patient's diet
PLASMA PREPARATION ERRORS

- Failure to collect specimen in correct additive.
- Failure to mix specimen with additive immediately after collection.
- Hemolysis or damage to red blood cells breakdown.
- Incomplete filling of the tube, thereby creating a dilution factor excessive for total specimen volume (QNS).
- Failure to separate plasma from cells within 30-45 minutes of draw.
- Failure to label transport tubes as "plasma".
- Failure to indicate type of anticoagulant (eg, "EDTA", "citrate", etc.)
URINE COLLECTION ERRORS

- Failure to obtain a clean-catch, midstream specimen.
- Failure to refrigerate specimen or store in a cool place.
- Failure to provide a complete 24-hour collection/aliquot.
- Failure to add the proper preservative to the urine collection container prior to collection of the specimen.
- Failure to provide a sterile collection container and refrigerate specimen when bacteriological examination of the specimen is required.
- Failure to tighten specimen container lids, resulting in leakage of specimen.
- Failure to provide patients with adequate instructions for 24-hr urine collection.
2. Transport of specimen to laboratory

- Inaccuracy in labeling of specimen
- Labeling lost during transport
- Transport media improperly prepared
- Specimen heated or cooled during transport
- Delays or inconsistent transport time
- Specimen lost during transport to laboratory
3. Specimen Processing

- Specimen Processing is one of the first critical pre-analytical steps in laboratory Science.

- Each specimen checked for proper identification, specimen integrity, and then accessioned into laboratory computer system.

- Specimens are processed per the study SOP, stored or distributed to the appropriate laboratory for testing.
Blood after centrifugation
Regardless of who collects the specimens, having an easy-to-follow instructions, color coding or numbering materials to match steps, and providing packets containing all materials and instructions together will reduce errors in specimen collection.

Determining the required storage conditions and tolerance for storage is an important component of protocol development.

A small amount of specimen stored in a large vial may result in specimen loss due to evaporation.

The label should be able to tolerate the storage conditions, because some labels fall off when a vial is frozen and thawed.
4. Storage, Repository or Biobank

The process of collecting, maintaining, and sharing biological samples is viewed as an essential tool in the modern landscape of the genetic, medical, and behavioral sciences and we should;

- Keep precious biological samples safe and in a secure place
- Check and log freezers temperature daily.
- Locate samples quickly and easily through computer base program to access sample reporting and retrieval easily.
- Retrieval and shipping of biological samples while maintaining the cold environment.
- Trained and certified personnel with compliance to IATA, DOT and ICAO regulations should ship the specimen.
Diagnostics test for infectious disease
5. Laboratory Test

- The laboratory test should reflect the true value and be valid and it depends on two major classes of errors.

1. Random error: It occurs without prediction or regularity.

Factors affecting the precision of an assay include:
- Temperature fluctuations
- Unstable instrumentation
- Change in reagents
- Manual techniques variation (pipetting, mixing, timing)
- Operator variation
2. Systemic error (bias): (occur one direction, over or under estimation)

It is defined as error that the results consistently low or high.

A) Constant error: If the error is consistently low or high by the same amount over the entire concentration range.

B) Proportional Error: If the error is consistently low or high by an amount Proportional to the concentration of the analyte. (e.g. incorrect assignment of calibrator)

Avoid of systemic error:
- Set of inclusionary and exclusionary criteria
- As similar as possible, collect, store, and process of specimen for all participants.
- Arranging and selecting a laboratory procedure so that any effects of storage and testing equally impact all specimens.
A Constant Error

Perfect agreement

y-intercept is not at zero (0).

B Proportional Error

Perfect agreement

Proportional error

Slope of the regression line is not close to 1.00.

C Random Error

The scatter about the regression line is large
UCLA Multicenter AIDS Cohort Study (MACS)

Repository
Quality Assurance
Blood Collection for MACS

A) 6 x 10mL Green top tube (sodium heparin) for local and national cells and plasma storage

B) 7 x 10 mL (SST) Red top or gold blood tube for local, national serum storage, HIV-1 and Chemistry testing.

C) 3x 5 mL Lavender top blood tube for cell phenotyping by Flow cytometry, Complete blood count and HbA1c testing.

D) 2 x10 mL lavender top blood tube for plasma storage and HIV viral load testing.
## Specimen allocation

<table>
<thead>
<tr>
<th></th>
<th>UCLA Repository</th>
<th>NIH Repository</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plasma</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heparin</td>
<td>3 vials of 3 mL</td>
<td>1 vial of 3 mL</td>
</tr>
<tr>
<td></td>
<td>3 vials of 1.0 mL</td>
<td>6 vials of 0.5 mL</td>
</tr>
<tr>
<td>EDTA</td>
<td></td>
<td>4 vials of 0.5 and 2 vials of 1.0 mL</td>
</tr>
<tr>
<td><strong>Serum</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 vials of 3 mL</td>
<td>2 vial of 3 mL</td>
</tr>
<tr>
<td></td>
<td>1 vial of 1 mL for HIV</td>
<td>6 vials of 0.5 mL</td>
</tr>
<tr>
<td><strong>PBMC</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Viable cells</td>
<td>3 vials at 10^7 cells/mL</td>
<td>3 vials at 10^7 cells/mL</td>
</tr>
<tr>
<td></td>
<td>2 vials at 2.5x10^6</td>
<td>2 vials at 2.5x10^6</td>
</tr>
<tr>
<td>Cell pellets</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
PBMC Quality Control

A) Internal QC

1. QC of cells counter:

Every other week two samples of PBMC are counted using Lab Z1 Coulter counter and CTRL Sysmex Hematology analyzer and result from both methods are evaluated and the %CV of both methods should be within 15.

2. QC of laboratory tech:

Every other week 10 mL of blood from a normal donor process and freeze and then thaw by the tech and evaluate for cell viability and viable cell recovery.
B. External Quality Assurance

1. To ensure the adequate recovery of functionally viable cells from each vial, the MACS established a prospective quality assessment (QA) program in July 2004 to evaluate the PBMC cryopreserved in the four MACS laboratories.

2. In this program, randomly selected vials of recently cryopreserved PBMC from all MACS laboratories are evaluated three times a year, not only for viable cell recovery but also for phenotype and function of the recovered cells.
Viable Cell
Proliferative responses of cryopreserved PBMC ($n = 28$ per group) to phytohemagglutinin (PHA).

The stimulation index was defined as the counts per minute in the presence of PHA divided by the counts per minute in the absence of PHA.
T lymphocyte phenotypes in fresh whole-blood and cryopreserved PBMC

<table>
<thead>
<tr>
<th>Percentage type</th>
<th>CD3⁺ (%)</th>
<th>CD3⁺ CD4⁺ (%)</th>
<th>CD3⁺ CD8⁺ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fresh</td>
<td>Cryopreserved</td>
<td>Fresh</td>
</tr>
<tr>
<td>Mean</td>
<td>76</td>
<td>76</td>
<td>35</td>
</tr>
<tr>
<td>Minimum</td>
<td>47</td>
<td>40</td>
<td>10</td>
</tr>
<tr>
<td>Maximum</td>
<td>89</td>
<td>92</td>
<td>63</td>
</tr>
<tr>
<td>Median</td>
<td>76</td>
<td>78</td>
<td>36</td>
</tr>
<tr>
<td>IQR</td>
<td>10.1</td>
<td>10.1</td>
<td>31.3</td>
</tr>
</tbody>
</table>

*IQR is the interquartile range, the difference between the 25th and 75th percentiles.*
Enzyme linked immunosorbent assay (ELISA).

As screen test

- Following viral exposure, the first antibody to appear is IgM, which is followed by a much higher titre of IgG.

- In cases of reinfection, the level of specific IgM either remain the same or rises slightly. But IgG shoots up rapidly and far more earlier than in a primary infection.

- Many different types of serological tests are available such as ELISA, RIA, etc.

- Newer techniques such as ELISA offer better sensitivity, specificity and reproducibility than classical techniques.
(ELISA continu)

• **Principle:**
  • Use of enzyme-labelled immunoglobulin to detect antigens or antibodies

  • signals are developed by the action of hydrolyzing enzyme on chromogenic substrate

  • optical density measured by micro-plate reader
(a) Indirect ELISA

1. Antigen-coated well
2. Add specific antibody to be measured
3. Add enzyme-conjugated secondary antibody
4. Add substrate (S) and measure color
5. Wash

(b) Sandwich ELISA

1. Antibody-coated well
2. Add antigen to be measured
3. Add enzyme-conjugated secondary antibody
4. Add substrate and measure color
5. Wash

(c) Competitive ELISA

1. Incubate antibody with antigen to be measured
2. Add Ag-Ab mixture to antigen-coated well
3. Add enzyme-conjugated secondary antibody
4. Add substrate and measure color
5. Wash
HIV Serological Profile

- **p24 Antigen**
- **IgM Antibody**
- **IgG Antibody**

**Concentration**

**Months Post Infection**
HIV-1/2 Plus 0 ELISA Screen test:

- Sandwich enzyme-linked immunosorbent assay (ELISA) HIV-1,2 +O based on the principle of the direct antibody sandwich.

- Microwell strip plates (Solid phase) are coated with purified antigens: gp 160 and p24 recombinant proteins derived from HIV1, peptide from HIV-2 (gp36) and synthetic ploypeptide of HIV-1 group O.

- Sample and controls are added to the wells along with specimen diluents and incubated.
1) **GS HIV Combo Ag/Ab EIA**
   The GS HIV Combo Ag/Ab EIA is an enzyme immunoassay kit for the simultaneous qualitative detection of HIV p24 antigen and antibodies to HIV Type 1 (HIV-1 groups M and O) and HIV Type 2 (HIV-2) in human serum and plasma.

2) **GS HIV-1/HIV-2 PLUS O EIA**
   This recombinant and synthetic peptide EIA test is used to detect antibodies to human immunodeficiency virus type 1 (HIV-1 groups M and O) and/or type 2 (HIV-2) in human serum, plasma, or cadaveric serum specimens.

3) **HIV-1 Western Blot Confirmatory Test**
Clear determination of positive and negative Results
GS HIV-1 Western Blot is a qualitative assay for the detection and identification of antibodies to human immunodeficiency virus type 1 (HIV-1) that allows same-day results.
HIV-1 Western blot results at three MACS clinic visits. K0, K1, and K2 are negative, weak positive, and strong positive controls, respectively. P1, P2, and P3 are before, during (6 months later), and 7 months after seroconversion, respectively. NC is an HIV-1 negative-control plasma sample.
**Interpretation:**

**NEGATIVE:** No bands are present

**POSITIVE :** At least TWO of the major bands: gp160/120, gp41 or p24 must be present. Bands must be at least as intense as the Low Positive control gp120 band (a reactivity score of + or greater) to be considered POSITIVE. The band at gp41 must be broad and diffuse.

**INDETERMINATE:** Any pattern of one or more bands are present but the blot does not meet the criteria for positive results. Indeterminate results should not be considered either POSITIVE or NEGATIVE.

Repeat the indeterminate immunoblot using the original specimen. If the sample is still indeterminate, retest using fresh sample drawn 2-4 weeks after and again three and six months after the original draw.
Quality Control

A) Internal QC

1) Three Kit controls (Negative, HIV-1 Low Positive Control and HIV-1 Positive Control)
2) Two Lab control (negative and HIV-1 high positive control)

B) External QC

CAP sends 5 serum/plasma samples quarterly
HIV Viral load test:

- Test is a nucleic acid amplification test for the quantitation of Human Immunodeficiency Virus Type 1 (HIV-1) RNA in human plasma

- This test can quantitate HIV-1 RNA over the range of 48 - 10,000,000 copies/mL

- Specimen volume required for this method is 1000 μL.

- Upon loading the sample in appropriate racks, nucleic acid extraction, amplification and detection are performed using the COBAS TaqMan HIV-1 Test, v2.0 software
COBAS® TaqMan® Analyzer

• COBAS® TaqMan® Analyzer or the COBAS® TaqMan® 48 Analyzer
## Four FDA-approved Rapid HIV Tests

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity (95% C.I.)</th>
<th>Specificity (95% C.I.)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Reveal G2</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- serum</td>
<td>99.8 (99.2 – 100)</td>
<td>99.1 (98.8 – 99.4)</td>
</tr>
<tr>
<td>- plasma</td>
<td>99.8 (99.0 – 100)</td>
<td>98.6 (98.4 – 98.8)</td>
</tr>
<tr>
<td><strong>Multispot</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- serum/plasma</td>
<td>100 (99.9 – 100)</td>
<td>99.9 (99.8 – 100)</td>
</tr>
<tr>
<td>- HIV-2</td>
<td>100 (99.7 – 100)</td>
<td></td>
</tr>
</tbody>
</table>
### Four FDA-approved Rapid HIV Tests

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity (95% C.I.)</th>
<th>Specificity (95% C.I.)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>OraQuick Advance</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- whole blood</td>
<td>99.6 (98.5 - 99.9)</td>
<td>100 (99.7-100)</td>
</tr>
<tr>
<td>- oral fluid</td>
<td>99.3 (98.4 - 99.7)</td>
<td>99.8 (99.6 – 99.9)</td>
</tr>
<tr>
<td>- plasma</td>
<td>99.6 (98.5 - 99.9)</td>
<td></td>
</tr>
<tr>
<td><strong>Uni-Gold</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Recombigen</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- whole blood</td>
<td>100 (99.5 – 100)</td>
<td>99.7 (99.0 – 100)</td>
</tr>
<tr>
<td>- serum/plasma</td>
<td>100 (99.5 – 100)</td>
<td>99.8 (99.3 – 100)</td>
</tr>
</tbody>
</table>
Reveal G2

- CLIA moderate complexity with serum, plasma
- Reconstitute and refrigerate reagents
- Screens for HIV-1
- Perform test in 5 minutes

Reactive

Negative

Read results immediately
Uni-Gold Recombigen

- CLIA-waived for finger stick, whole blood; moderate complexity with serum, plasma
- Store at room temperature
- Screens for HIV-1
- Results in 10 minutes

Read results in 10 - 12 minutes
OraQuick Advance HIV-1/2

- CLIA-waived for finger stick, whole blood, oral fluid; moderate complexity with plasma
- Store at room temperature
- Screens for HIV-1 and 2
- Results in 20 minutes
Collect oral fluid specimens by swabbing gums with test device.

Gloves optional; waste not biohazardous
Insert device; test develops in 20 minutes

Read results in 20 – 40 minutes
Multispot HIV-1 and HIV-2 Rapid Test

Image showing two test devices:
- Left device: Reactive Control
- Right device: Recombinant HIV-1, Peptide HIV-2, Peptide HIV-1

Bottom text:
- Negative
- HIV-1 & HIV-2 Positive
UCLA MACS Laboratory

Shaun Hsueh, Chantel Delshad, Yegermal Asnake
Wilshire LAMS Clinic
Photo N/A

MACS Harbor UCLA Clinic
Carlos Aquino, Lisa Siqueiros, Pedro Chavez

Los Angeles GLC
Ray Mercado, Eduardo Mercado, GLC staffs