MTN-003 Endpoint Confirmations Algorithm for Specimen Testing

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What is endpoint confirmation?

- Women who become HIV-infected while in VOICE are considered ENDPOINTS.
- The goal of VOICE is to determine the effectiveness of active product vs placebo by measuring # of seroconverters in each arm.
- The NL independently verifies all participants identified as being HIV infected.
NL Role in Endpoint Confirmations

- The NL tests:
  - A 10% random sample of participants’ specimens:
    - Study Entry
    - PUEV
    - Termination Visit
  - Seroconverters identified by SCHARP
  - An equal # of matched study entry and Follow-Up specimens from a random sample of uninfected participants
Endpoint Confirmation Process

Request shipment based on visit cutoff

Prepare shipments
Send to NL

Arrive in Pittsburgh
QA/QC

Send CRFs with results

Further testing if needed
Send corrected CRF to SCHARP

Conduct investigation if needed.

Send final investigation reports to SCHARP
Virology CORE Algorithm

Endpoint Confirmation and Follow-Up Testing for VOICE

START
Sample of unknown status received from site*

EIA

EIA

EIA x 2

NEGATIVE

INVESTIGATION

WB

Results do not match site report.

Results match site report.

SCHARP notifies NL with PTIDs of samples requiring further testing.**
Western Blot

- Confirm status of samples with positive or discordant EIA’s

- Test Characteristics
  - **POSITIVE**: 2 major bands at intensity of Low + gp120 band
    - gp160 and/or gp120
    - gp41
    - p24
  - **INDETERMINATE**: 1 or more bands present
    - Doesn’t meet Positive criteria
  - **NEGATIVE**: No bands present

- Early infection v. Chronic Infection
HIV-1 RNA

- Determine viral loads of patient specimens
- Cannot be used to diagnose HIV infection
- The role of VL testing in detecting infected participants at enrollment

- Which Samples get Viral Load testing?
  - Samples identified by SCHARP
  - Visit 3.0 of all seroconverters
Now you get to be NL…

- Results will be presented for each PTID
- Interpret the results for each case.

Think about:
- What test should be done next?
- What is the final HIV status?
- Is further testing necessary before it can be decided?
- Does this case require investigation?
CASE 1

- NL received plasma for PTID 1.
  What test is done first?
CASE 1

- EIA Results:

<table>
<thead>
<tr>
<th>Patient Code</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1 v3.0</td>
<td>0.039</td>
</tr>
<tr>
<td>Patient 1 v6.0</td>
<td>3.500 R+</td>
</tr>
</tbody>
</table>

Cutoff calculation: NCX + 0.250 = 0.283  NCX = 0.033
PC1X = 2.328  PC2X = 2.022  PC3X = 1.891

What tests should be done next?
**CASE 1**

**PTID 1 v3.0**

Do REPEAT EIA IN DUPLICATE

<table>
<thead>
<tr>
<th>Patient Code</th>
<th>Result</th>
<th># Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1 v3.0</td>
<td>0.039</td>
<td>Single</td>
</tr>
<tr>
<td>Patient 1 v3.0</td>
<td>0.062</td>
<td>DUPL</td>
</tr>
<tr>
<td>Patient 1 v3.0</td>
<td>0.053</td>
<td>DUPL</td>
</tr>
</tbody>
</table>

Cutoff calculation: NCX + 0.250 = 0.283  NCX = 0.033
PC1X = 2.320  PC2X = 2.022  PC3X = 1.891

**PTID 1 v6.0**

Do Western blot

What is the HIV status of this participant?
Virology CORE Findings

- Enrollment (v 3.0):
  - EIA Results indicate participant was NEGATIVE

- Follow-up Visit (6.0):
  - EIA Results indicate participant was POSITIVE
  - WB Results indicate participant is POSITIVE

- Conclusion:
  - Participant is HIV-positive at follow up.

- ARE WE FINISHED?
CASE 1

- Must do VL on v3.0 sample to ensure participant was not infected at enrollment.

- VL result v3.0:
  Target Not Detected
  <40 copies/ml
CASE 1

- What if the VL result for v3.0 was:
  - 487,884 copies/ml
  - Limit of detection 40 copies/ml

- Participant was:
  - Infected at enrollment
  - NOT a seroconverter

- NL must:
  - Send report to SCHARP for EAC evaluation
Virology CORE Algorithm

Endpoint Confirmation and Follow-Up Testing for VOICE

START
Sample of unknown status received from site*

EIA

- or ind

WB

Results do not match site report.

Positive

Results match site report.

SCHARP notifies NL with PTIDs of samples requiring further testing.**

INVESTIGATION

-/+ or +/+
CASE 2

- NL received plasma for PTID 2.
- EIA Results:

<table>
<thead>
<tr>
<th>Patient Code</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 2 v3.0</td>
<td>*3.500  R+</td>
</tr>
<tr>
<td>Patient 2 v5.0</td>
<td>*3.500  R+</td>
</tr>
</tbody>
</table>

Cutoff calculation: NCX + 0.250 = 0.281  NCX = 0.031

What tests should be done next and on what samples?
Now for the investigation...

What test should be done?
**CASE 2**

### EIA Results

<table>
<thead>
<tr>
<th>Patient Code</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 2 v3.0</td>
<td>*3.500 R+</td>
</tr>
<tr>
<td>Patient 2 v3.0</td>
<td>*3.500 R+</td>
</tr>
<tr>
<td>Patient 2 v5.0</td>
<td>*3.500 R+</td>
</tr>
<tr>
<td>Patient 2 v5.0</td>
<td>*3.500 R+</td>
</tr>
</tbody>
</table>

### WB Results

![WB Results Image]

**What’s next?**
Virology CORE Findings

- **At Enrollment (v 3.0):**
  - EIA Results indicate participant was **POSITIVE**
  - WB Results indicate participant is **NEGATIVE**
  - VL Result indicates participant is **POSITIVE**

- **At Follow-up (V 5.0):**
  - EIA Results indicate participant was **POSITIVE**
  - WB Results indicate participant is **POSITIVE**

- **Conclusion:**
  - Participant was **HIV-POSITIVE** at enrollment (v3.0)
NL received plasma for PTID 3.

EIA Results:

<table>
<thead>
<tr>
<th>Patient Code</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 4 v3.0</td>
<td>1.033 R+</td>
</tr>
<tr>
<td>Patient 4 v4.1</td>
<td>*3.500 R+</td>
</tr>
</tbody>
</table>

Cutoff calculation: NCX + 0.250 = 0.285
NCX = 0.035
PC1X = 2.300
PC2X = 2.768
PC3X = 2.127

What tests should be done next?
### CASE 3

**EIA Results**

<table>
<thead>
<tr>
<th>Patient Code</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 4 v3.0</td>
<td>1.033 R+</td>
</tr>
<tr>
<td>Patient 4 v4.1</td>
<td>3.500 R+</td>
</tr>
</tbody>
</table>

**Cutoff calculation:**

\[
\text{NCX} + 0.250 = 0.205 \quad \text{NCX} = 0.035
\]

\[
\text{PC1X} = 2.300 \quad \text{PC2X} = 2.768 \quad \text{PC3X} = 2.127
\]

Is the v 4.1 WB Positive?  YES

What would you do next?

**Increases to:**

\[
\text{NCX} + 0.250 = 3.005
\]
CASE 3

- With a low positive result for v 3.0 EIA and a negative result for the v 3.0 WB, what further testing would you consider?
- Viral Load on v 3.0

**VL Results (v 3.0)**

Copies / mL:

383,643
What happens if the NL results do not match the site report? (e.g. the site declared a participant to be HIV-negative at enrollment)

Must confirm that the site did not identify a false negative

Repeat Rapid Test of v3.0 at NL to compare with site results

Why does the Rapid Test give a negative result if the patient is HIV-positive?
Virology CORE Findings

- At Enrollment (v 3.0):
  - EIA Results indicate participant was POSITIVE
  - WB Results indicate participant is NEGATIVE
  - Rapid Test Results indicate participant is NEGATIVE
  - VL Result indicates participant is POSITIVE

- At Follow-up (V 4.1):
  - EIA Results indicate participant was POSITIVE
  - WB Results indicate participant is POSITIVE

- Conclusion:
  - Participant was HIV-POSITIVE at enrollment (v3.0)
Acknowledgements

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