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:
  - A10% random sample of participants'
    - 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

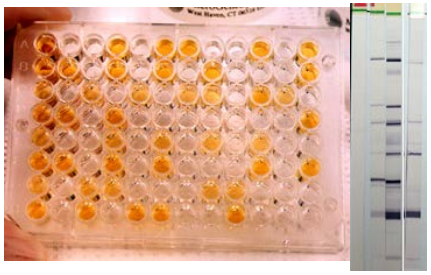
Endpoint Confirmation Process

Statistical Center for HIV/AIDS Research & Prevention
SCHARP

Request shipment based on visit cutoff

Prepare shipments
Send to NL

Arrive in Pittsburgh
QA/QC



Follow NL endpoint confirmation algorithm

Send CRFs with results

Statistical Center for HIV/AIDS Research & Prevention
SCHARP

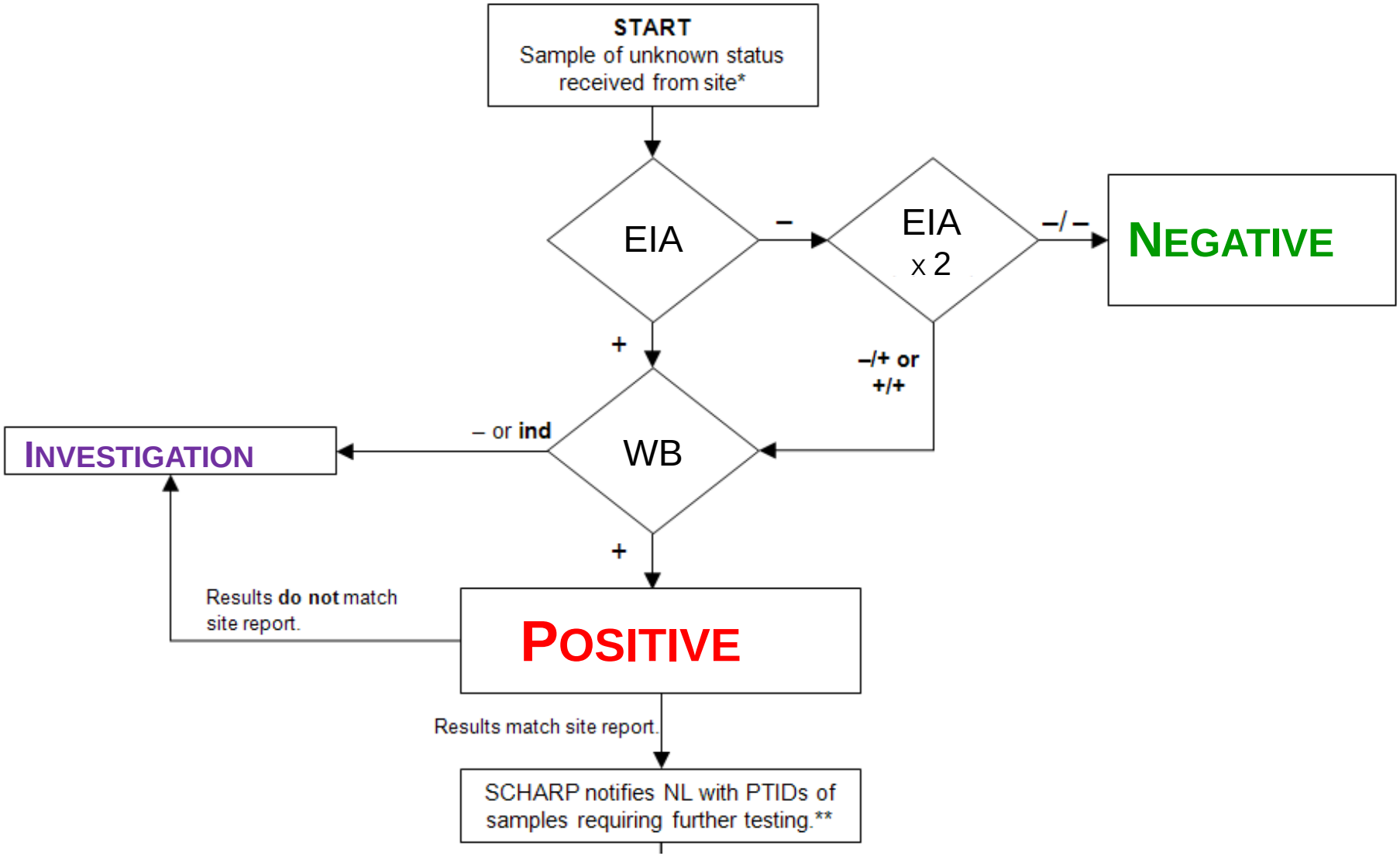
Further testing if needed
Send corrected CRF to SCHARP

Send final investigation reports to SCHARP

Conduct investigation if needed.

Virology CORE Algorithm

Endpoint Confirmation and Follow-Up Testing for VOICE

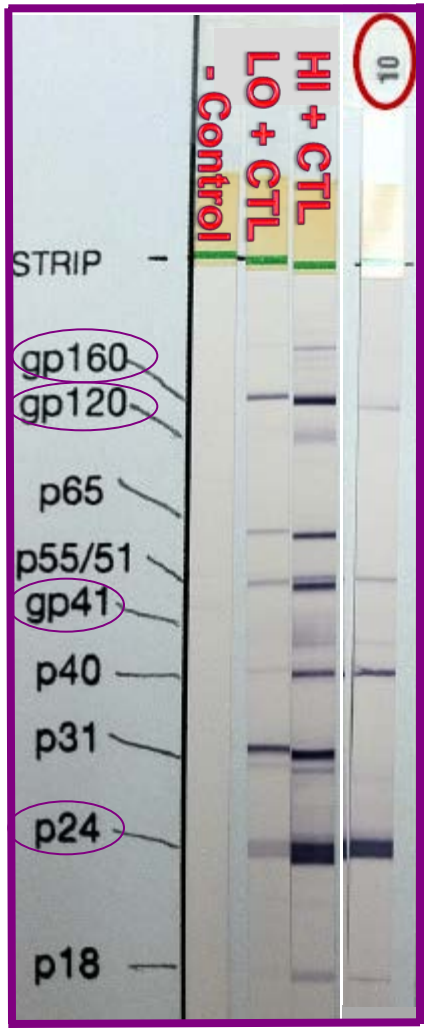


Western Blot

- **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:

Patient Code	Result	
Patient 1 v3.0	0.039	-
Patient 1 v6.0	*3.500	R+

Cutoff calculation: $\overline{NCX}$+0.250 = 0.283 $\overline{NCX}$ = 0.033

$\overline{PC1X}$ = 2.328 $\overline{PC2X}$ = 2.022 $\overline{PC3X}$ = 1.891

What tests should be done next?

CASE 1

PTID 1 v3.0

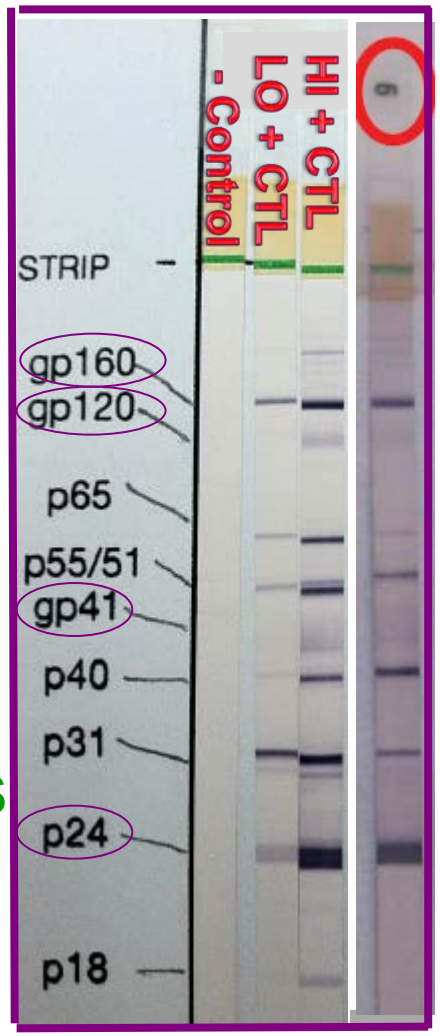
Do REPEAT EIA IN DUPLICATE

Patient Code	Result	# Test
Patient 1 v3.0	0.039 -	Single
Patient 1 v3.0	0.062 -	DUPL
Patient 1 v3.0	0.053 -	DUPL

Cutoff calculation: NCX+0.250 = 0.283 NCX = 0.033

PC1X = 2.328 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

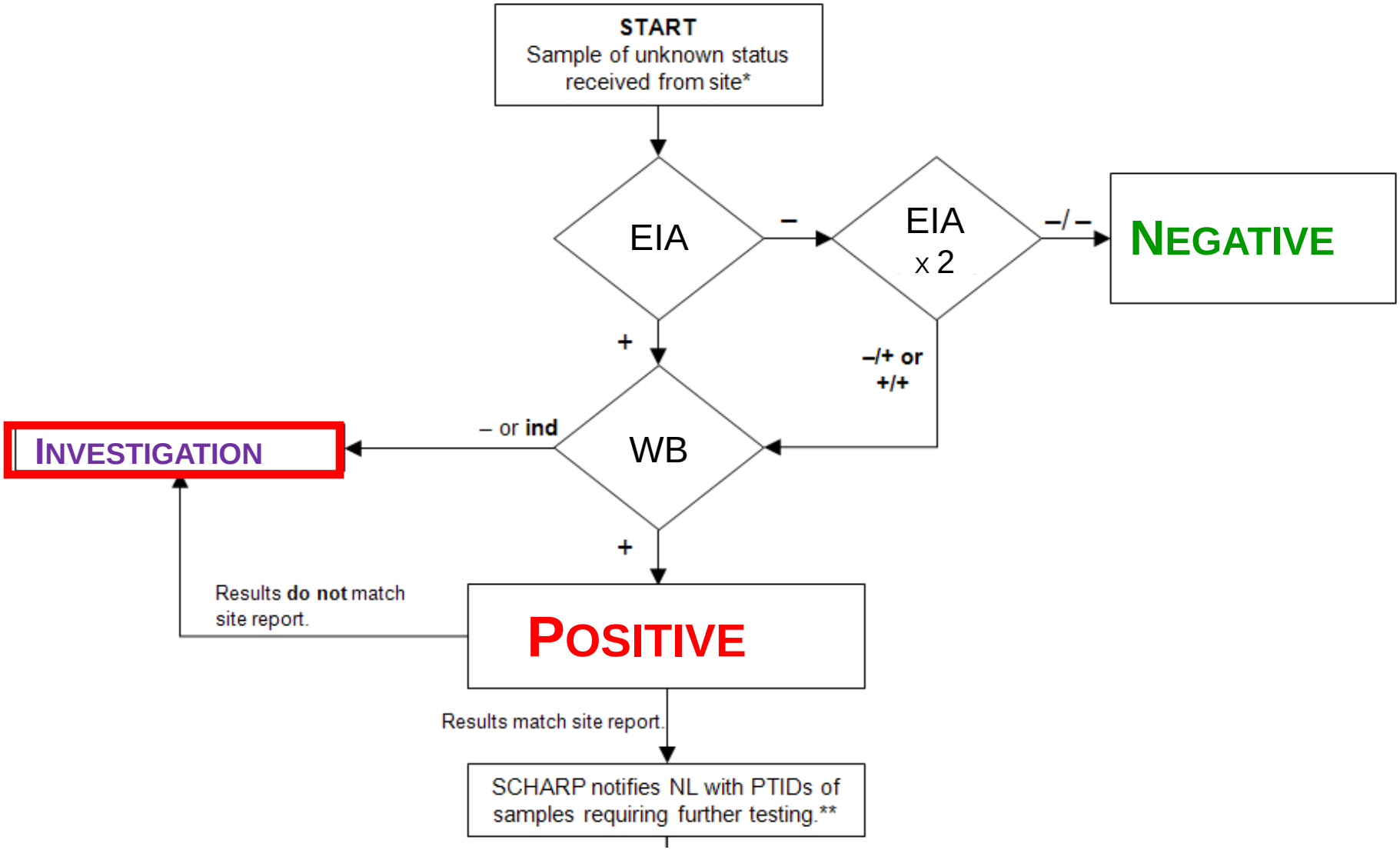
  <40 copies/ml

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



CASE 2

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

Patient Code	Result
Patient 2 v3.0	*3.500 R+
Patient 2 v5.0	*3.500 R+

Cutoff calculation: $\overline{NCX}$+0.250 = 0.281 $\overline{NCX}$ = 0.031

$\overline{PC1X}$ = 2.350 $\overline{PC2X}$ = 1.637 $\overline{PC3X}$ = 1.738

What tests should be done next and on what samples?

CASE 2

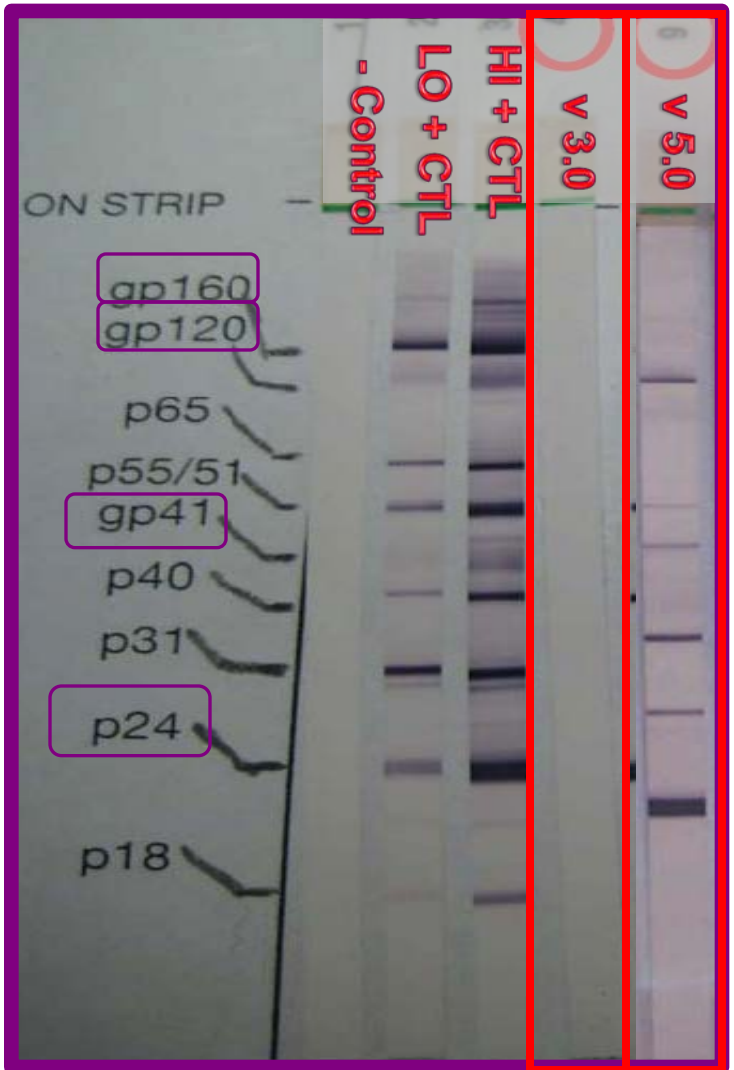
EIA Results

Patient Code	Result
Patient 2 v3.0	*3.500 R+
Patient 2 v5.0	*3.500 R+

Now for the investigation...

What test should be done?

WB Results



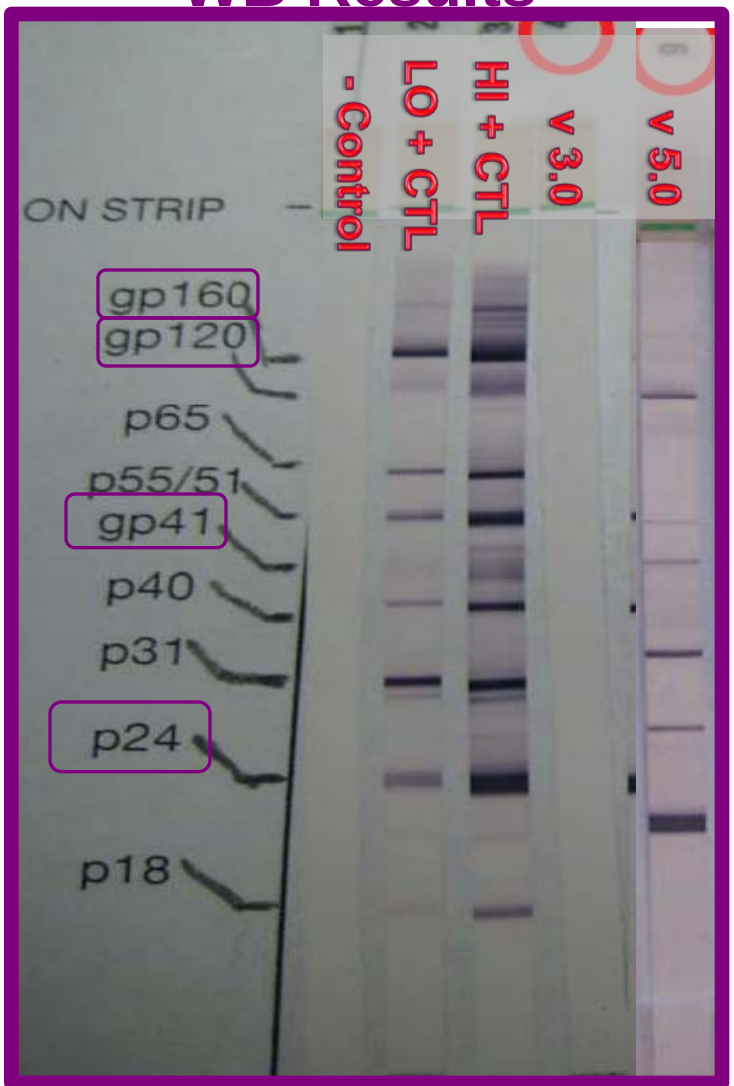
CASE 2

EIA Results

Patient Code	Result
Patient 2 v3.0	*3.500 R+
Patient 2 v3.0	*3.500 R+
Patient 2 v5.0	*3.500 R+
Patient 2 v5.0	*3.500 R+

What's next?

WB Results



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)

CASE 3

- NL received plasma for PTID 3.
- EIA Results:

Patient Code	Result
Patient 4 v3.0	1.033 R+
Patient 4 v4.1	*3.500 R+

Cutoff calculation: NCX+0.250 = 0.285 NCX = 0.035

PC1X = 2.380 PC2X = 2.768 PC3X = 2.127

What tests should be done next?

CASE 3

EIA Results

Patient Code	Result
Patient 4 v3.0	1.033 R+

Patient 4 v4.1 *3.500 R+

Cutoff calculation: NCX+0.250 = 0.285 NCX = 0.035

PC1X = 2.380 PC2X = 2.768 PC3X = 2.127

Is the v 4.1 WB Positive? YES

What would you do next?

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

CASE 3

- 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?

Rapid Test Result:

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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