Scenario 4: Mysterious HIV Algorithm Results

Materials and Directions:

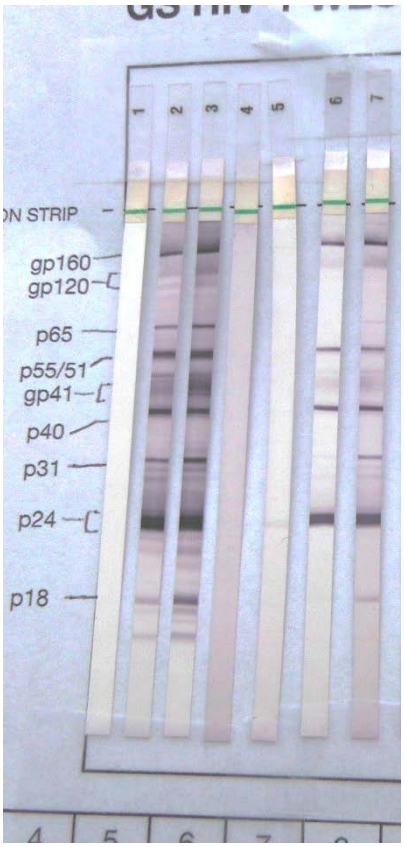
- ASPIRE Follow-Up Algorithm
- COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test Error Codes Appendix
- Scenarios: Read and complete each part separately. The next part will be given to you once the previous part has been completed.

Part 1:

An ASPIRE participant comes in for her monthly visit, and the technologist performs 2 rapid tests as part of HIV testing. Determine came out to be POSITIVE and Unigold came out to be NEGATIVE. To follow the HIV algorithm, the participant then sent a vial of plasma to the site's Central Lab to be tested for Western blot.

The technician from the Central Lab came in with these Western blot results:

Figure 1. Western blot results from Central Lab for Participant with Discordant Rapids



Western blot strips:

1 = negative control
2 = low positive control
3 = high positive control
4 = Participant X
5 = Our participant we are interested in
6 = Participant Y
7 = Participant Z

Question: Based on the Western blot test results, is the participant HIV positive? Why or why not? What is the test result? What should be done next?

No. The participant cannot be considered HIV positive from this Western Blot result. 2 major bands are required for a positive result: see BioRAD product insert below:

Interpret the immunoblot as NEGATIVE, INDETERMINATE, or POSITIVE based on the pattern that is present.

POSITIVE	At least TWO of the major bands: gp160 and/or gp120, gp41, or p24 must be present. (The presence of gp160 and/or gp120 qualifies as one major band.) 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	One or more bands are present but the blot does not meet the criteria for a POSITIVE result as described above.
NEGATIVE	No bands are present.*

The participant would be considered INDETERMINATE. Following MTN algorithm, HIV-RNA test for viral load should be run next.

Part 2:

The technician decides to send the sample to the site's Central Lab for HIV-1 RNA (viral load) testing. This site uses the Roche TaqMan machine. All of the controls perform according to the package insert and testing guidelines, but the participant whose sample tested indeterminate for Western blot (hereafter referred to as Sample 1) has an INVALID result with the flag of PRECHECK. See Figure 1.

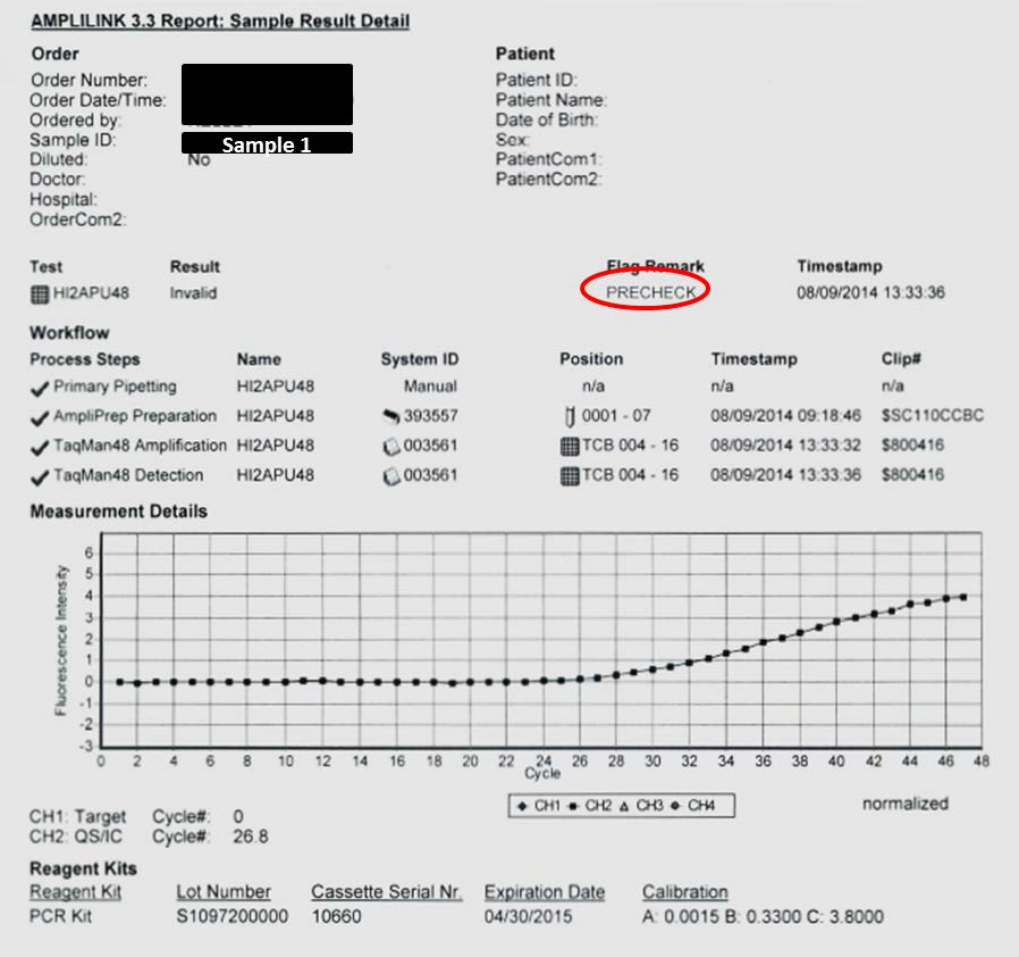
Figure 1. Sample 1 Roche TaqMan Test Results

Rack ID	Timestamp	Batch ID	Comment
0001	08/09/2014 07:39	2014-08-09	

T	T #	Order/Lot Number	Sample ID	Test	Result	Flag Remark	Timestamp
S	07	ORDER #1	Sample 1	HI2APU48	Invalid	PRECHECK	08/09/2014 13:33:36

The technician first pulls up the raw data for Sample 1 and for another participant's sample that did not have an error code (Sample 2). This is what the technician finds:

Figure 2. Sample 1 Detailed Results



AMPLILINK 3.3 Report: Sample Result Detail

Order

Order Number: [redacted]
Order Date/Time:
Ordered by:
Sample ID: Sample 1
Diluted: No
Doctor:
Hospital:
OrderCom2:

Patient

Patient ID:
Patient Name:
Date of Birth:
Sex:
PatientCom1:
PatientCom2:

Test	Result	Flag Remark	Timestamp
HI2APU48	Invalid	PRECHECK	08/09/2014 13:33:36

Workflow

Process Steps	Name	System ID	Position	Timestamp	Clip#
Primary Pipetting	HI2APU48	Manual	n/a	n/a	n/a
AmpliPrep Preparation	HI2APU48	393557	0001 - 07	08/09/2014 09:18:46	$SC110CCBC
TaqMan48 Amplification	HI2APU48	003561	TCB 004 - 16	08/09/2014 13:33:32	$800416
TaqMan48 Detection	HI2APU48	003561	TCB 004 - 16	08/09/2014 13:33:36	$800416

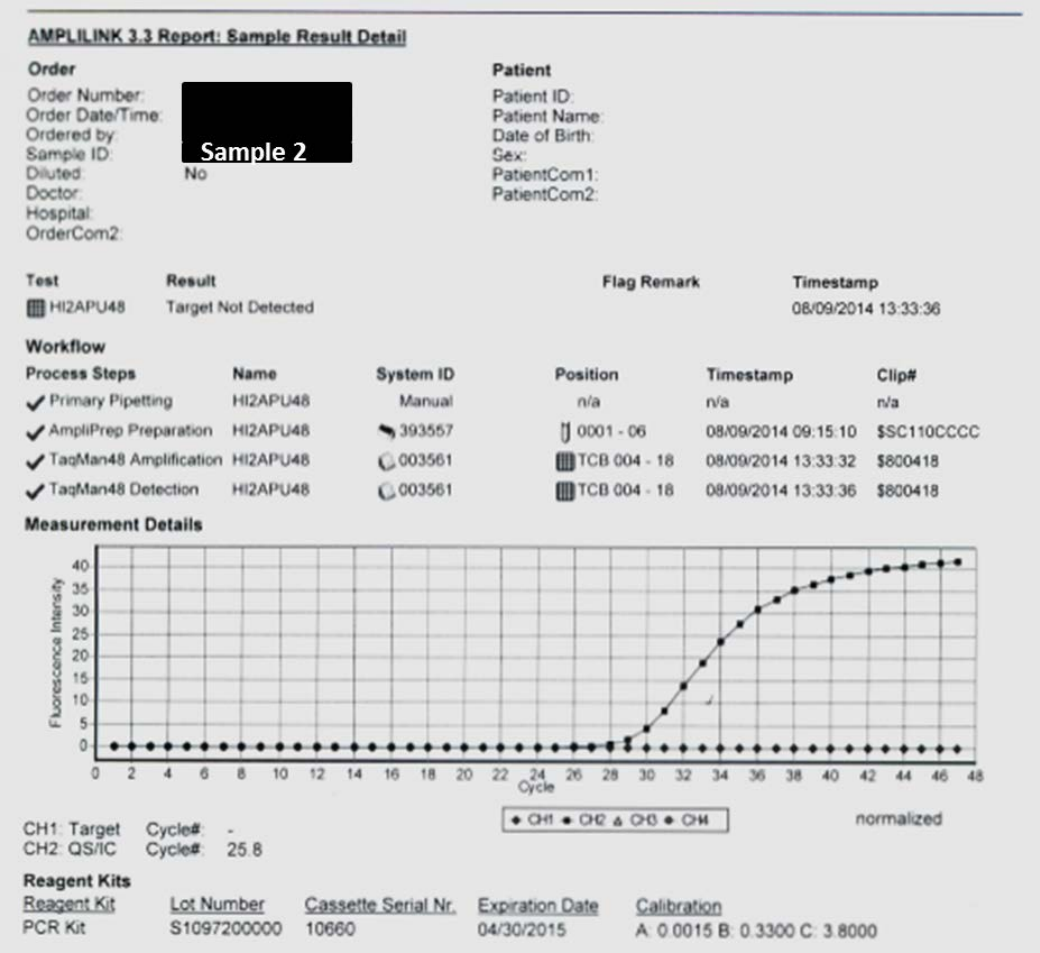
Measurement Details

CH1: Target Cycle#: 0
CH2: QS/IC Cycle#: 26.8

Reagent Kits

Reagent Kit	Lot Number	Cassette Serial Nr.	Expiration Date	Calibration
PCR Kit	S1097200000	10660	04/30/2015	A: 0.0015 B: 0.3300 C: 3.8000

Figure 3. Results from another participant whose viral load did not fail testing.



AMPLILINK 3.3 Report: Sample Result Detail

Order

Order Number: [redacted]
Order Date/Time:
Ordered by:
Sample ID: Sample 2
Diluted: No
Doctor:
Hospital:
OrderCom2:

Patient

Patient ID:
Patient Name:
Date of Birth:
Sex:
PatientCom1:
PatientCom2:

Test	Result	Flag Remark	Timestamp
HI2APU48	Target Not Detected		08/09/2014 13:33:36

Workflow

Process Steps	Name	System ID	Position	Timestamp	Clip#
Primary Pipetting	HI2APU48	Manual	n/a	n/a	n/a
AmpliPrep Preparation	HI2APU48	393557	0001 - 06	08/09/2014 09:15:10	$SC110CCCC
TaqMan48 Amplification	HI2APU48	003561	TCB 004 - 18	08/09/2014 13:33:32	$800418
TaqMan48 Detection	HI2APU48	003561	TCB 004 - 18	08/09/2014 13:33:36	$800418

Measurement Details

CH1: Target Cycle#: -
CH2: QS/IC Cycle#: 25.8

Reagent Kits

Reagent Kit	Lot Number	Cassette Serial Nr.	Expiration Date	Calibration
PCR Kit	S1097200000	10660	04/30/2015	A: 0.0015 B: 0.3300 C: 3.8000

QUESTIONS: Do the result details and the error code table give an indication of what might have happened? (view valid sample as comparison)

The results indicate that there is an issue with the PCR. Although the QC passed for the sample with the PRECHECK error, fluorescence intensity for the QC on this sample is relatively low. Also, the PCR curve does not exemplify exponential amplification (the curve is flat). PRECHECK ERRORS can occur for a number of reasons; including physical issues with the sample or reagents, PCR inhibitors carried over from phlebotomy (heparin), or having too much template (VL is TOO high to measure without dilution)

What should the technician do to resolve this issue? Should he report the problem to his supervisor or just repeat the sample? Who could he contact for assistance?

The technician should consult their supervisor. Sample could be repeated. If the problem persists, the company (ROCHE) should be consulted. Virology Core lab is also available for assistance with technical issues.

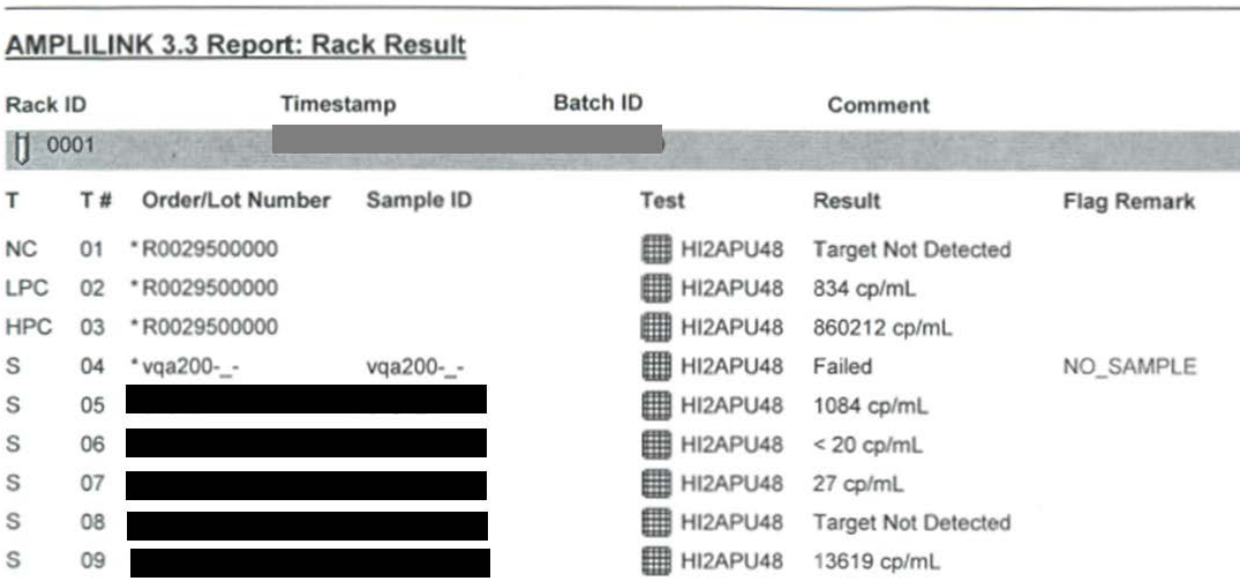
Part 3: **(ROCHE recommends running @ a dilution)**

The technician decided to re-run the sample using a VQA-approved dilution of 1:20. The result came out to be 761,848 copies/ml.

What diluent did the technician use in order to perform the dilution according to VQA guidelines? BM53 (ultra-purified HIV negative human plasma)
What was the problem in the last run? Too much template in the PCR reaction. VL was too high to measure.
What is the actual viral load result for this participant? 15,236,960 copies per mL

Unfortunately, although the error code for Sample 1 now disappeared, the technician noticed another problem with the viral load run. See Figure 4:

Figure 4:



AMPLILINK 3.3 Report: Rack Result

Rack ID	Timestamp	Batch ID	Comment
0001	[redacted]		

T	T #	Order/Lot Number	Sample ID	Test	Result	Flag Remark
NC	01	*R0029500000		HI2APU48	Target Not Detected	
LPC	02	*R0029500000		HI2APU48	834 cp/mL	
HPC	03	*R0029500000		HI2APU48	860212 cp/mL	
S	04	*vqa200-_-	vqa200-_-	HI2APU48	Failed	NO_SAMPLE
S	05	[redacted]		HI2APU48	1084 cp/mL	
S	06	[redacted]		HI2APU48	< 20 cp/mL	
S	07	[redacted]		HI2APU48	27 cp/mL	
S	08	[redacted]		HI2APU48	Target Not Detected	
S	09	[redacted]		HI2APU48	13619 cp/mL	

What is the new problem? Is this run valid? What should be done next? Can the results from this run be reported out? The VQA 200 copy control has failed. ☹ The run will have to be repeated. None of these results can be reported out.

IF the run is repeated and the results are confirmed (with no control failures), the participant would be determined to be HIV POSITIVE (indeterminate rapid tests, indeterminate WB, positive HIV RNA = POSITIVE)