Critical Thinking with Rapid Waived and Non-waived Molecular Tests

April 4, 2023

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Disclosure

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No relevant financial relationships to disclose.

SSMHealth

Objectives

- Discuss how clinical laboratory test are classified in terms of complexity
- Show the importance of critical thinking skills in interpreting laboratory test results
- Illustrate why molecular tests are so sensitive
- Demonstrate some of the challenges associated with using molecular tests

Audience Question

Do you perform nucleic acid amplification tests in you laboratory?

1. Yes

2. No

QUOTE: “Most microbiology lab testing is like a microwave dinner,” says Mina. “It comes as one kit, you push the button and you get your result. Nobody can screw it up. The CDC test kit for COVID-19 is more like Blue Apron. It comes with a box of ingredients that you can go to any store and buy, but it’s nicely packaged up for you, and comes with a recipe.”

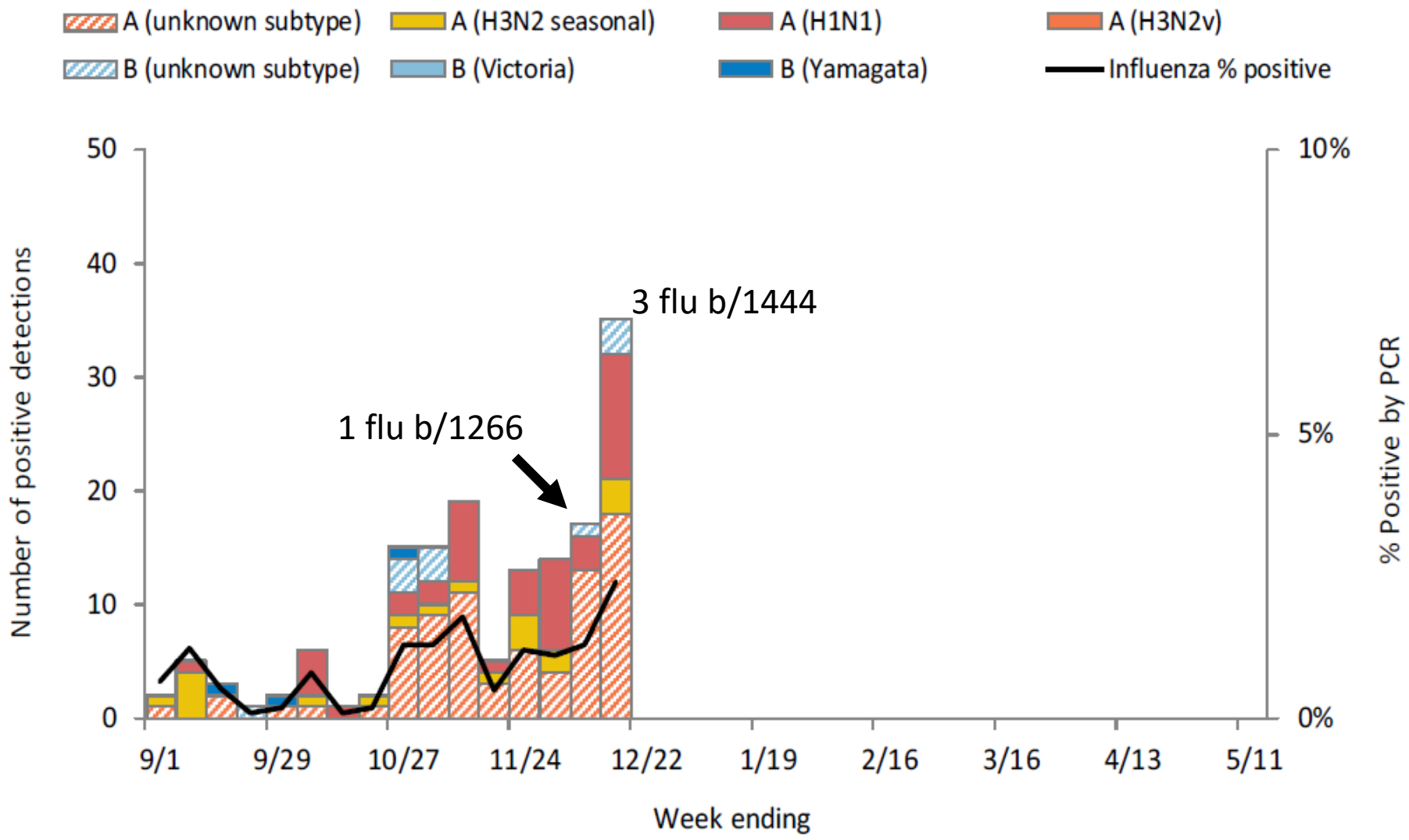
Dr. Michael Mina, MD, Ph.D.

Hospital Lab Waived Rapid Molecular Influenza Test

- **Between December 12, 2018 and December 28, 2018 Hospital Laboratory X detected influenza B from five patient specimens via molecular testing.**

- **At this time only very scattered cases of influenza A were reported by the WSLH and very rare influenza B cases.**

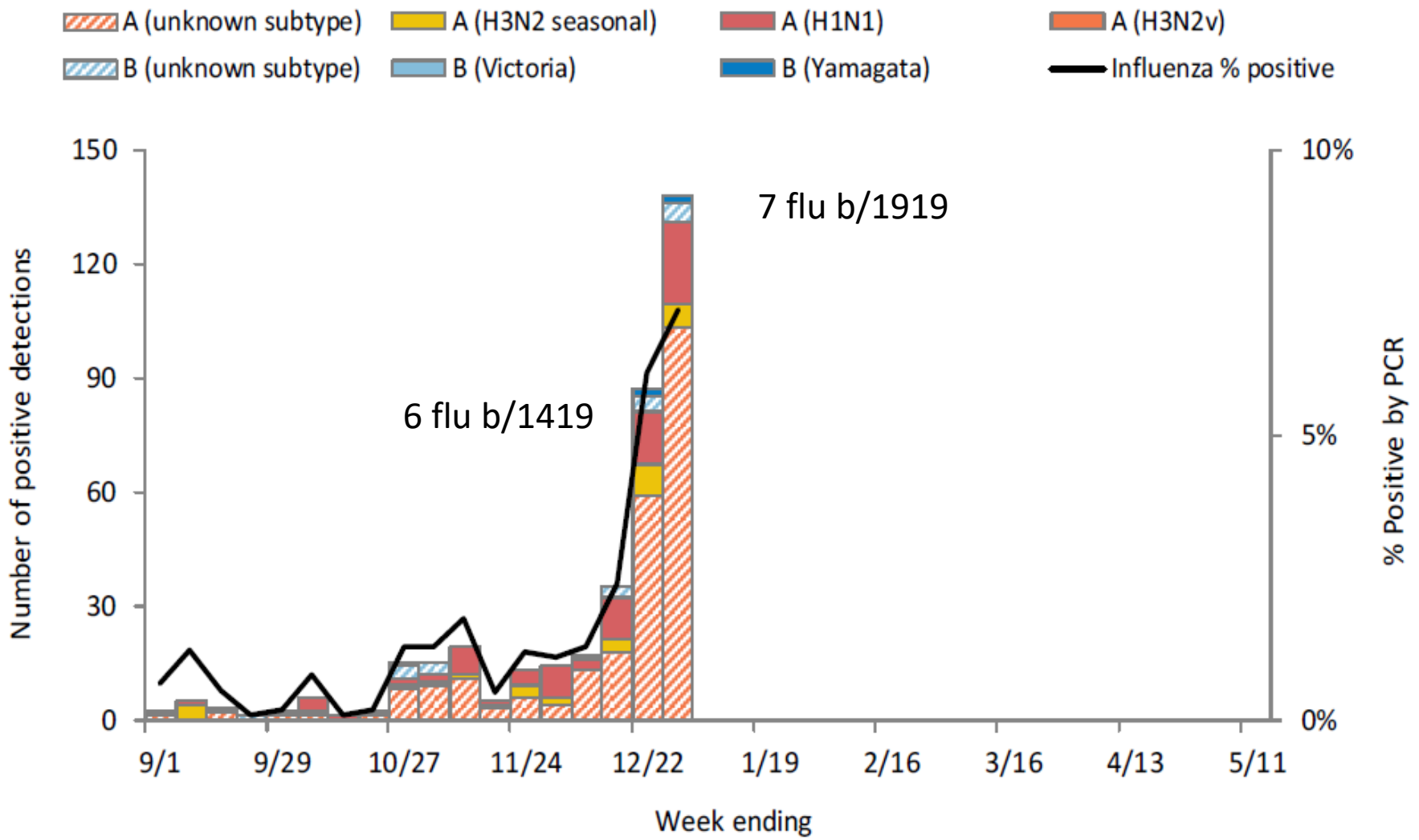
Wisconsin positive influenza results and subtypes by PCR



Hospital Lab Waived Rapid Molecular Influenza Test

- **Between December 12, 2018 and December 28, 2018 Hospital Laboratory X detected influenza B from five patient specimens via molecular testing.**
- **At this time only very scattered cases of influenza A were reported by the WSLH and very rare influenza B cases.**
- **During this time period Laboratory X tested 69 specimens total with 13 positive for RSV and 1 positive for influenza A.**
- **The rest of the region tested 206 specimens in this time period and reported 2 positive for influenza A, 33 positive for RSV, and 0 positive for influenza B.**

Wisconsin positive influenza results and subtypes by PCR



WSLH Results On These 5 Specimens

#1 No Influenza A or B Detected

#2 No Influenza A or B Detected

#3 No Influenza A or B Detected

#4 No Influenza A or B Detected

#5 No Influenza A or B Detected

What Happened?

- **Waived Complexity Test – All In-Tube Controls Passed**
- **Waived Complexity Test – All LIAT Instrument Controls Passed**
- **Waived Complexity Test – Yet We Did Not Get the Correct Result!**

"Most microbiology lab testing is like a microwave dinner," says Mina. "It comes as one kit, you push the button and you get your result. Nobody can screw it up.

Dr. Michael Mina, MD, Ph.D.

Audience Question

Do you receive, and review in a timely fashion, the regularly disseminated *Respiratory Virus Surveillance Report* from the Wisconsin Department of Health Services/Division of Public Health/Bureau of Communicable Diseases/Communicable Diseases Epidemiology Section and the *Laboratory Surveillance Report* from the Wisconsin State Laboratory of Hygiene?

1. **Yes, every time I receive one.**
2. **I review most right away, but sometimes I miss a few.**
3. **On occasion I review them right away, but not often.**
4. **I am so busy I never get a chance to review these in a timely fashion.**
5. **I do not receive these reports.**

How are Lab Tests Classified?

Tests that are Waived by regulation under 42 CFR 493.15(c), or cleared or approved for home use, are categorized as waived.
Criteria. Test systems are simple laboratory examinations and procedures which -

(1) Are cleared by FDA for home use;
(2) Employ methodologies that are so simple and accurate as to render the likelihood of erroneous results negligible; or
(3) Pose no reasonable risk of harm to the patient if the test is performed incorrectly.

For all other tests, the FDA determines the test's complexity by reviewing the package insert and using a scorecard to categorize a test as moderate or high complexity (42 CFR 493.17).

Each test is graded for level of complexity by assigning scores of 1, 2, or 3 for each of the seven criteria on the scorecard.

1 - Knowledge

2 - Training and experience

3 - Reagents and materials preparation

4 - Characteristics of operational steps

5 - Calibration, quality control, and proficiency testing materials

6 - Test system troubleshooting and equipment maintenance

7 - Interpretation and judgment

≤12 = Moderate complexity

>12 = High complexity

CLIA Test Complexity

CLIA waived tests

- Examples include: urine pregnancy tests, rapid strep tests, dipsticks for urine chemistry testing, glucometers, etc.

Moderately complex tests are typically found in clinical laboratory instrumentation. Facilities using these types of tests need to complete a proper instrument validation process to show proof of accurate testing, which includes precision, accuracy, verification of reportable range, and reference intervals or reference range for the patient population within the geographical area

- Examples include: chemistry panels, complete blood counts (CBC), some molecular (PCR) testing, urine dipsticks, urine drug screens and automated immunoassay tests

High complexity tests require clinical laboratory expertise beyond normal automation to perform

- Examples include: lipid chromatography-mass spectrometry (LCMS), cytology, flow cytometry, gel electrophoresis and molecular diagnostic tests that include gene chip array, dot blots, viral loads, expression arrays, some large capacity analyzers, and modification of FDA cleared tests or laboratory developed tests

Waived Nucleic Acid Amplification Test Systems - POC

mesabiotech

Roche Liat

Abbott ID NOW

SARS-CoV-2, Influenza A and B/RSV, GAS, *C. difficile* toxin

GeneXpert Xpress

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Waived complexity ≠ Failsafe

MOLECULAR-BASED MICROBIOLOGY TESTING-WAIVED TESTS

The requirements in this section apply to molecular-based microbiology tests classified as waived performed in a limited service laboratory setting. Microbiology testing performed by nonwaived molecular-based methods must be inspected with the Microbiology Checklist.

***REVISED** **10/24/2022*

LSV.45740 Quality Monitoring Statistics **Phase I**

The laboratory monitors for the presence of false positive results (eg, due to nucleic acid contamination) for all molecular microbiology tests.

NOTE: Examples include: review of summary statistics (eg, monitoring percentage of positive results relative to current local and regional rates and increased positive Strep results above historical rate within a run or over multiple runs), performance of wipe (environmental) testing, and review and investigation of physician inquiries. Based on monitoring data, the laboratory may implement additional mitigation strategies to minimize the risk of contamination, such as process controls.

Evidence of Compliance:

✓ Records of data review, wipe testing, statistical data evaluation and corrective action if indicated

LSV.45745 Final Report **Phase I**

The final report includes a summary of the test method and information regarding clinical interpretation if appropriate.

NOTE: For tests that may be performed by either direct antigen or molecular-based methods (PCR), including the test method in the report is important for interpretation of the results. The report must include a brief description of the method if the methodology is not explicit in the test name.

MOLECULAR-BASED MICROBIOLOGY TESTING-WAIVED TESTS

MIC.63252 **Quality Monitoring** **Phase I**

The laboratory monitors for the presence of false positive results (eg, due to nucleic acid contamination) for all molecular microbiology tests.

NOTE: Examples include review of summary statistics (eg, monitoring percentage of results that are positive for Chlamydia trachomatis and/or Neisseria gonorrhoeae for an increase above historical positive rate within a run or over multiple runs), unexpected increase in positive results for seasonal pathogens outside of the standard epidemiology, performance of wipe testing, and review and investigation of physician complaints on false positive results. Based on monitoring data, the laboratory may implement additional mitigation strategies to minimize risk of contamination, such as process controls.

Evidence of Compliance:

✓ Records of data review, wipe testing, statistical data, and evaluation and corrective action if indicated

MOLECULAR-BASED MICROBIOLOGY TESTING-WAIVED TESTS

MIC.63318 **Specimen Handling** **Phase II**

The laboratory uses appropriate processes to prevent specimen loss, alteration, or contamination during collection, transport, processing and storage.

NOTE: Specimen collection, processing and storage must follow manufacturer's instructions and limit the risk of preanalytical error. For example, there must be a procedure to ensure absence of cross-contamination of samples during processing/testing for amplified molecular testing using liquid based cervical cytology (LBC) specimens; alternatively, an aliquot can be removed for amplified molecular testing prior to LBC processing.

MIC.66100 **Final Report** **Phase I**

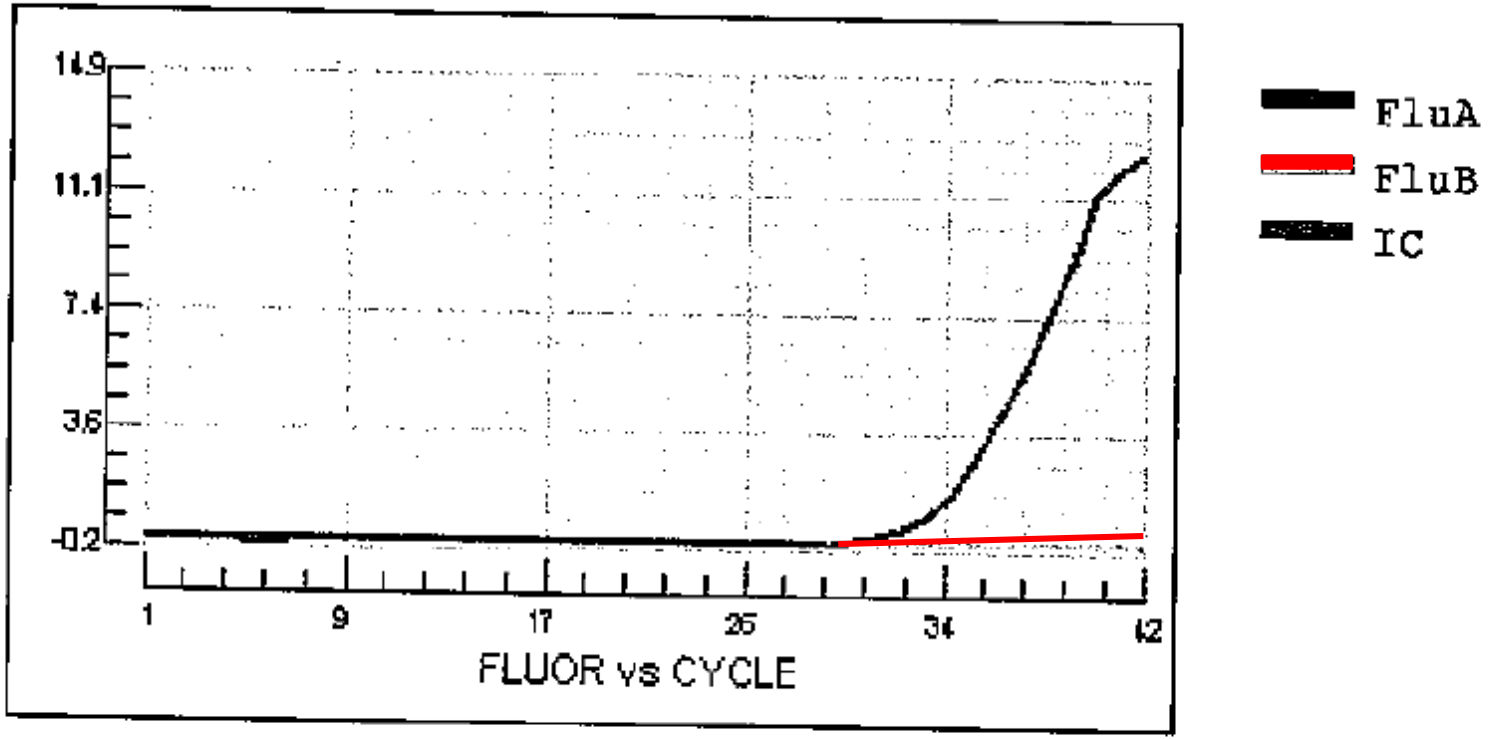
The final report includes a summary of the test method and information regarding clinical interpretation if appropriate.

NOTE: For example, when a test may be performed by either direct antigen or PCR, including the test method in the report is important information for interpreting the results.

Hospital Lab Waived Rapid Molecular Influenza and RSV Testing

Back To Our Story

cobas Liat Analyzer
Report of Liat Influenza Assay



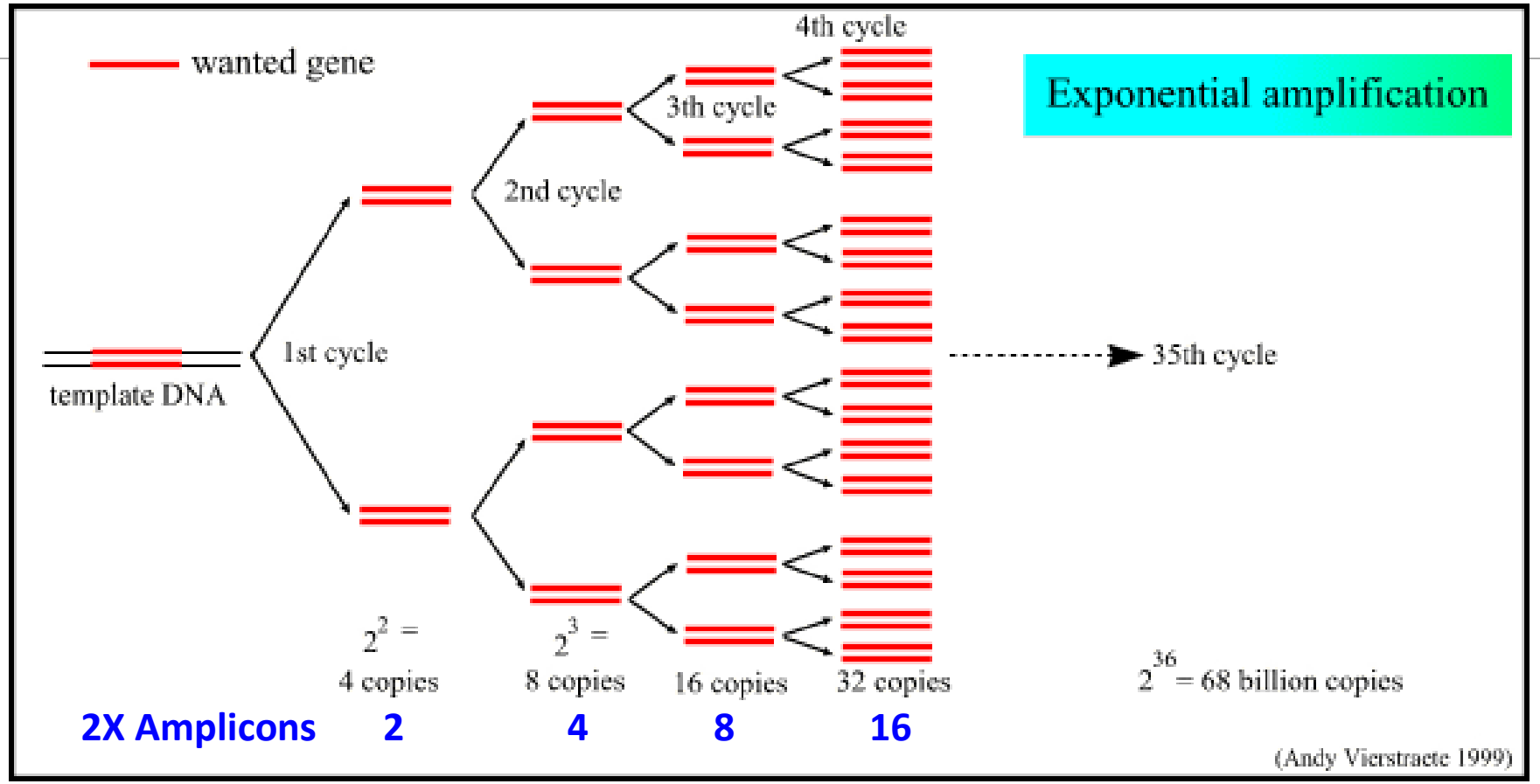
cobas Liat Result Report

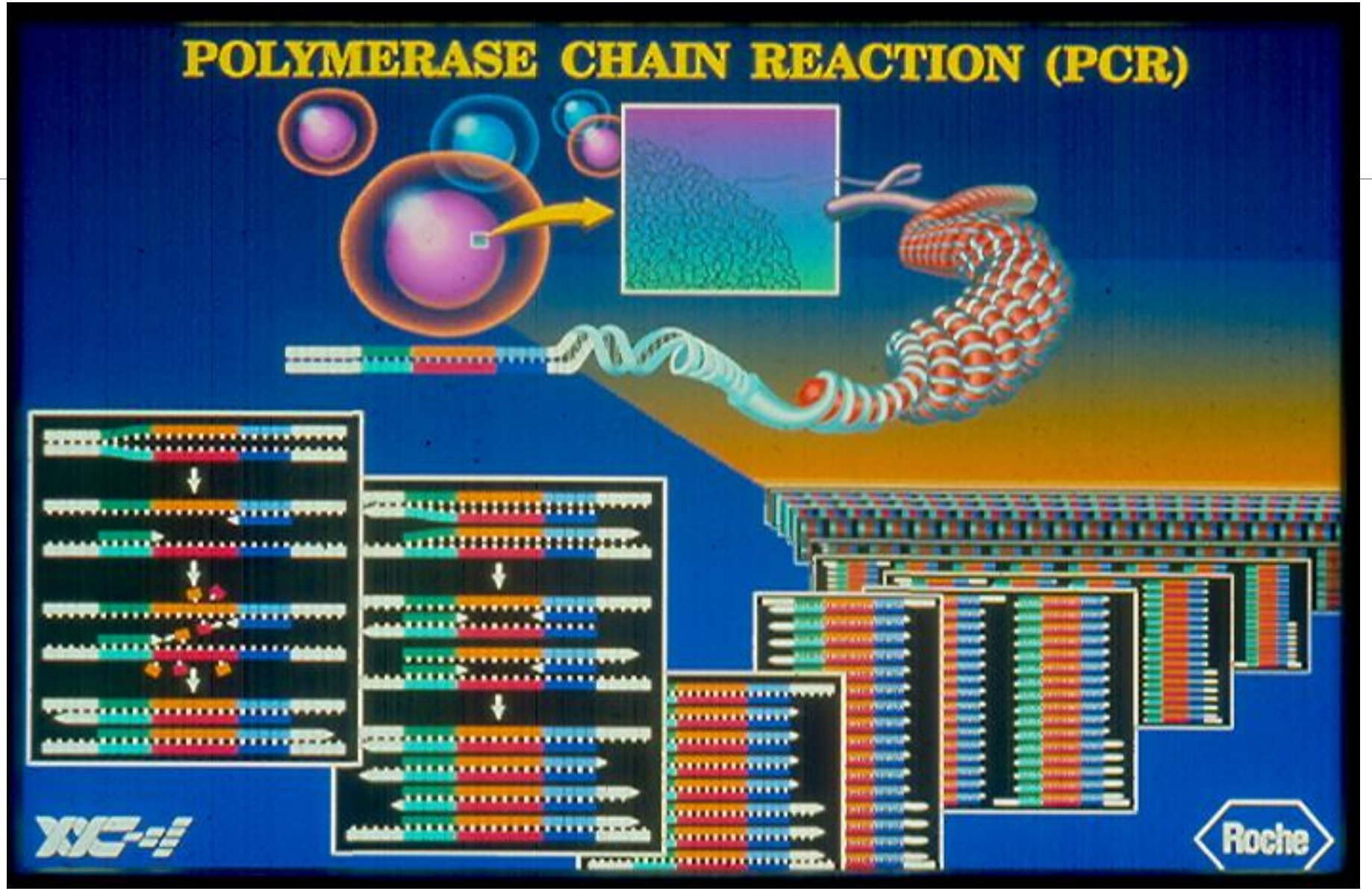
Report Results:

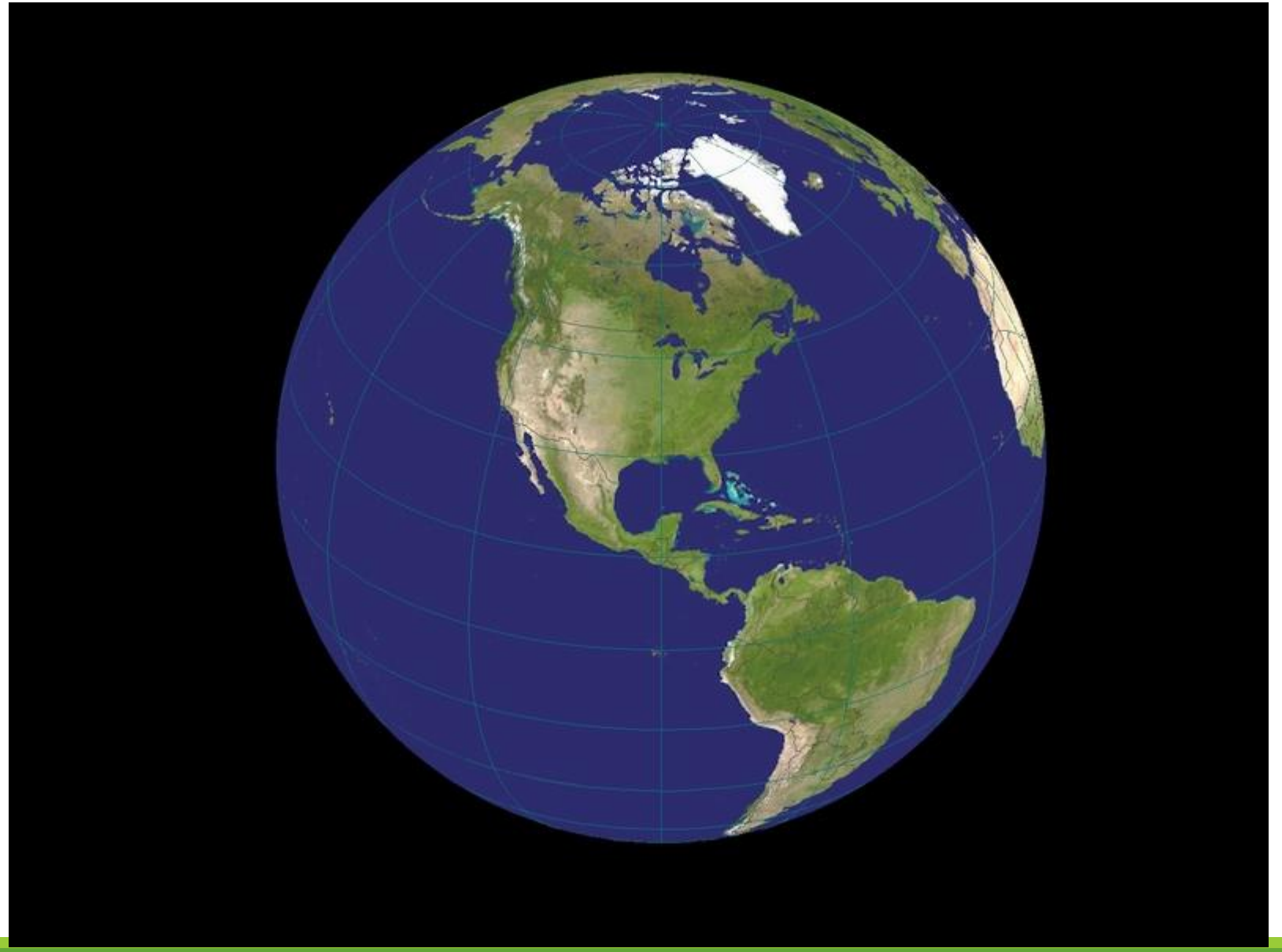
Influenza A Not Detected
Influenza B Detected

Details:

The Power of Nucleic Acid Amplification Based Tests




POLYMERASE CHAIN REACTION (PCR)
XC-i
Roche



Sample Collection- Why Molecular?

The power of sample amplification

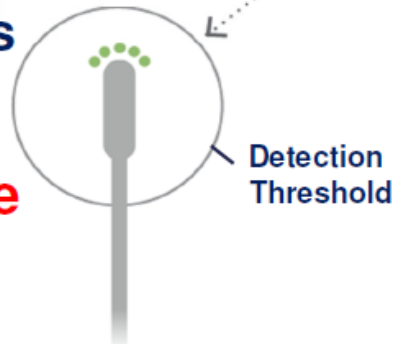
- Conventional non-molecular methods can have sub-optimal limits of detection.
- Samples with low viral or bacterial load could result in a false negative.

Positive Patient Sample

- With molecular, even a few hundred infectious particles can be amplified billions of times!
- Amplification increases likelihood of detection, and may compensate for sub-optimal sample collection.

Conventional Non-Molecular Methods

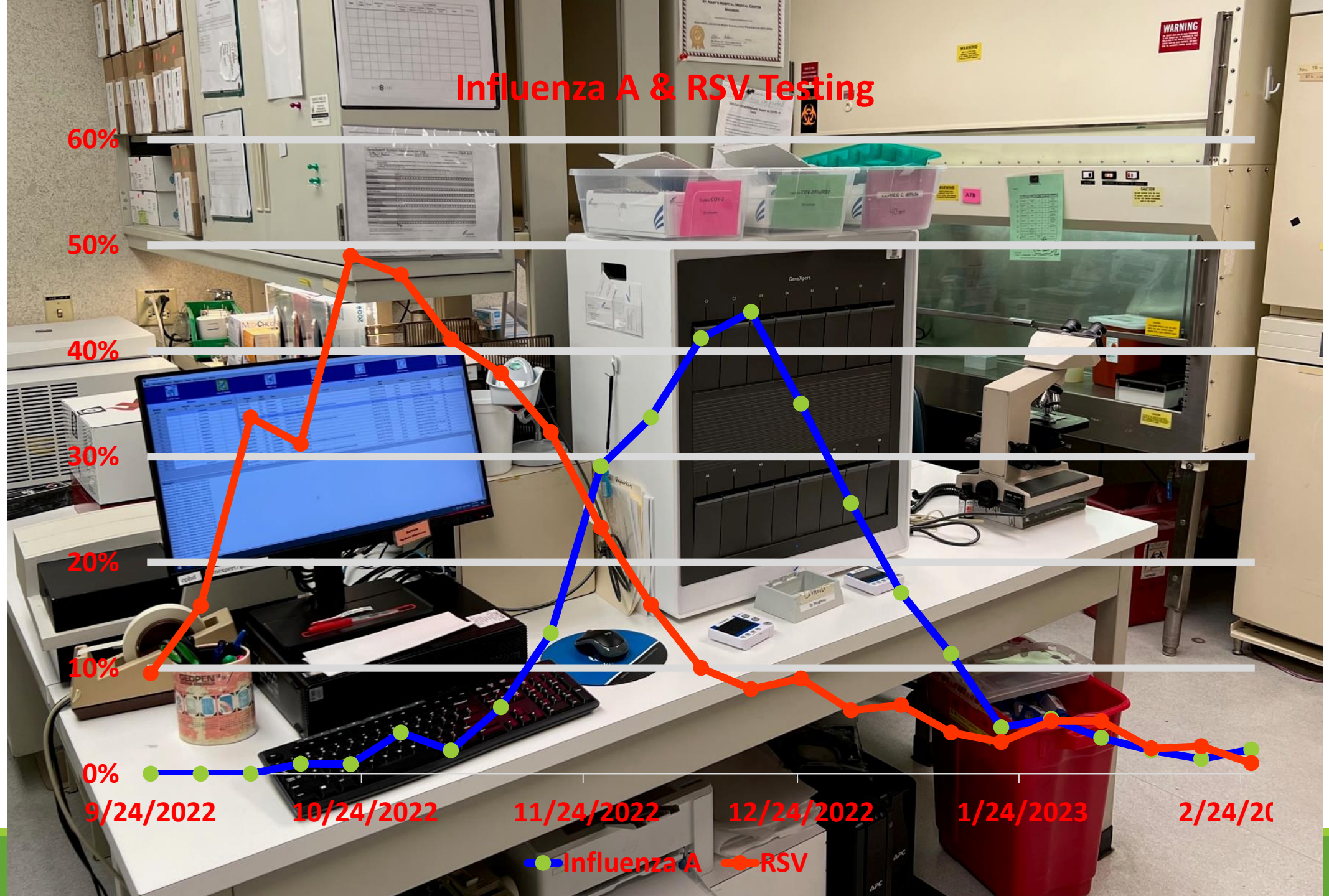
False Negative



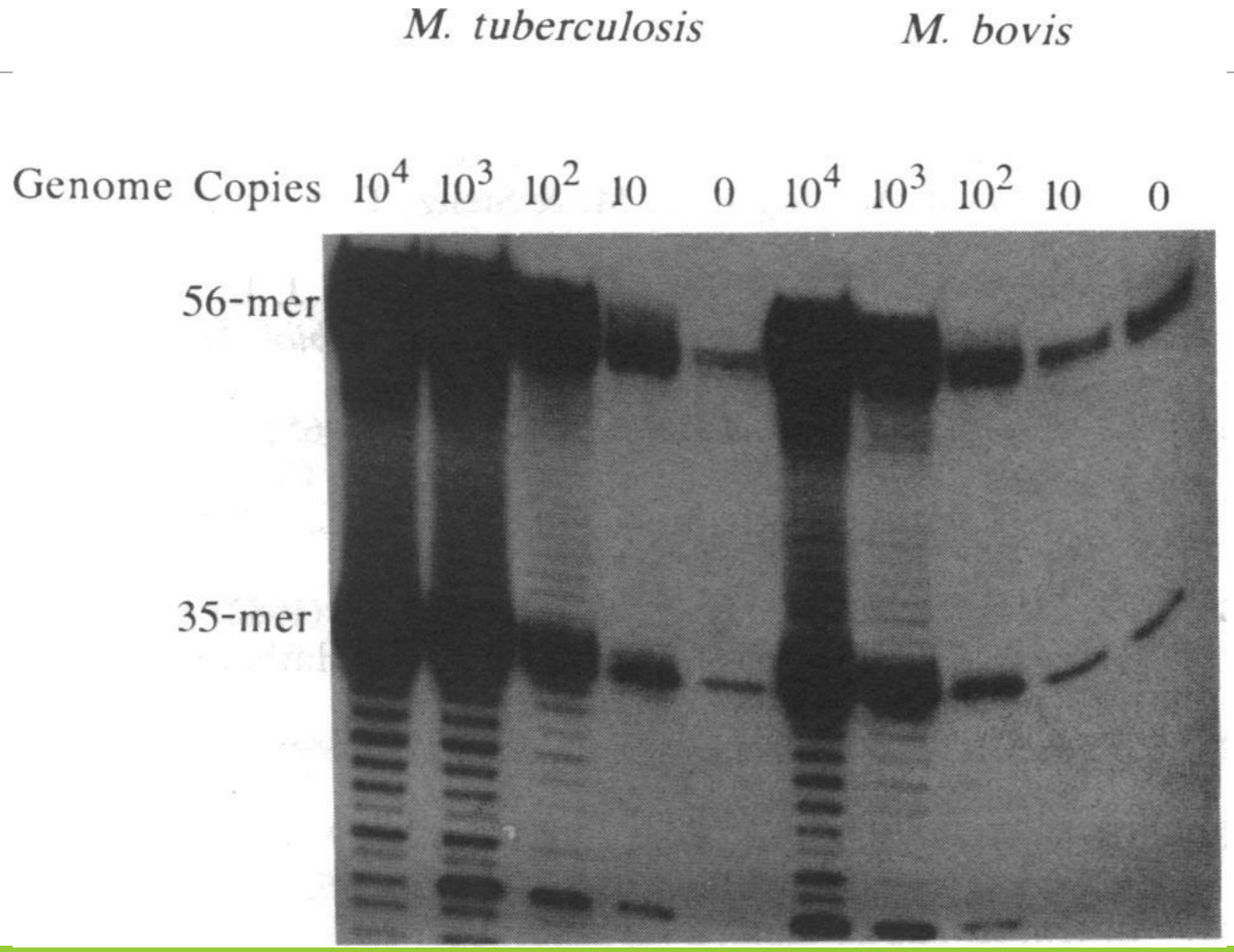
Molecular Amplified

True Positive


WARNING
GeneXpert
BIOHAZARD
FLAMMABLE
CAUTION
PEDPEN


Influenza A & RSV Testing
60%
50%
40%
30%
20%
10%
0%
9/24/2022
10/24/2022
11/24/2022
12/24/2022
1/24/2023
2/24/2(
Influenza A
RSV

Ouch! Those Nucleic Acid Amplification Tests Sure Are Sensitive!



Proc. Nati. Acad. Sci. USA Vol. 89, pp. 392-396, January 1992

Contamination

- Generation and spread of aerosols of PCR amplicons, positive control, proficiency samples, patient samples, pipette contamination, environment contaminated with target NA, or positive specimens.

- Contamination from hands, clothes, and hair.

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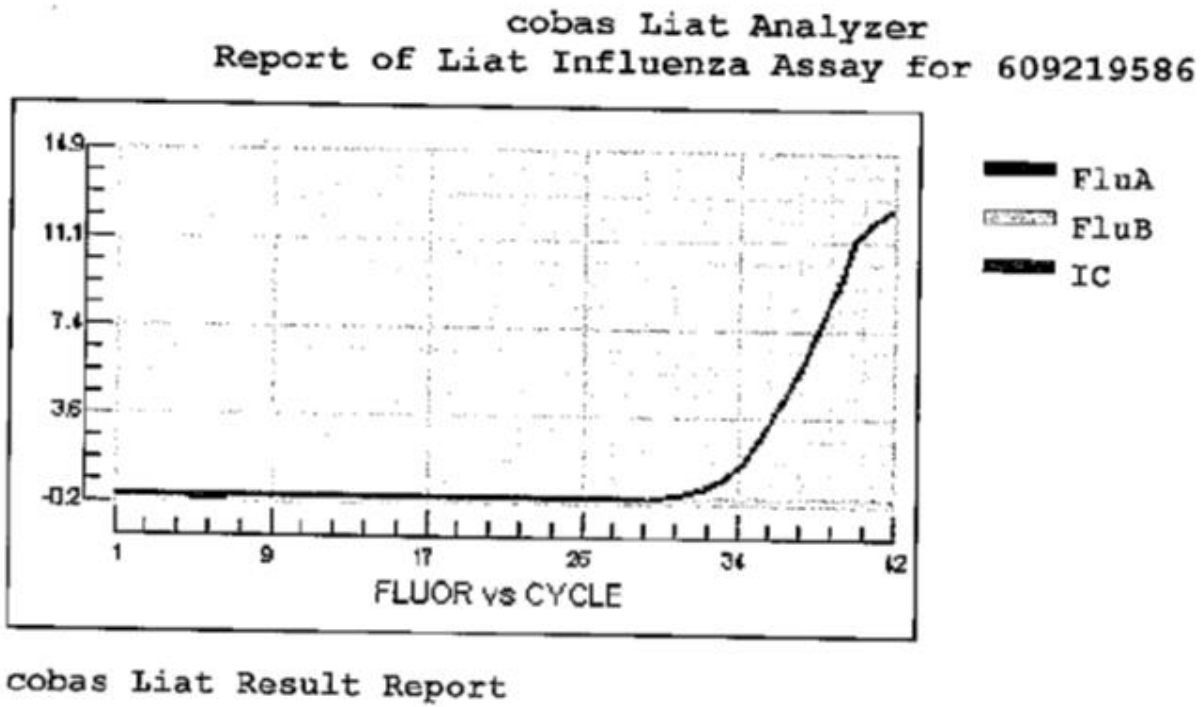
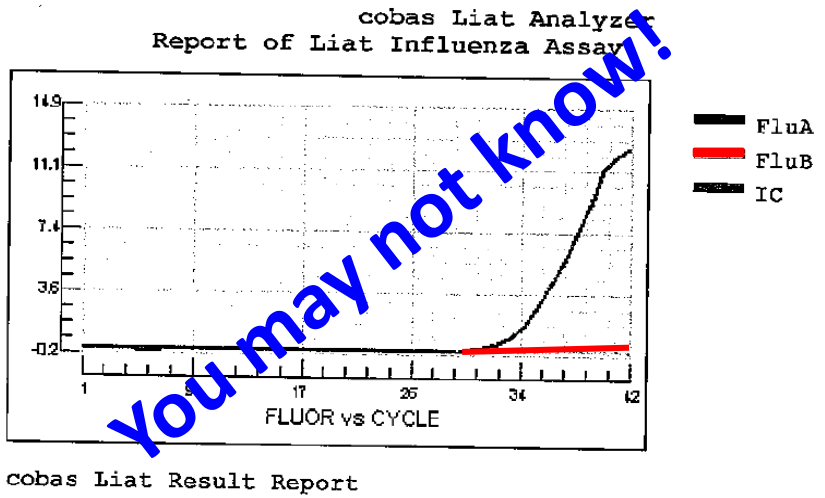


How Do You Know You Have Contamination?

- Negative Controls are positive
- Unexpected number of positives
- High Ct values
- Wipe test results
- Confirmatory testing negative



Case Study

65 year old oncology patient admitted for dehydration and possible septicemia due to WBC >35,000.

Blood cultures collected on admission

Set 1 positive at 13 hours → Gram negative rod

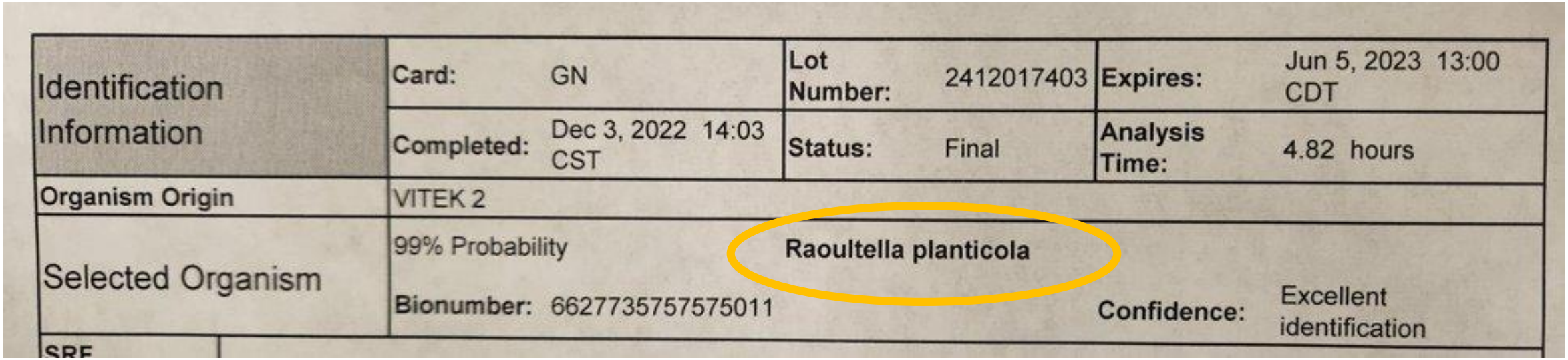
Gram Negative Blood Culture panel set up on Verigene system

BLOOD GRAM NEGATIVE PANEL

Res	Component	Value
1	Acinetobacter	Not detected
1	Citrobacter	Not detected
1	Enterobacter	Not detected
1	Proteus	Not Detected
1	Escherichia coli	Not Detected
1	Klebsiella pneumoniae	Not Detected
1	Klebsiella oxytoca	Detected
1	Pseudomonas aeruginosa	Not Detected
1	CTX-M	Not detected
1	KPC	Not detected
1	NDM	Not detected
1	VIM	Not detected
1	IMP	Not detected
1	OXA	Not detected

Blood Culture bottle subbed to media for Vitek ID/Sensitivity

Vitek ID



Identification Information	Card:	GN	Lot Number:	2412017403	Expires:	Jun 5, 2023 13:00 CDT
	Completed:	Dec 3, 2022 14:03 CST	Status:	Final	Analysis Time:	4.82 hours
Organism Origin	VITEK 2					
Selected Organism	99% Probability	Raoultella planticola				
	Bionumber:	6627735757575011			Confidence:	Excellent identification
SRF						

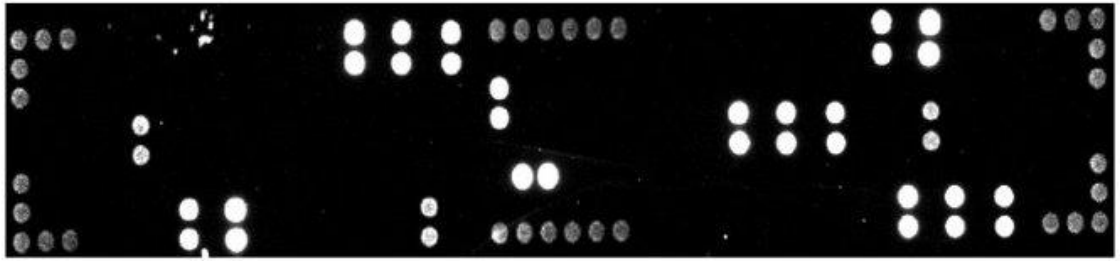
Which one is wrong?

VERIGENE® System

- Sample To Answer
- Modular Processing Capacity
- Touchscreen Interface
- Moderate CLIA Complexity

NanoGrid Technology

- Extraction
- Fragmentation or Amplification
- Hybridization
- Imaging/Analysis



Luminex
complexity simplified.
Package Insert

VERIGENE® Gram-Negative Blood Culture Nucleic Acid Test (BC-GN)

Kluyvera ascorbata, *Raoultella ornithinolytica*, *Raoultella planticola*, and *Cedecea davisae* cross-react with BC-GN *Klebsiella oxytoca* probes, which will cause a false positive "K. oxytoca Detected" result.

Comment added to Gram Negative Blood Culture Panel in chart to state that *Raoultella planticola* can cause false-positive results with K. oxytoca probe.

This helped to inform the provider that none of our equipment failed and that the quality of results not in question.

54 y/o Male Presents to ED Twice

- 54 y/o male, ED with AMS, left sided facial pain and paresthesia (tingling)
- Admitted, extensive testing, nothing found, discharged home
- Children say he is still “out of it”
- Next day back to a different ED for evaluation
- LP – CSF is clear, colorless, 2 nucleated cells/µL, 2 RBCs/µL, glucose 85 mg/dL (serum 144 mg/dL), protein 102 mg/dL.

- CSF cytospin Gram stain no PMNs and NOS
- M/E panel positive for HHV-6 only
- MRI brain show numerous foci in periventricular and subcortical white matter, not specific for any particular condition
- Admitted because of facial and upper extremity paresthesia, confusion and a diagnosis of viral meningitis
- Condition resolved and discharged after 48 hours with neurology follow up

CSF quant. HHV-6B, 14,900 copy/mL
Serum quant. HHV-6B, > 999,000 copy/mL

HHV-6B causes roseola, >90% pop. infected early in life. Reactivation can cause encephalitis. Latent or integrated virus can confound interpretation of DNA detection.

Our Next M/E Panel Test – 5 days Later

- 13 y/o M, outside ED with severe headache of several days
- Fever
- LP – CSF is clear, colorless, 1 nucleated cells/µL, 412 RBCs/µL, glucose 61 mg/dL (serum 95 mg/dL), protein 38 mg/dL.
- CSF Gram stain NOS

- CSF referred to us for M/E panel testing – positive for HHV-6 only
- **Repeat M/E panel on different tube of CSF – positive for HHV-6 only**
- No HHV-6 viral loads or typing performed

M/E Panel Laboratory Precautions

Process all specimens in a biological safety cabinet.

To guard against contamination of the specimen or test pouch due to nucleic acid/organism from the operator, other specimens, or cultured organisms in the laboratory.

Dedicated use of biological safety cabinet for sample preparation and pouch loading.

Cabinet should not be used for culture setup, examination of growing cultures, and is ideally used for molecular testing only.

Process one specimen and pouch at a time.

To prevent sample to sample contamination.

M/E Panel Post Test Review

Correlate the results of CSF testing on the M/E panel with other laboratory data, clinical presentation and patient history.

- **All unexpected or atypical results should be examined carefully and repeat M/E panel testing may be warranted.**

Audience Question

If I get an erroneous result from my nucleic acid amplification test it is always the test reagents or the instruments fault?

1. **True**

2. **False**

Points to Remember

1. **Critical thinking skills are important for the interpretation of even the most simple laboratory tests.**

2. **Timely review of respiratory virus reports from public health and the Wisconsin state laboratory can help determine the validity of certain test results.**

3. **A working knowledge of a test's *Instructions For Use* may be able to help resolve discrepancies between test results.**

4. **Nucleic acid amplification tests are very sensitive assays and require special care to insure the accuracy of the test results.**

5. **Just because a test is waived complexity does not mean that it is foolproof.**

6. **Microbiology testing is not like preparing a microwave dinner!!**


The End