HIV Trends, Guideline Recommendations, and the Evolution of Rapid Screening Tests

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Learning Objectives

- Identify differences in HIV testing methodologies
- Review current CDC and HRSA guidelines for HIV testing/screening/analysis and importance of early detection
- Determine the patient population that can benefit from rapid point-of-care testing for HIV antigen/antibody
- Develop strategies within one's own institution to increase screening for HIV
- Apply current guidelines and best practices to improve the care of patients who are HIV positive and HIV negative

Case Study

- 25-year-old female presents with fever, cough, malaise
- Has had these symptoms for the past two weeks
- No known sick contacts
- Found to have lymphadenopathy on physical exam
- Among other tests a rapid HIV fourth-generation test is ordered
- Rapid HIV fourth-generation test was reactive

Case Study

- When presented with the results the patient is distraught
- Reveals she did just acquire a new sexual partner in the past month
- Physician tells her the results require additional confirmatory testing which should be completed in approximately 1-2 weeks
- Collects a blood sample to send for Western blot confirmatory testing to his nearest reference lab

Human Immunodeficiency Virus (HIV)

- Enveloped single stranded RNA retrovirus
- Infects CD4 positive T cells leading eventually to immune deficiency and autoimmune deficiency syndrome (AIDS)
- Two major viral species of HIV:

  HIV-1
  - Derived from chimpanzees
  - Responsible for AIDS worldwide pandemic
  - Eventually leads to profound immunosuppression in most patients

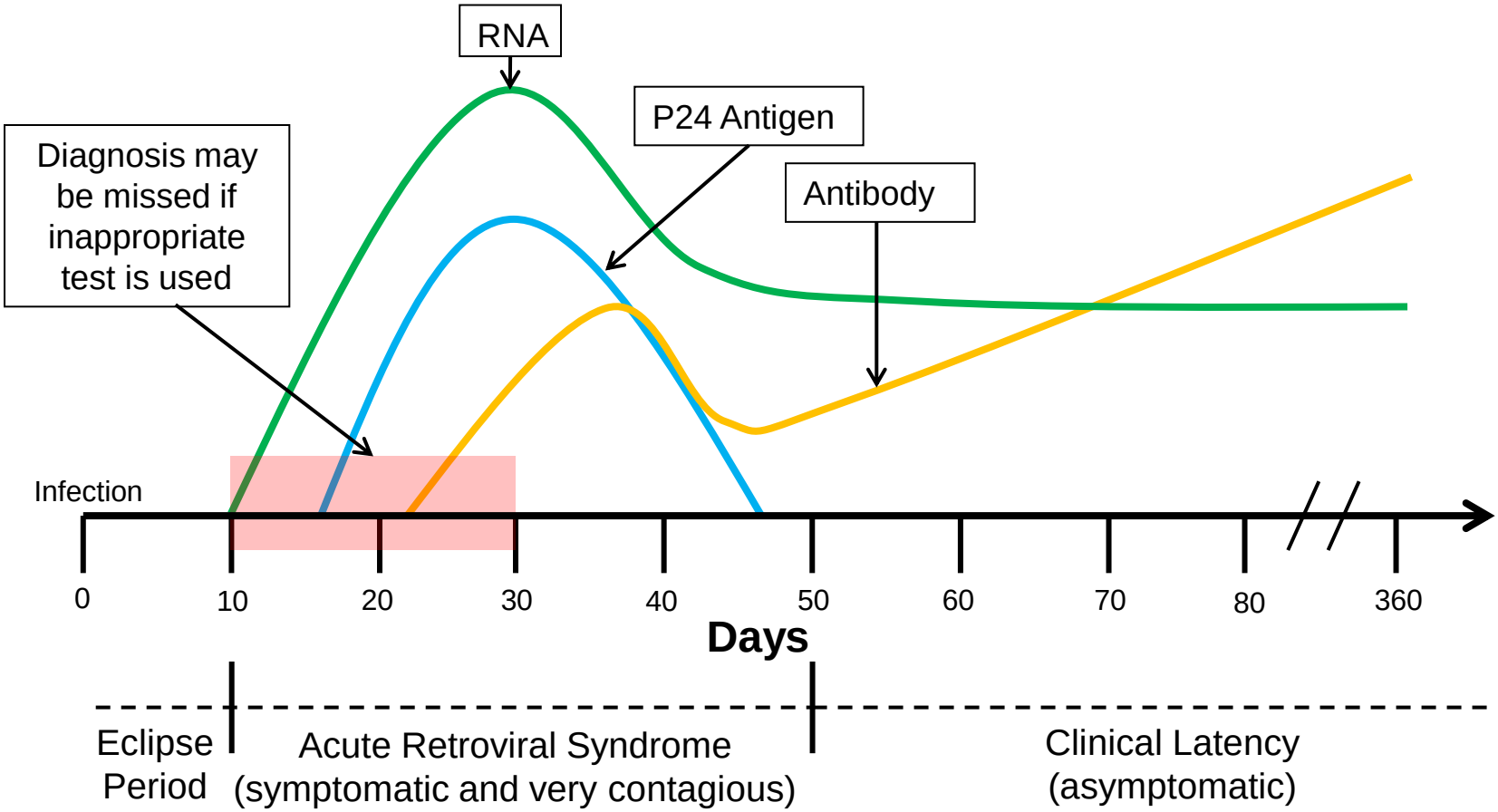
  HIV-2
  - Derived from sooty mangabeys
  - Limited geographic distribution (predominantly Africa and parts of Europe)
  - May be less severe than HIV-1, though also capable of profound immunosuppression

HIV Epidemiology

- Incidence is still high despite advances in knowledge and education
  - 44,073 people were diagnosed with HIV in the United States during 2014
- Prevalence is high
  - Approximately 1.2 million people are infected with HIV worldwide
  - 1 in 8 of infected patients do not know they are infected
  - 44% of people aged 13-24 do not know they are infected

Centers for Disease Control and Prevention. HIV Surveillance Report. 2014;26. http://www.cdc.gov/hiv/library/reports/surveillance/ Published November 2015. Accessed 7/15/2016.

HIV Progression

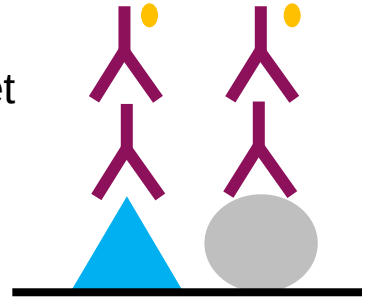


Adapted from Laboratory Testing Recommendations for the Diagnosis of HIV, Updated Recommendations, Centers of Disease Control and Prevention. June 2014

Advances in Serology

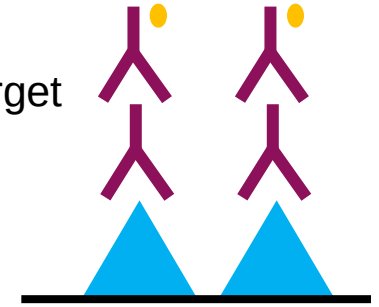
First Generation

Viral lysate antigen target
Detect IgG only
Only ~95% specific
8-10 week window



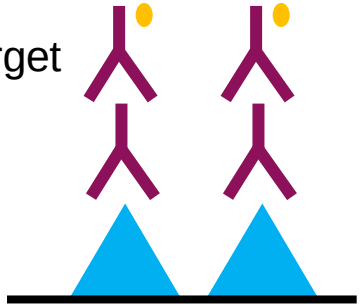
Third Generation

Recombinant antigen target
Detect IgG and IgM
99.5% specific
2-3 week window



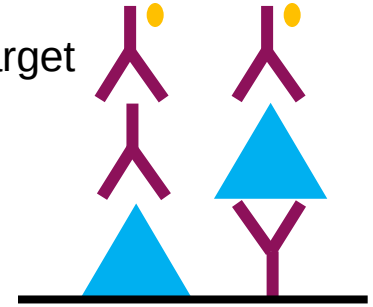
Second Generation

Recombinant antigen target
Detect IgG only
99% specific
4-6 week window



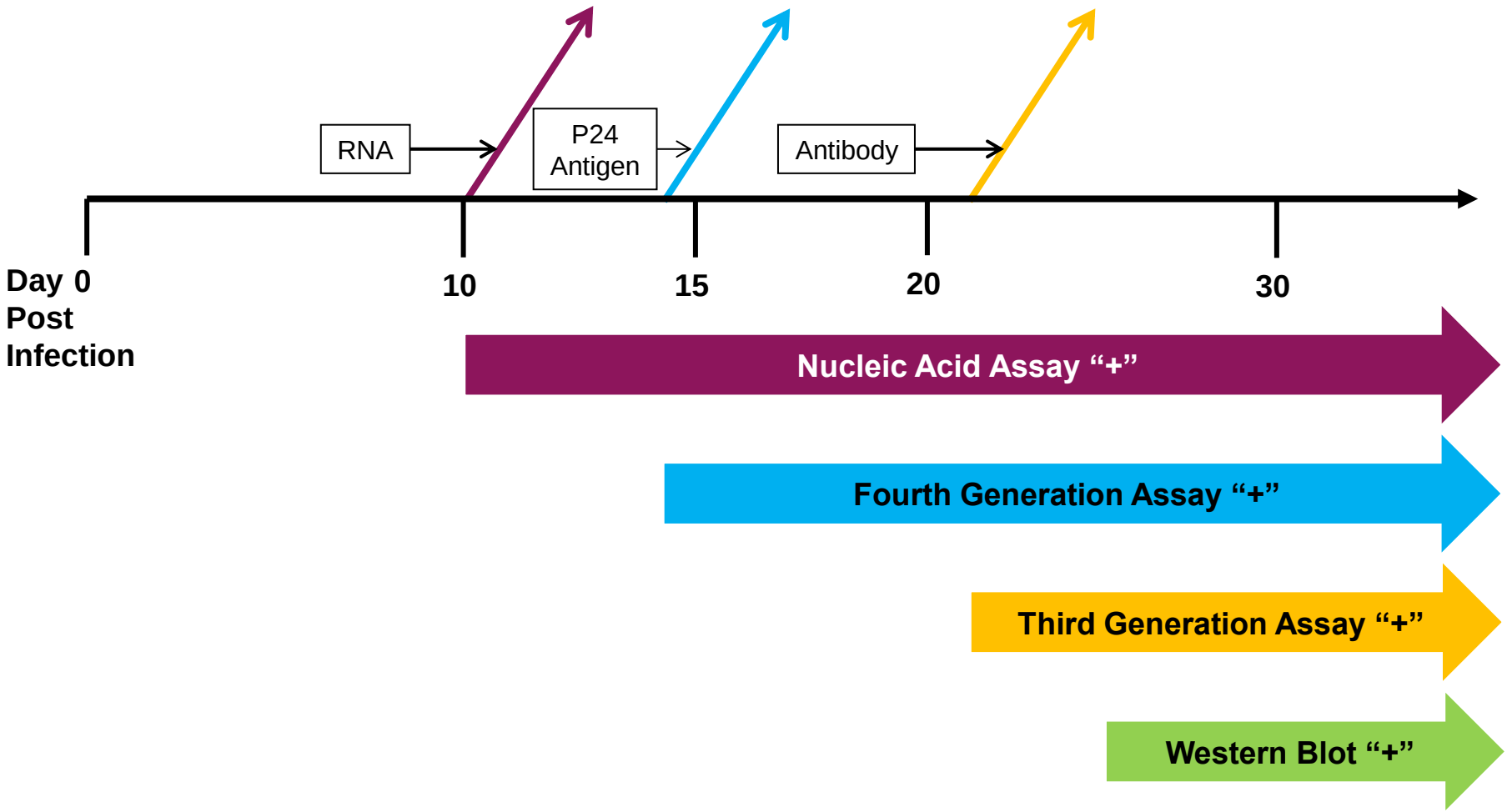
Fourth Generation

Recombinant antigen target
Detect p24 antigen
Detect IgG and IgM
99.5% specific
2 week window



Adapted from Chappel R J, Wilson K M, Dax E M. *Future Microbiol*. 2009;4(8):963-82.

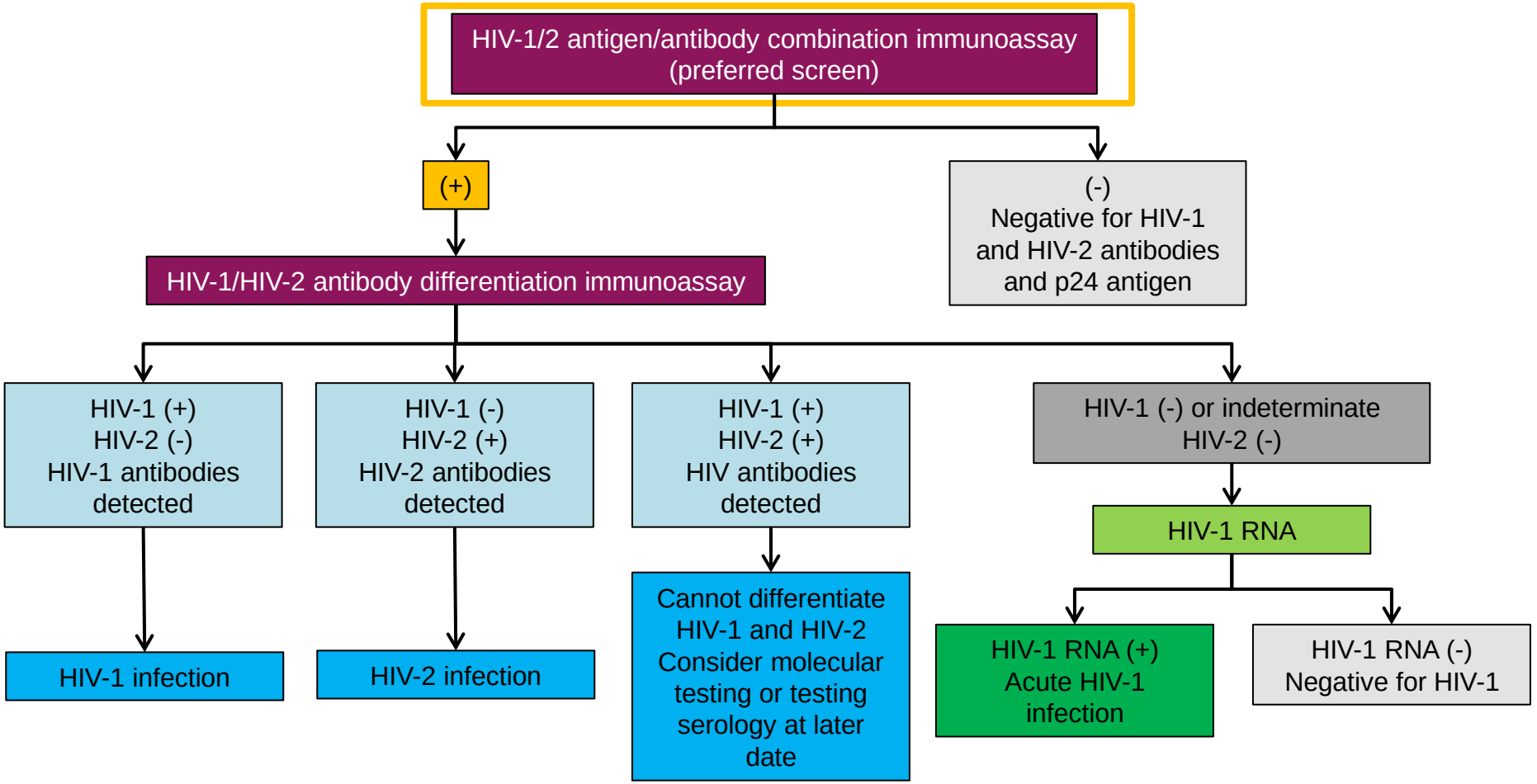
Lab Result Timeline



Available Diagnostics

- Traditional screening performed using a third-generation enzyme immunoassay (EIA)
  - Tests for presence or absence of HIV specific antibodies
- Traditional confirmation performed by Western blot immunoassay
  - Discerns antibody specificity to immobilized HIV proteins
  - Must have antibodies to multiple key proteins to be interpreted as positive
- Novel “fourth-generation assays” detect both antibody and p24 antigen
  - Allow for earlier diagnosis than serology alone
  - Currently recommended by Centers for Disease Control (CDC) for routine screening
  - Still require confirmatory testing

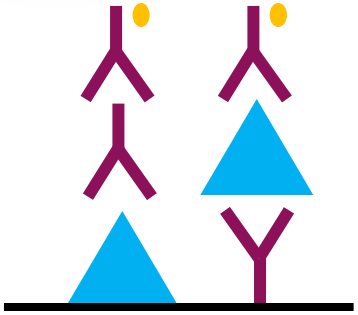
Fourth-Generation Algorithm



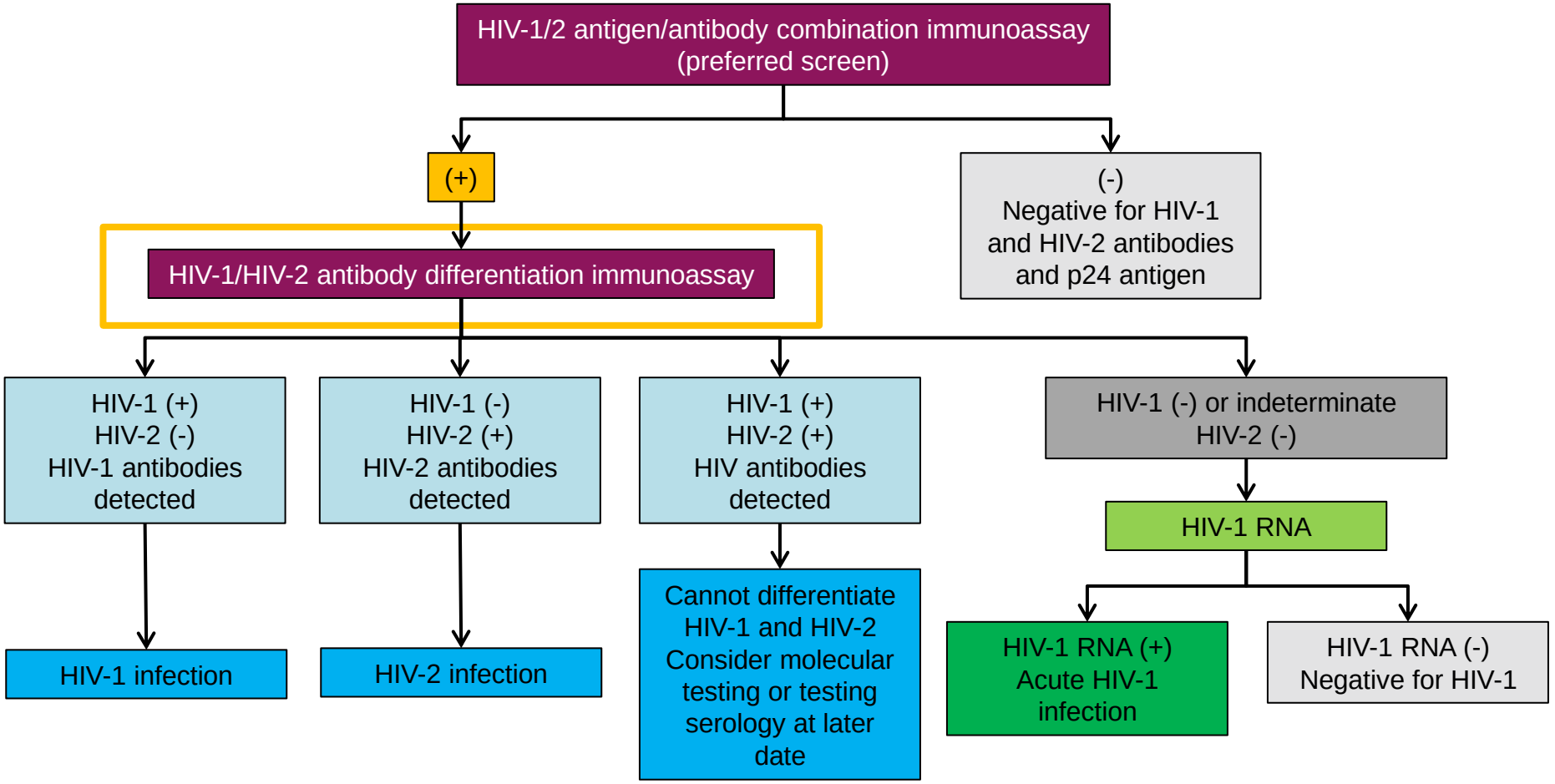
Adapted from: http://www.cdc.gov/hiv/pdf/hivtestingalgorithmrecommendation-final.pdf

Fourth Generation Antigen/Antibody Assays

- Detects all immunoglobulin classes to HIV-1 and HIV-2
- Detects p24 expressed by HIV-1 and HIV-2
- Increased sensitivity and specificity compared to many third-generation assays
- Most performed on large chemistry lab analyzers
  - ADVIA Centaur: < 1 hour run time
  - Abbott Architect: < 30 minute run time
  - Bio-Plex
    - 45 minute run time
    - Capable of differentiation between p24 and HIV-/2 antibodies
- Positive results require further confirmation



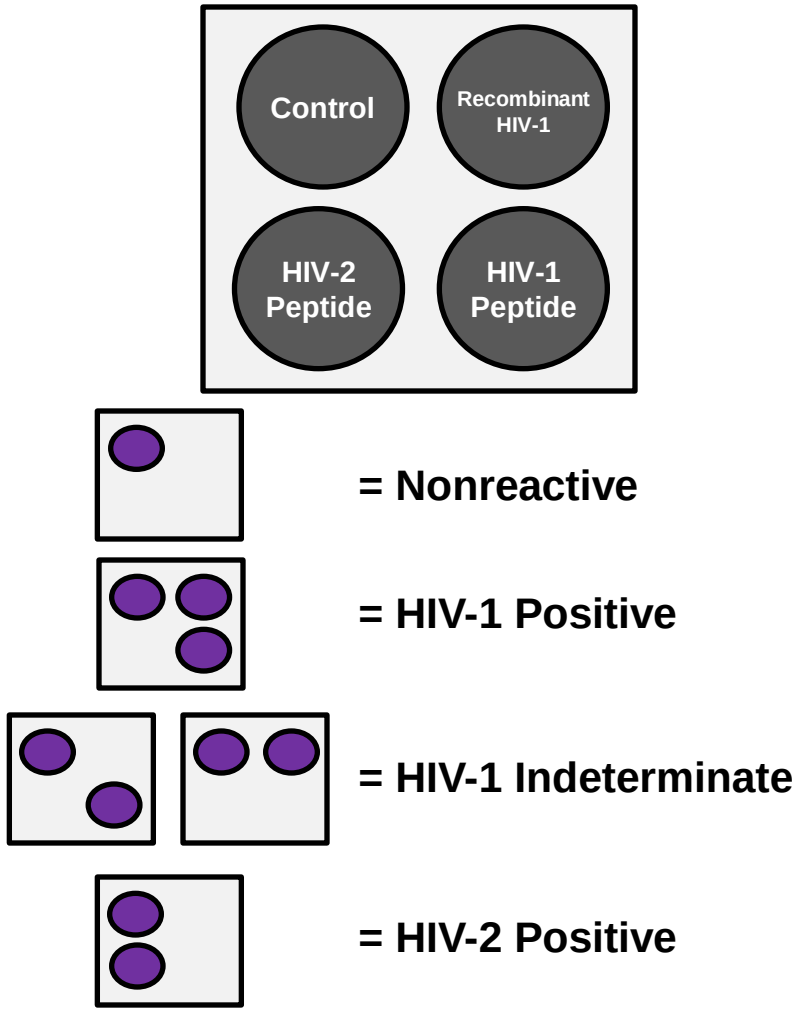
Fourth-Generation Algorithm



Adapted from: http://www.cdc.gov/hiv/pdf/hivtestingalgorithmrecommendation-final.pdf

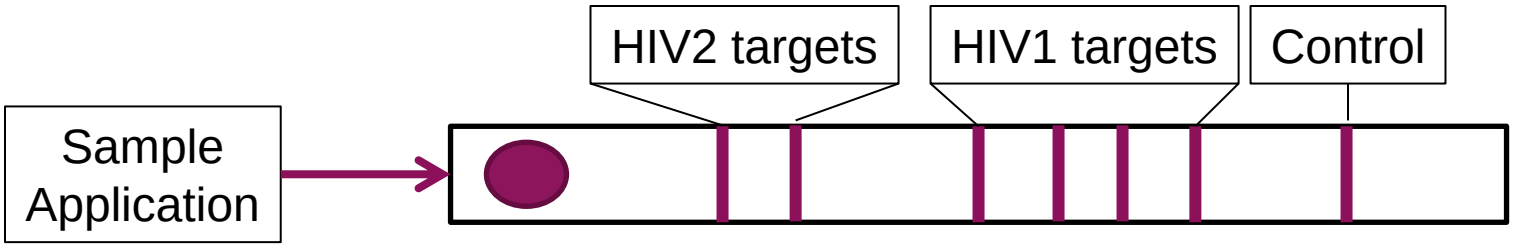
Multispot HIV-1/HIV-2 Rapid Test

- A.k.a. HIV-1/2 Differentiation Assay
- Automatically performed following positive antigen/antibody screen (not orderable)
- Second-generation assay
- Detects only antibody
- Steps
  - Immobilized HIV-1 and HIV-2 antigens treated with patient serum
  - After washing alkaline phosphatase labeled goat antihuman IgG is added
  - Developer is added and positive test spot turn purple



Geenius™ HIV ½ Supplemental System

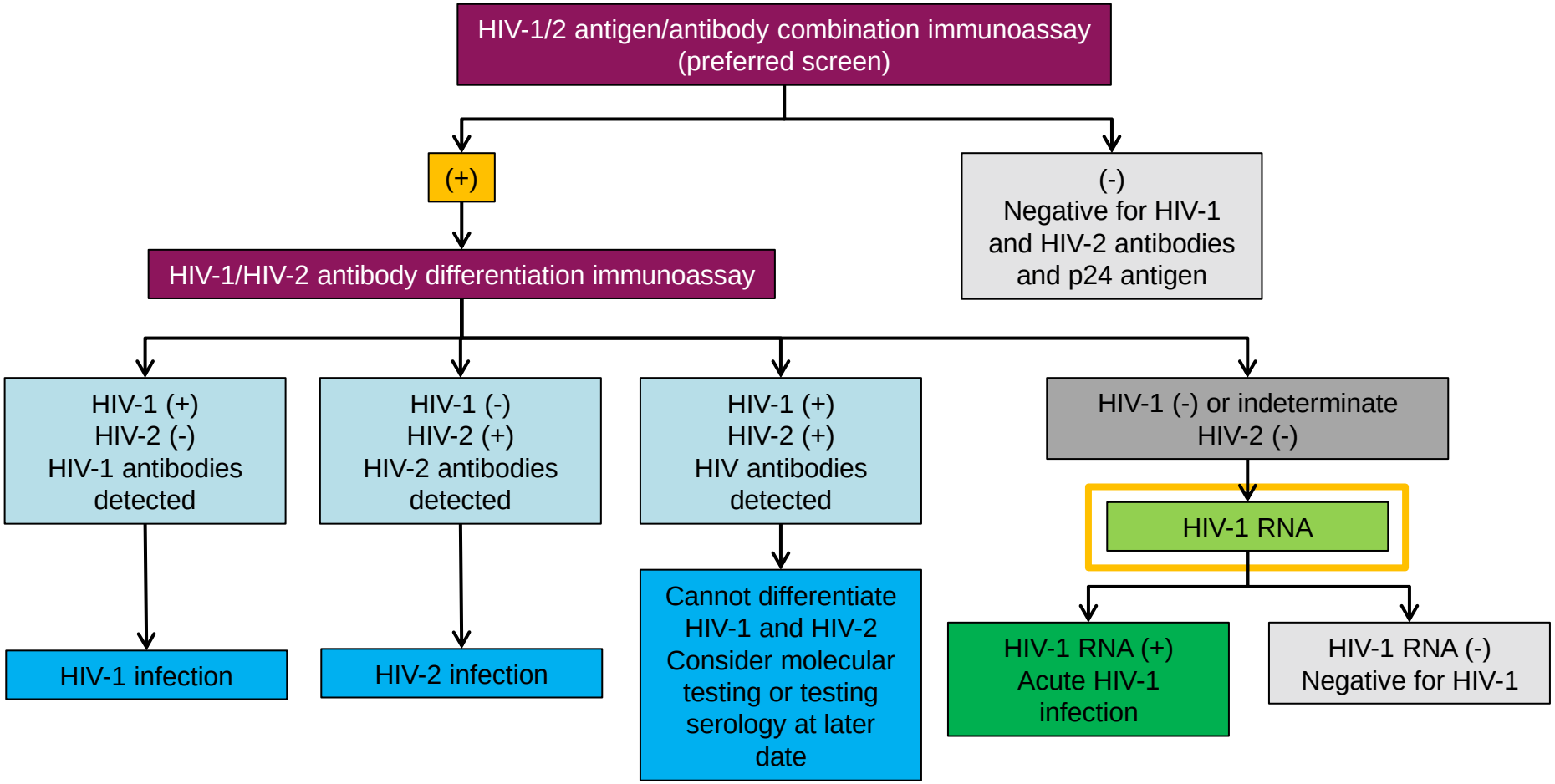
- FDA approved supplemental HIV test
- Successor to the Multispot HIV-1/HIV-2 Rapid Test
  - Multispot no longer in production by manufacturer
- Immunochromatographic assay
- Tests for antibodies against
  - 4 HIV-1 proteins
  - 2 HIV-2 proteins
- Results interpreted by an automated reader
  - Helps prevent user error



Geenius Validation Data

- 46 specimens previously tested by the Multispot were tested by the Geenius
- 22 Multispot negative specimens
  - All 22 tested negative by Geenius
- 24 Multispot HIV-1 positive specimens
  - 22 tested HIV-1 positive
  - **2 (8.3%) tested HIV-1 positive with HIV-2 crossreactivity**
- 7 Multispot HIV-2 positive specimens
  - 1 tested HIV-2 positive
  - **5 (71%)tested HIV-2 positive with HIV-1 crossreactivity**
  - 1 tested as undifferentiated

Fourth-Generation Algorithm



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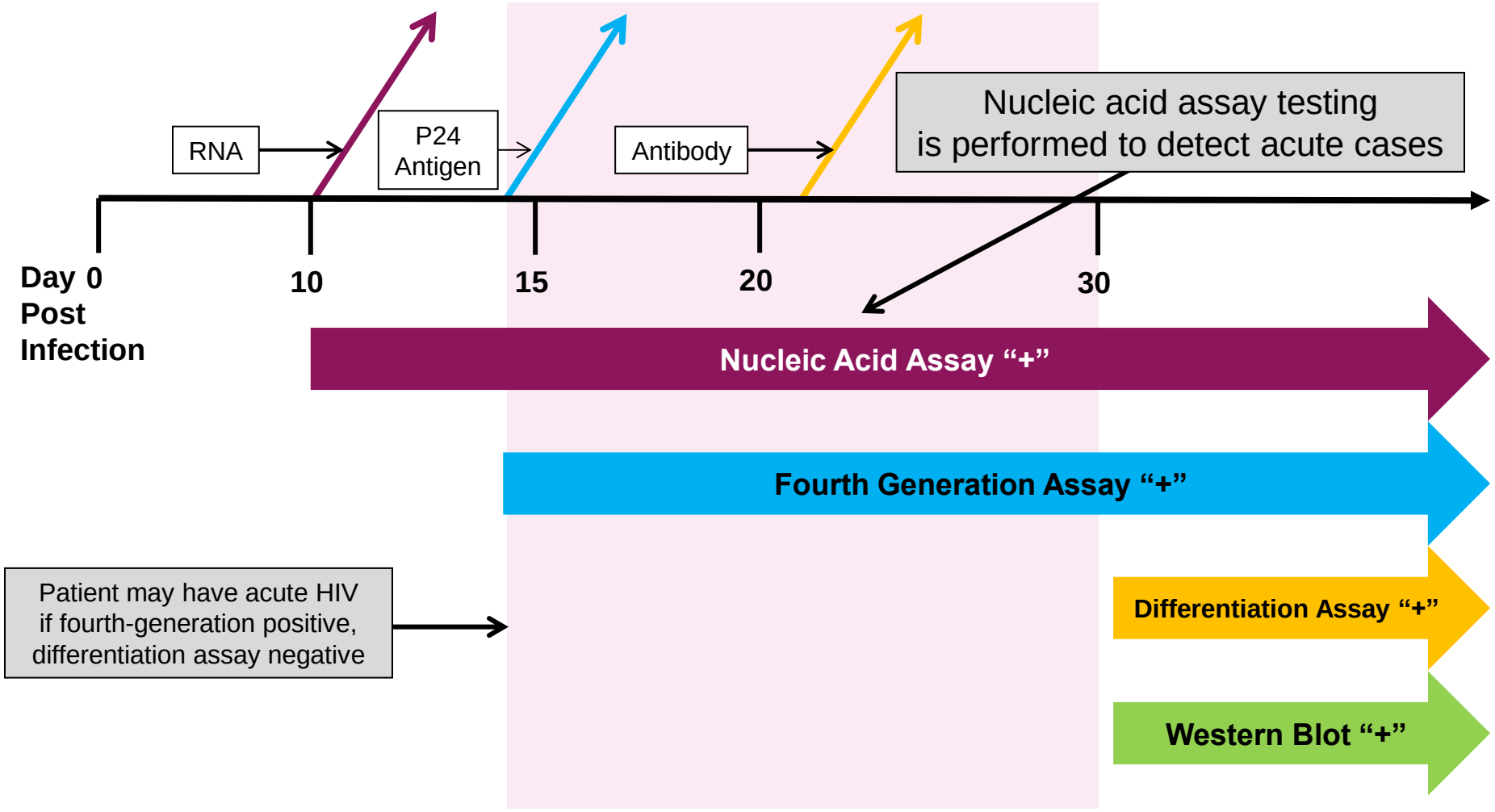
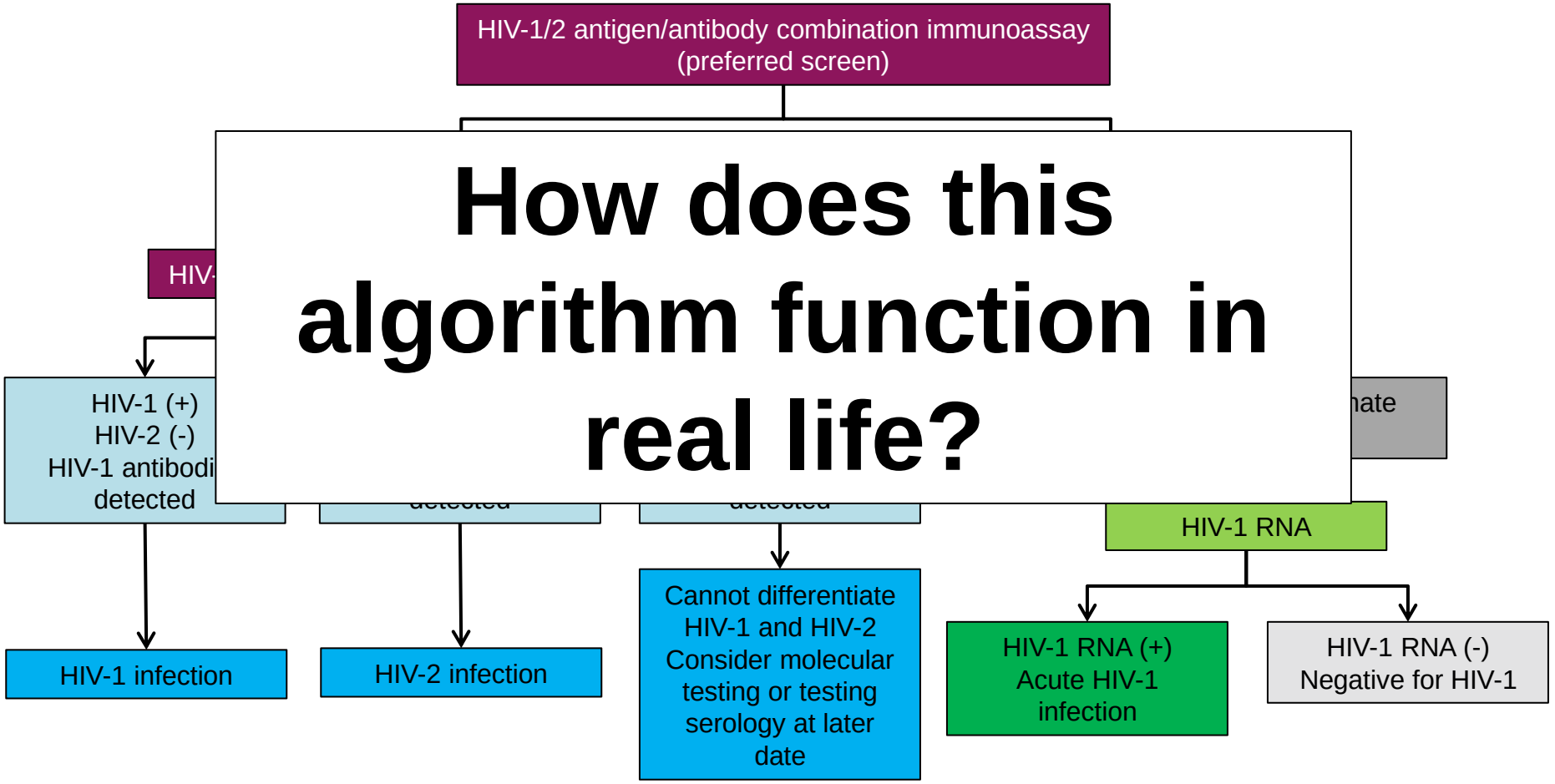
Lab Result Timeline



HIV PCR Role in Diagnosis

- Currently only one test is FDA approved for HIV-1 diagnosis
  - Aptima HIV-1 RNA Qualitative Assay
  - Qualitative and targets viral RNA
  - Uses transcription mediated amplification rather than PCR
- In practice, quantitative tests are often used as part of a diagnostic algorithm
  - These tests are FDA-approved for monitoring, not diagnosis (low rate of false positives)
  - If a patient is positive by molecular testing alone, serologic conversion should be demonstrated for a definitive diagnosis
- **All FDA-approved HIV PCR tests only detect HIV-1 (need separate testing if HIV-2 suspected)**

Fourth-Generation Algorithm



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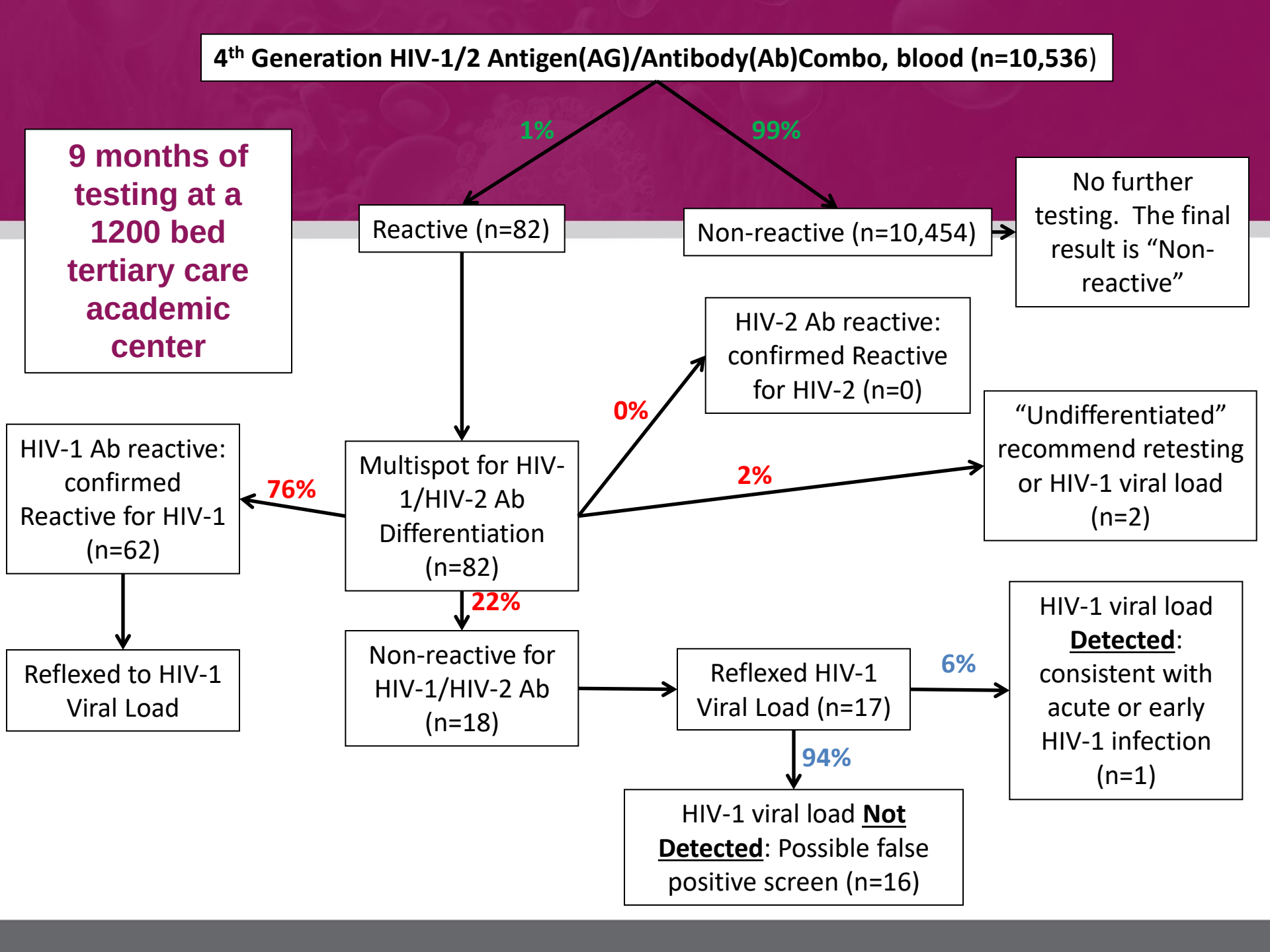

4th Generation HIV-1/2 Antigen(AG)/Antibody(Ab)Combo, blood (n=10,536)
1%
99%
9 months of testing at a 1200 bed tertiary care academic center
Reactive (n=82)
Non-reactive (n=10,454)
No further testing. The final result is "Non-reactive"
HIV-2 Ab reactive: confirmed Reactive for HIV-2 (n=0)
0%
"Undifferentiated" recommend retesting or HIV-1 viral load (n=2)
2%
HIV-1 Ab reactive: confirmed Reactive for HIV-1 (n=62)
76%
Multispot for HIV-1/HIV-2 Ab Differentiation (n=82)
Reflexed to HIV-1 Viral Load
22%
Non-reactive for HIV-1/HIV-2 Ab (n=18)
Reflexed HIV-1 Viral Load (n=17)
6%
HIV-1 viral load Detected: consistent with acute or early HIV-1 infection (n=1)
94%
HIV-1 viral load Not Detected: Possible false positive screen (n=16)

False Positive Antibody Screens

- Approximately 25% of antigen/antibody screens were false positives
  - Is this too high???
- A study of 10,014 life insurance applicants of low seroprevalence were tested by this algorithm
  - 13 patients were positive on initial testing **(85% false positives)**
- A study of 51,935 Florida patients in a high seroprevalence setting were tested by the algorithm
  - 1089 patients were positive on initial testing **(7.2% false positives)**
- **Take home- Population sero-prevalence affects positive predictive value!**

-Nasrullah, Muazzam et al. “Performance of a Fourth-Generation HIV Screening Assay and an Alternative HIV Diagnostic Testing Algorithm.” *AIDS (London, England)* 27.5 (2013): 731–737. *PMC*. Web. 15 Sept. 2017.
-B. Bennett, D. Neumann, S. Fordan, R. Villaraza, S. Crowe, L. Gillis**Performance of the new HIV-1/2 diagnostic algorithm in Florida's public health testing population: a review of the first five months of utilization** J. Clin. Virol., 58 (Suppl. 1) (2013), pp. e29-33,

False Positive Antibody Screens

- Conditions implicated with false positives
  - Rheumatoid arthritis, lupus, Sjogren's and other autoimmune conditions
  - Cross reacting viruses
  - Pregnancy
- Chart review of patients with false positive screens (n=14)
  - 7/14 patients were either pregnant (n=3) or had a documented autoimmune disorder (n=4)
  - 2/14 had identified risk factors (IVDU)
    - Both positive for HCV, though ultimately HIV negative
  - Remaining five patients included
    - Patient with alcoholic pancreatitis
    - Patient with sepsis
    - Patient with FUO that spontaneously resolved
    - Patient with cystic fibrosis s/p lung transplant
    - Patient with unknown medical history

Role of Laboratory in HIV Testing

- Fourth-generation algorithm is relatively new
  - First formally recommended by the CDC in 2014
- Since diverse groups of physicians are ordering HIV testing there WILL be mistakes!
- The laboratory has a duty to educate and guide appropriate testing
- Can be accomplished through:
  - Published algorithms
  - Automatic reflexive testing
  - Clinical decision support
  - Limiting inappropriate testing

Common Testing Challenges

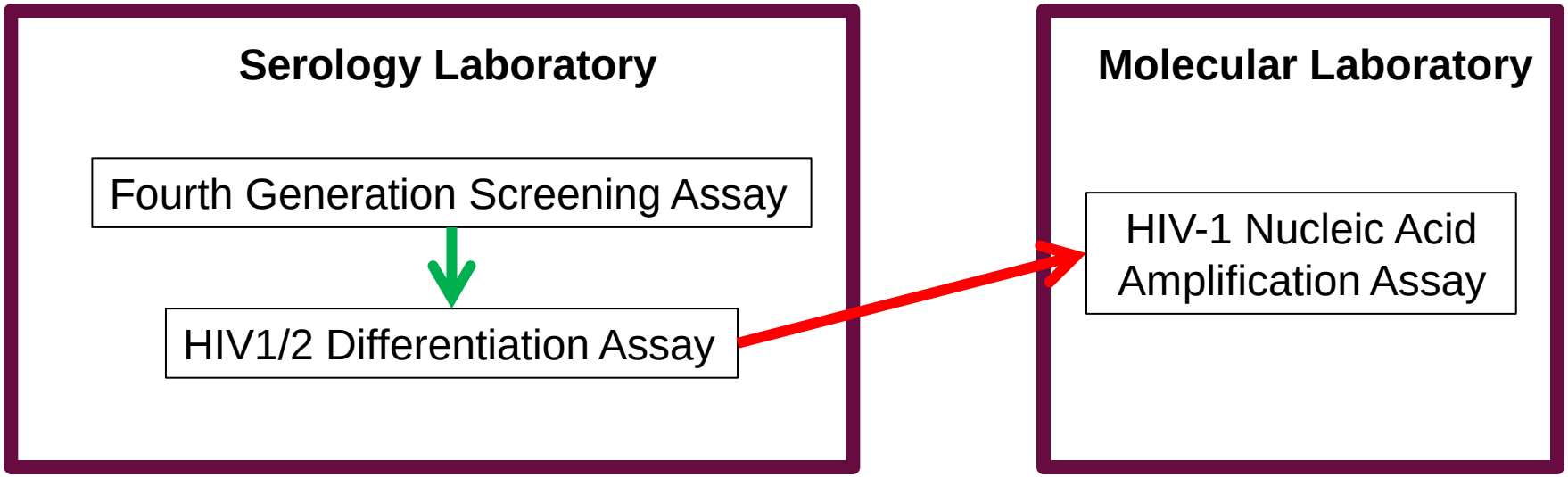
- **Proper result reporting**
- **Assuring follow-up testing happens**
- **Testing outside of the recommended algorithm**

Testing reporting- Using the right language

- Laboratory MUST specify assay used
- Laboratories MAY issue preliminary results before completing algorithm
  - If they do, reports should include what follow-up testing is needed
- Reporting fourth generation screening assays
  - “Reactive” and “Nonreactive” should be used
- Reporting HIV1/2 differentiation assays
  - “HIV-1 positive”, “HIV-1 negative”, “HIV-2 positive”, “HIV-2 negative” should be used
- **A final interpretation of algorithm results should always be provided**

Assuring follow-up testing happens

- Ideally algorithmic testing works best when it can be performed automatically on a single specimen
- Only works if all testing performed at same facility!
- Even when testing is available all in one facility this is challenging…



Contamination Commonly Occurs in Chemistry Laboratories

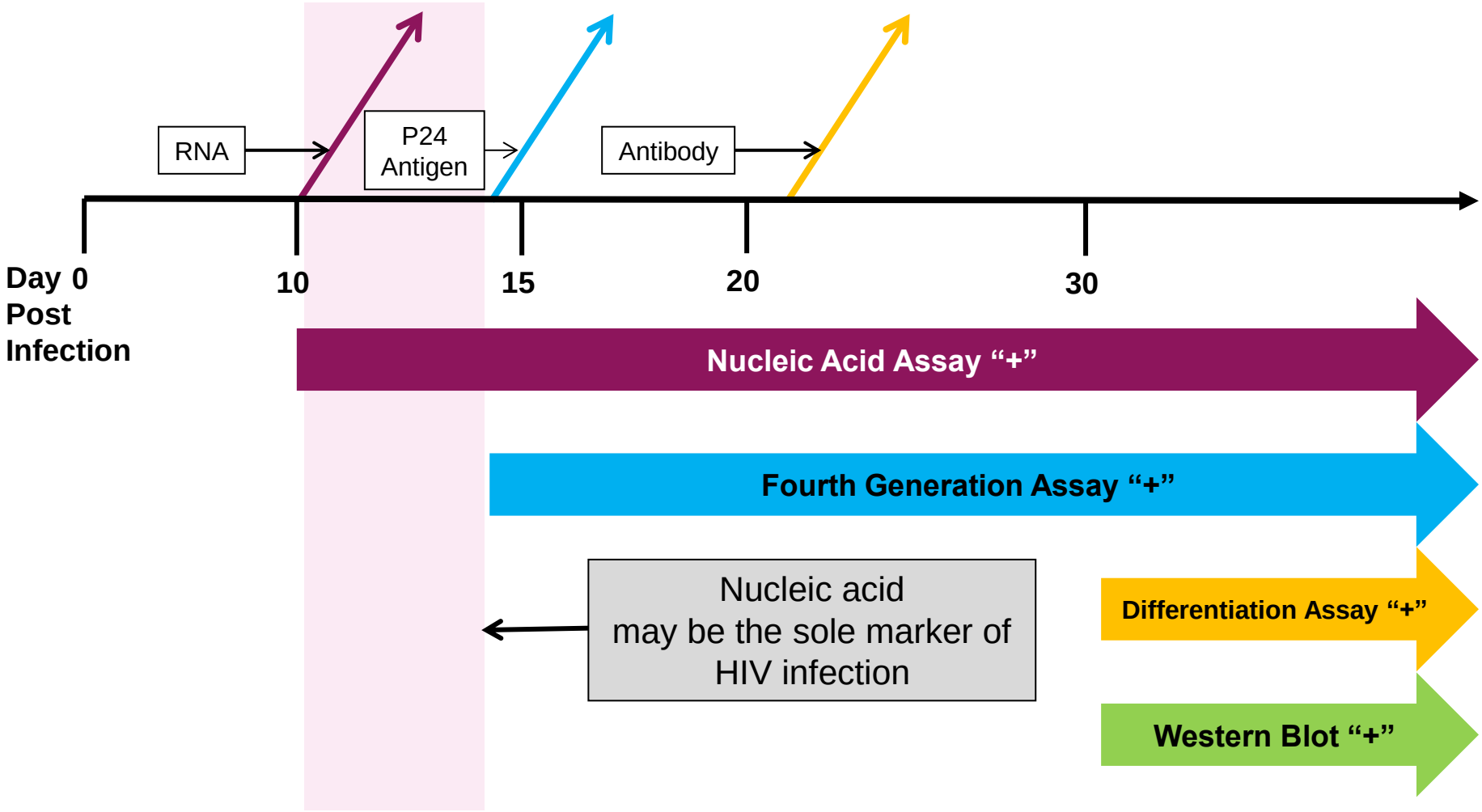
- 2016 publication in Clinical Chemistry by Bryan and colleagues
- Performed environmental sampling of their total laboratory automation system for HCV and HBV to assess for contamination
  - Of the 79 baseline swabs, 10 were positive for HBV and 8 for HCV
  - Positive sites included specimen decapper and centrifuge rotor
- Ran high titer HCV sample through a routine chemistry analyzer
  - Demonstrated additional sites of HCV contamination

Bryan A, Cook L, Atienza EE, Kuypers J, Cent A, Baird GS, et al. Bloodborne viral patho gen contamination in the era of laboratory automation. Clin Chem 2016;62: 973-981.

Molecular and Serology Testing do not Mix Well

- CAP checklist item
  - "There are written procedures to prevent specimen loss, alteration, or contamination."
  - *"Special precautions must be taken to avoid sample cross-contamination that may not affect culture-based methods but may lead to false positive results when tested using molecular amplification methods."*
- Many laboratories have adopted a policy of requiring specific dedicated specimens for molecular testing
- **A system must be in place to assure a second specimen is obtained if molecular testing is needed**
  - **Report, phone-call, physician alert, etc.**

Screening with Molecular Testing



Screening with Molecular Testing

- Screening with an HIV molecular testing may be appropriate when acute HIV is suspect
- Several significant limitations
  - FDA approved assays are limited in availability (currently only 1)
  - Expensive
  - Misses HIV-2
  - Very susceptible to false positives
- Should always be accompanied by appropriate serologic testing

Analysis of HIV NAAT Ordering

- Retrospective analysis of NAAT ordering over a ten month period at a 1200 bed tertiary care academic center
- Examined how many patients without a previous diagnosis of HIV were tested by
  - NAAT
  - Serology
  - Serology and NAAT
- Examined patient charts to discern indication for test ordering
- NAAT test available- COBAS Ambliprep/COBAS Taqman HIV RNA Assay
- Serology test available- Abbott architect fourth generation assay

Analysis of HIV NAAT Ordering

	n
Total Screened for HIV Diagnosis	14,766
Total Screened with Serology Alone	14,513 (98.3%)
Total Screened with NAAT Alone	119 (0.8%)
Total Screened with both Serology and NAAT Initially	134 (0.9%)

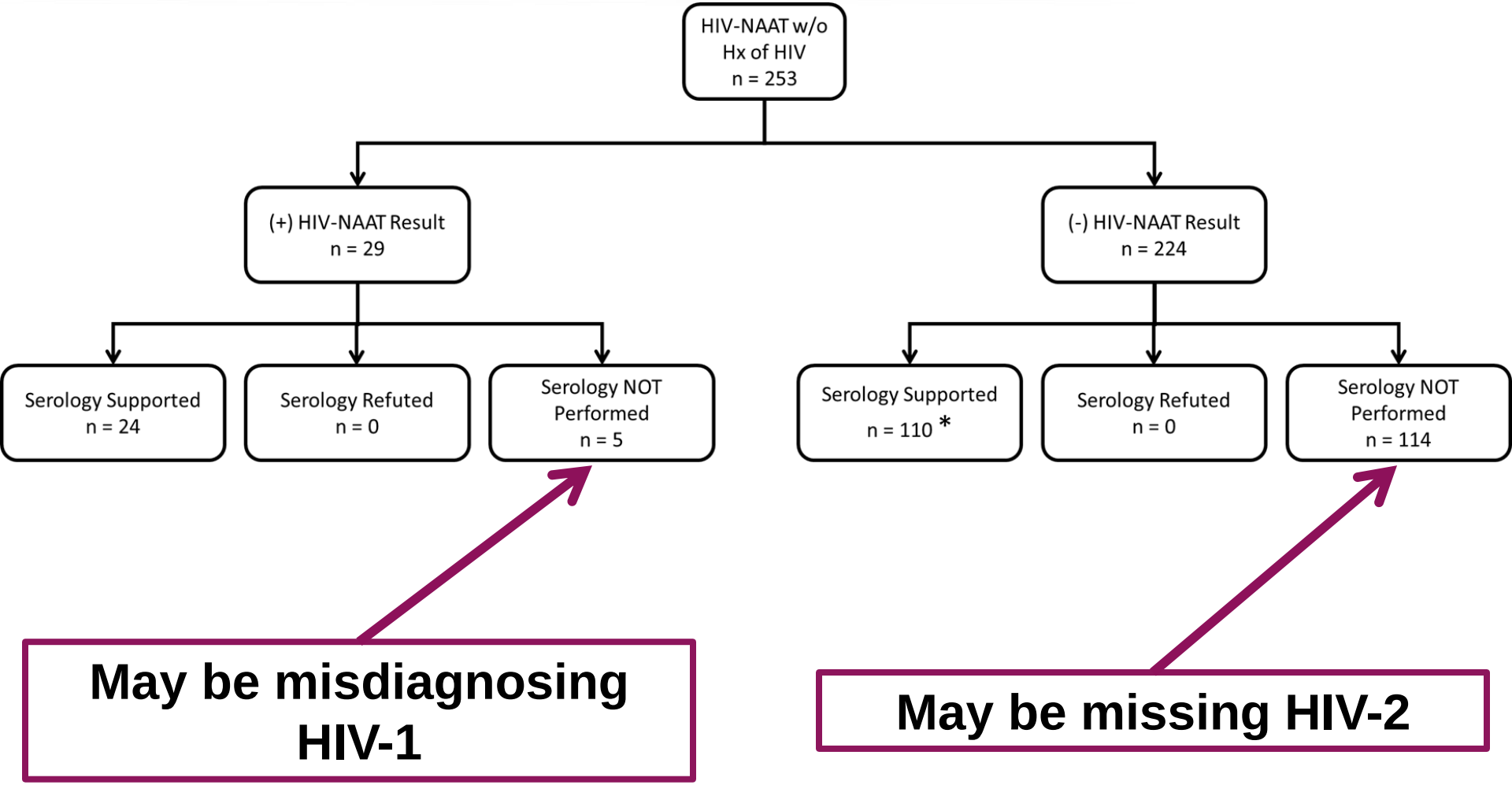
	NAAT (+)	NAAT (-)	Total
Inpatient	12 (41.4%)	136 (60.7%)	150 (59.3%)
Outpatient	8 (27.6%)	79 (35.3%)	87 (34.4%)
Emergency Unit	9 (31.0%)	4 (1.8%)	13 (5.1%)
Unknown	0 (0.0%)	5 (2.2%)	5 (2.0%)
Total	29	224	253

Indication for NAAT Ordering

	(+) NAAT	(-) NAAT	Total
Fever of Unknown Origin	3 (10.3%)	28 (12.5%)	31 (12.2%)
Altered Mental Status	4 (13.8%)	31 (13.8%)	35 (13.8%)
Respiratory Symptoms	3 (10.3%)	11 (4.9%)	14 (5.5%)
Other Symptoms c/w HIV *	8 (27.6%)	43 (19.2%)	51 (20.2%)
Patients with High Risk **			38 (15.0%)
History of IV Drug Abuse			16 (6.3%)
High Risk Sexual Behavior	9 (31.0%)	13 (5.8%)	22 (8.7%)
Transplant Patient	1 (3.4%)	39 (17.4%)	40 (15.8%)
Asymptomatic & No Risk Factors	3 (10.3%)	70 (31.3%)	73 (28.9%)
Unknown History	2 (6.9%)	17 (7.6%)	19 (7.5%)
Total Patients in Study Group	**29**	**224**	**253**

Potentially inappropriate orders

NAAT Ordering with Serologic Followup



NAAT Orders Without Serology

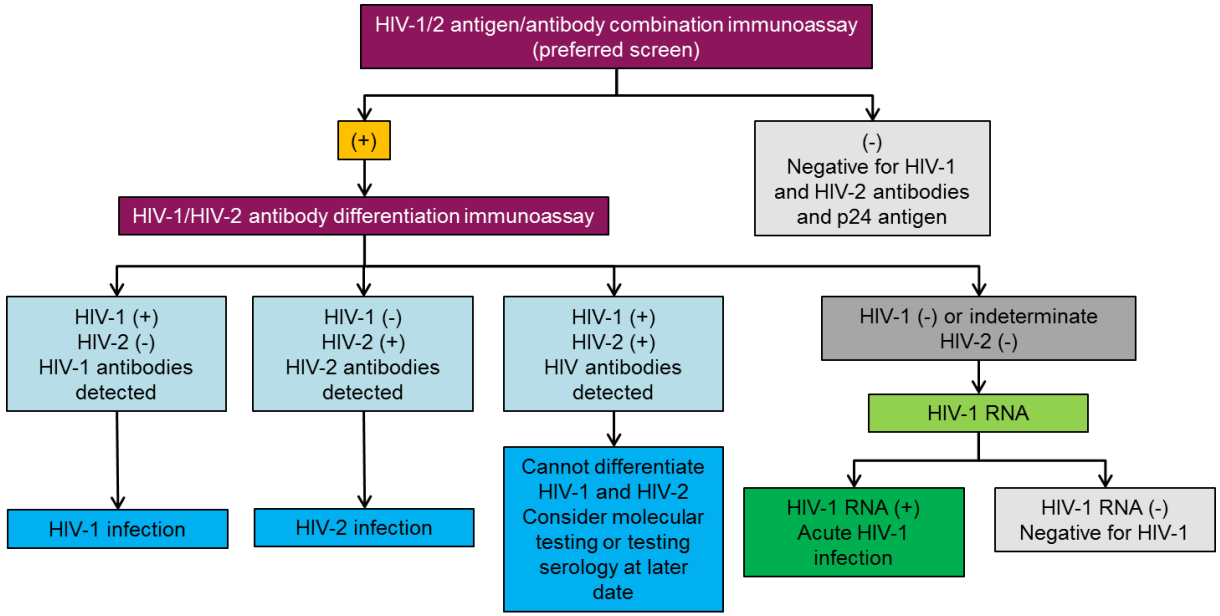
Patient	Viral Load (copies/mL)	Symptoms and/or Relevant History
1	497,000	Undergoing evaluation for Bone Marrow Transplant
2	114,000	Respiratory Infection, History of MSM
3	<20.0	Altered Mental Status
4	145,000	Progressive Blindness, History of IV Drug Use
5	74,800	Altered Mental Status

NAAT Screening Recommendations

- May be appropriate if acute HIV is suspected
- If a laboratory offers molecular HIV testing they really should pay attention to how the test is being used
- While highly specific, false positives with these tests do occur
- **Follow-up testing to document sero-conversion should be conducted if diagnosis is based on molecular test alone**

Alternative Algorithms

- Multiple common HIV tests are not included in the fourth generation algorithm
  - Third generation assays
  - Western blots
  - Rapid antibody tests
- These can very challenging to interpret!



Adapted from: http://www.cdc.gov/hiv/pdf/hivtestingalgorithmrecommendation-final.pdf

Alternative Screening

- The CDC recommends using a fourth generation screening assay for routine patient screening
- Not all laboratories have access to a fourth generation screening assay
- Many patients are still screened using in-lab third generation assays
- How should these tests be interpreted?
- What type of follow-up testing is needed?

Third Generation Screening Recommendations

- Main limitation
  - Testing using a third generation assay is not as sensitive as fourth generation testing
- It should be clearly reported that the patient was tested with a third generation assay
- The limitations of this approach should also be stated

- **When using a third generation test as an initial screen follow-up testing should be performed using the rest of the fourth generation algorithm**
  - **HIV1/2 differentiation assay and molecular testing if appropriate**

Alternative Confirmation

- Confirmation should be performed using HIV1/2 differentiation assay, though these may not be available at most labs
- What about confirmation using Western Blot?
- DON'T DO IT!!!!
  - This strategy is inadequate for the diagnosis of new infections
  - This strategy has a higher likelihood of leading to indeterminate results
  - This strategy has a longer turnaround time
- **Many of the same reference labs that offer Western Blots also offer HIV1/2 differentiation assays…so there is no reason to send out testing for a Western Blot!**

Back to the Case…

- Upon ordering the Western blot, the physician was contacted by the lab and told they do not send out Western blots anymore
- Rather, they use the fourth-generation algorithm and can get an answer to the physician within a day
- The physician is grateful though confused
  - “How do rapid tests fit into the fourth-generation algorithm?”

Rapid HIV Tests

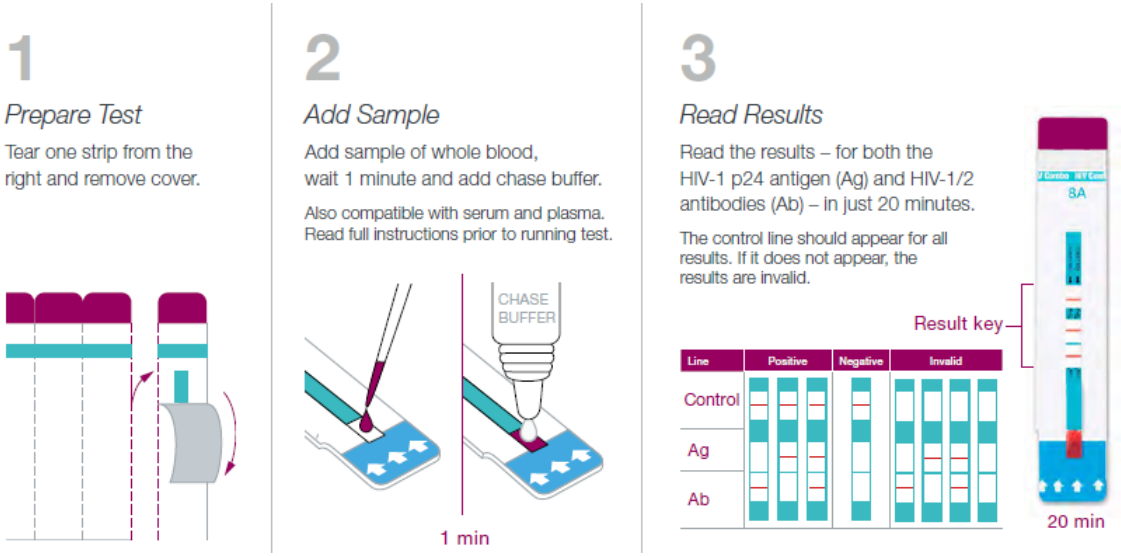
- Variety of different formats
  - Some detect IgG only (second-generation)
  - Some detect IgG/IgM (third-generation)
  - Some detect IgG/IgM and p24 antigen (fourth-generation)
- Advantages
  - Easy to perform
  - Results often in under 30 minutes
  - Many are CLIA- waived, so can be used at the point-of-care
  - Can use a variety of specimens (i.e. saliva, blood, etc.)

CLIA-Waived HIV Rapid Tests

Test	Detects
Chembio DPP HIV-1/2	HIV IgG antibody (second-generation)
Clearview COMPLETE HIV-1/2	
Clearview HIV-/2 STAT-PAK	
OraQuick ADVANCE Rapid HIV-1/2 Antibody Test	
Uni-Gold Recombigen HIV-1/2	HIV IgG/IgM antibody (third-generation)
INSTI HIV-1/HIV-2 Antibody Test	
Determine HIV-1/2 Ag/Ab Combo Test	HIV IgG/IgM antibody and antigen (fourth-generation)

Fourth-Generation Rapid Tests

- Important advance in HIV testing
- Allows for a rapid and highly accurate diagnosis
- Better accuracy in patients with acute HIV than other rapid tests
- Currently only Alere Determine HIV-1/2 Ag/Ab Combo Test FDA-approved
  - CLIA-waived for fingerstick whole blood
  - FDA-approved for whole blood, fingerstick whole blood and plasma

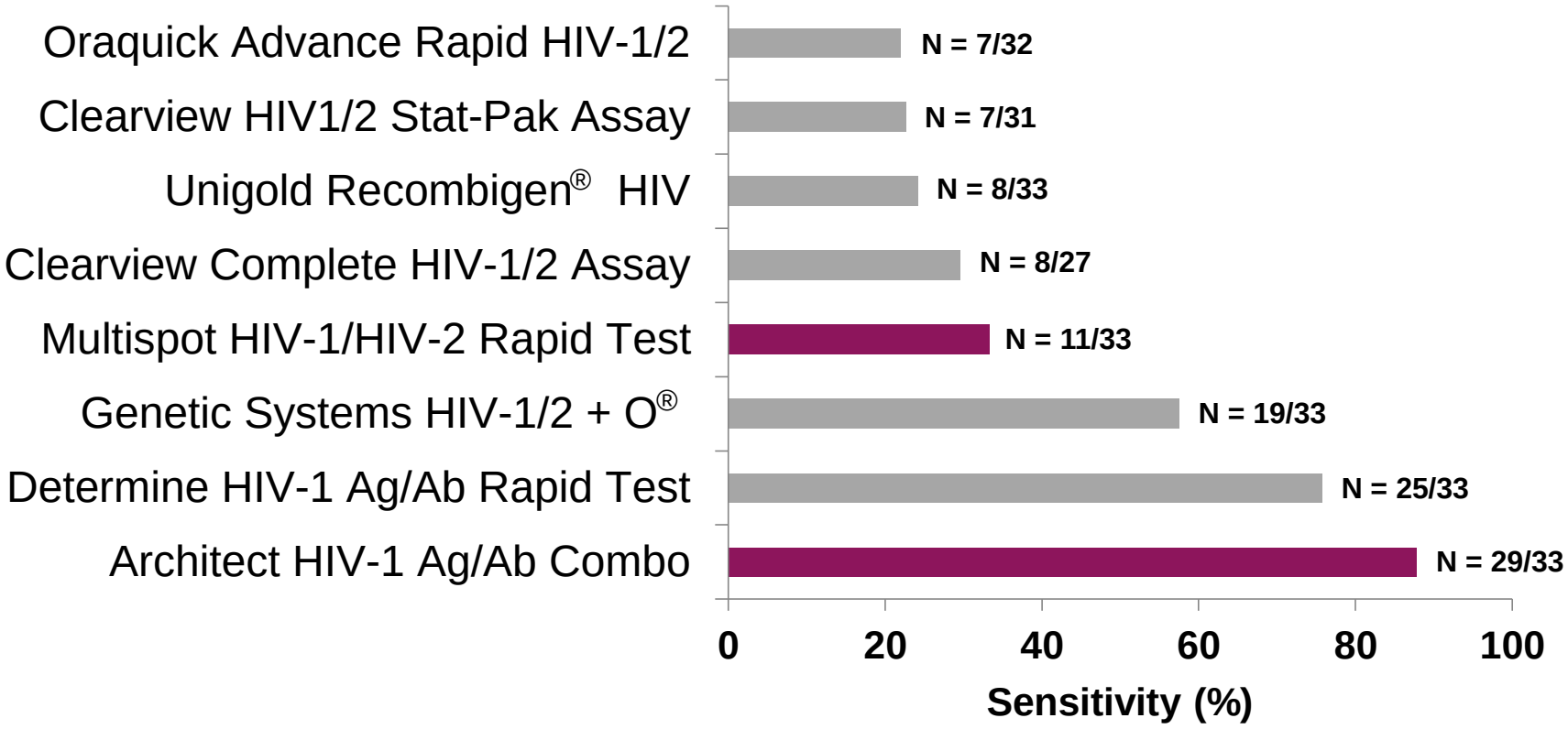


Performance of Rapid Tests Compared to In-lab Tests

Test	Type of Test	Time Positive Before Western Blot
Aptima	Molecular	-26 days
Abbott Architect	In-lab fourth-generation	-20 days
BioRad Combo	In-lab fourth-generation	-18.5 days
Determine Combo	Rapid fourth-generation	-15.5 days
Advia Centaur	In-lab third-generation	-14 days
Vitros	In-lab third-generation	-13 days
Uni-Gold	Rapid third-generation	-2 days
Multispot	In-lab second-generation	-7 days
OraQuick	Rapid second-generation	-1 day

Masciotra S, Luo W, Youngpairoj AS, et al. *J Clin Virol.* 2013;58(Suppl 1):e54-e58.

Screening in Early/Acute HIV



Sensitivity in patients with positive nucleic acid amplification test and negative/indeterminate Western blot

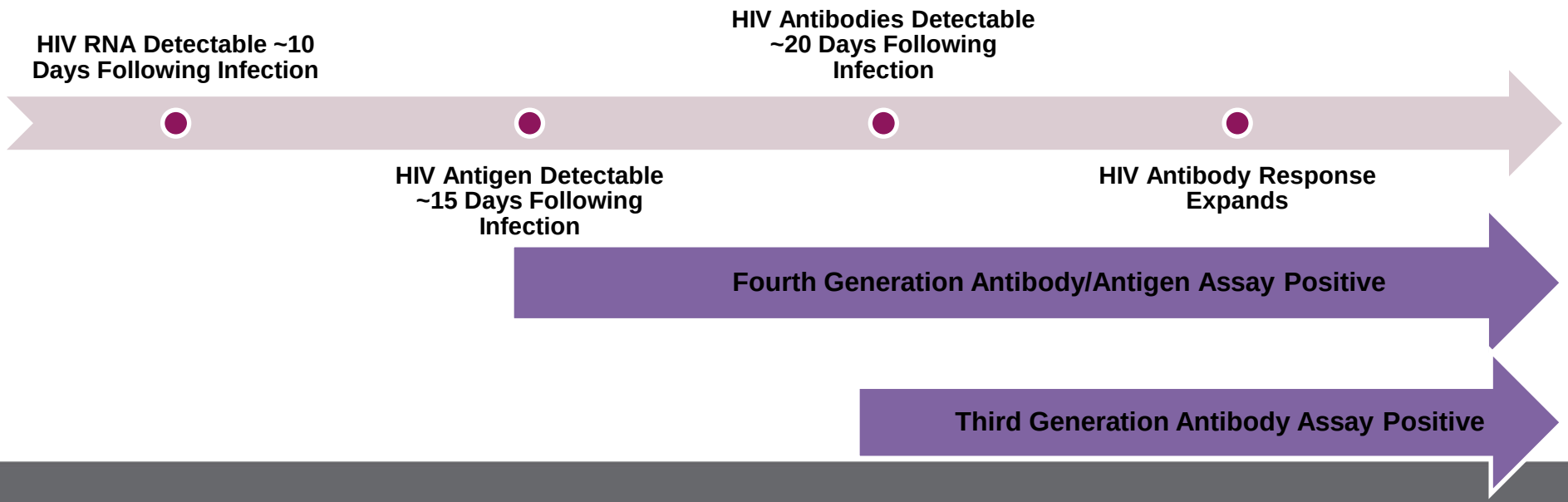
Patel P, Bennett B, Sullivan T, et al. *J Clin Virol*. 2012;54:42-47.

Confirmation of Rapid Tests

- Western blot or immunofluorescence assay was previously recommended to confirm rapid tests
  - This was because certain rapid tests were actually more sensitive than in-lab immunoassays
- This has changed with fourth generation testing
  - Fourth-generation in-lab tests are more sensitive and specific than currently available rapid tests (even rapid fourth generation tests)

Rapid HIV Tests

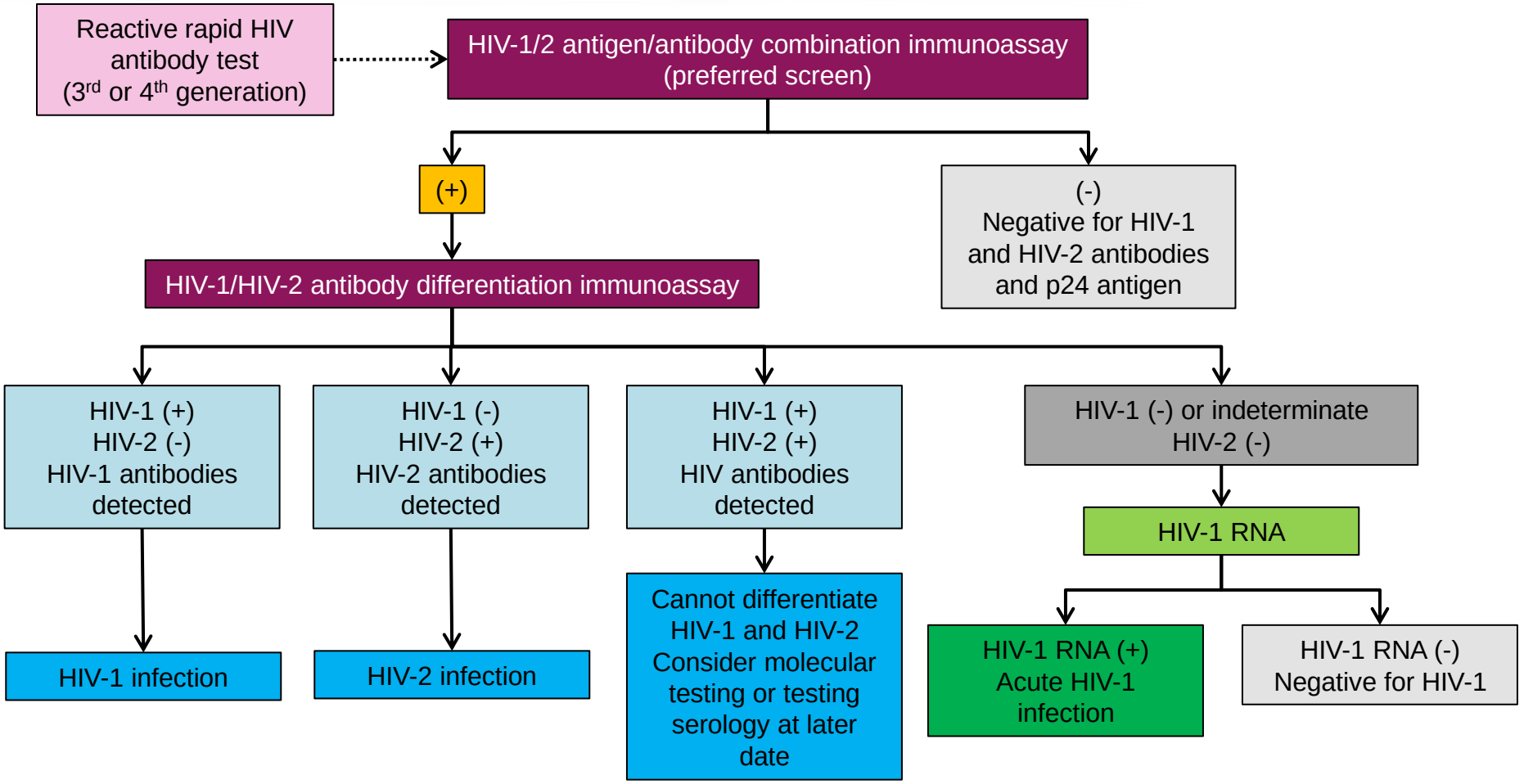
- Fourth gen antigen/antibody tests have much greater sensitivity than third gen tests **(should ALWAYS be positive if third gen test is true positive)**



Current CDC Recommendations

- Any reactive rapid antigen test should be tested by the fourth-generation algorithm starting at the beginning
- Supplemental testing is NOT required for any patients positive by rapid antigen, and negative by fourth-generation
- **The role of the rapid test is to screen for those who should get fourth-generation testing**

Fourth-Generation Algorithm



Adapted from: http://www.cdc.gov/hiv/pdf/hivtestingalgorithmrecommendation-final.pdf

Back to our Case: How the Lab Helped

- Rather than being sent for Western blot, the patient's sample was tested by an in-lab fourth-generation assay
  - Reported as: Reactive, confirmatory testing required
- Reflex testing by HIV-1/2 differentiation assay was automatically performed
  - Reported as: Negative for HIV-1 and HIV-2 antibodies, additional confirmatory testing required by a molecular method
- Qualitative viral load was performed
  - Reported as: Positive for HIV-1, recommend baseline viral load
- **Final diagnosis: ACUTE HIV**

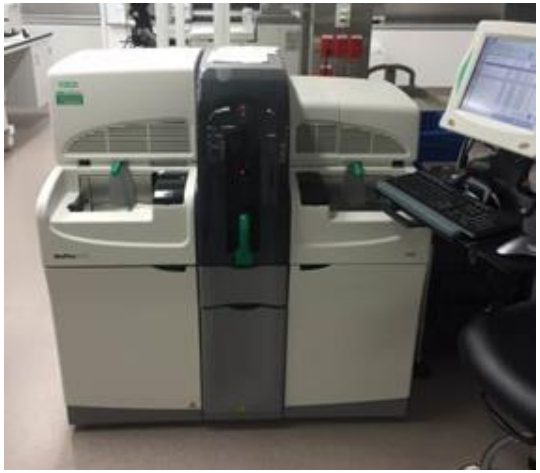
How the Rapid Helped

- Without the rapid HIV test
  - Physician would have sent the patient home with a diagnosis of viral infection while awaiting results
- Positive results obtained in the office allowed for a discussion about HIV
  - Able to take a more directed risk history
  - Able to provide counseling about infectivity during acute infection
  - Able to advise testing of partner
  - May have prevented further transmission

FUTURE DIRECTIONS FOR HIV TESTING

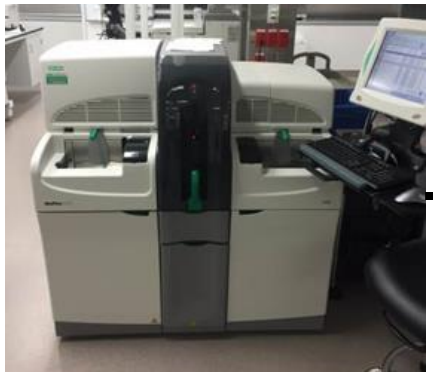
5th Generation Testing?

- Bioplex (5th generation HIV testing)
  - Tests separately and differentiates HIV 1 ab, HIV 2 ab, and p24
  - Acceptable 4th gen screening assay though technically also an HIV1/2 differentiation assay
  - Could change testing algorithm dramatically…



5th Generation Algorithm?

THEORETICAL!!!!



5th Generation Assay

HIV-1 AB -, p24 - → Negative

HIV-1 AB +, p24 - → Additional testing required

HIV-1 AB -, p24 + → Proceed to molecular testing

HIV-1 AB +, p24 + → Abbreviated additional testing required?

What should be used for confirmatory testing?

What is specific enough to establish a diagnosis?

More data is needed regarding the performance of the Bioplex and potential 5th generation algorithms

The Rise of Molecular?

- Rapid qualitative molecular testing
  - Cepheid Xpert HIV-1 Qualitative test has been approved for use outside US
  - 90 minute run time and amenable to near-POC
- Greater availability of molecular testing may make it more attractive for screening
- Same limitations and considerations of other molecular HIV diagnostics
  - Need to confirm results with seroconversion!

The Rise of Rapids?

- Fourth generation rapid testing is currently not included in CDC fourth generation algorithm
- However, inclusion of fourth generation rapid testing as an acceptable screen is attractive
  - Would allow for initiation of fourth generation algorithm at the point of care
- Data is still being gathered regarding the performance of fourth generation rapid serology tests and possible inclusion into the CDC algorithm

QUESTIONS?