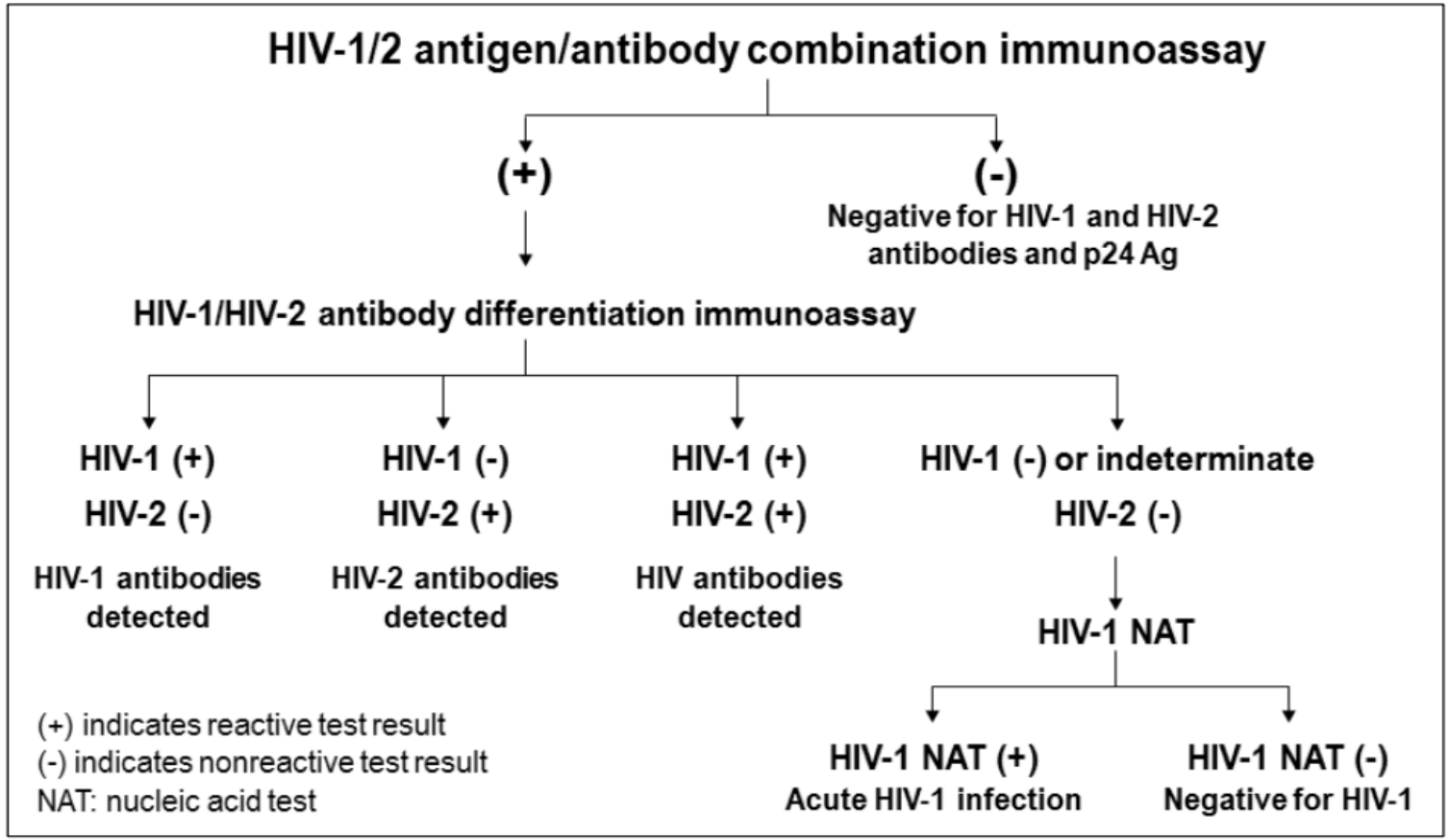
Diagnosis of HIV-2 infection by an HIV-2 total nucleic acid qualitative assay using the Abbott m2000 platform

Ming Chang, PhD, ASCP(MB)
University of Washington, Seattle
minchang@uw.edu

The CDC algorithm for HIV diagnosis requires HIV-2 NAT

Box 1. Recommended Laboratory HIV Testing Algorithm for Serum or Plasma Specimens

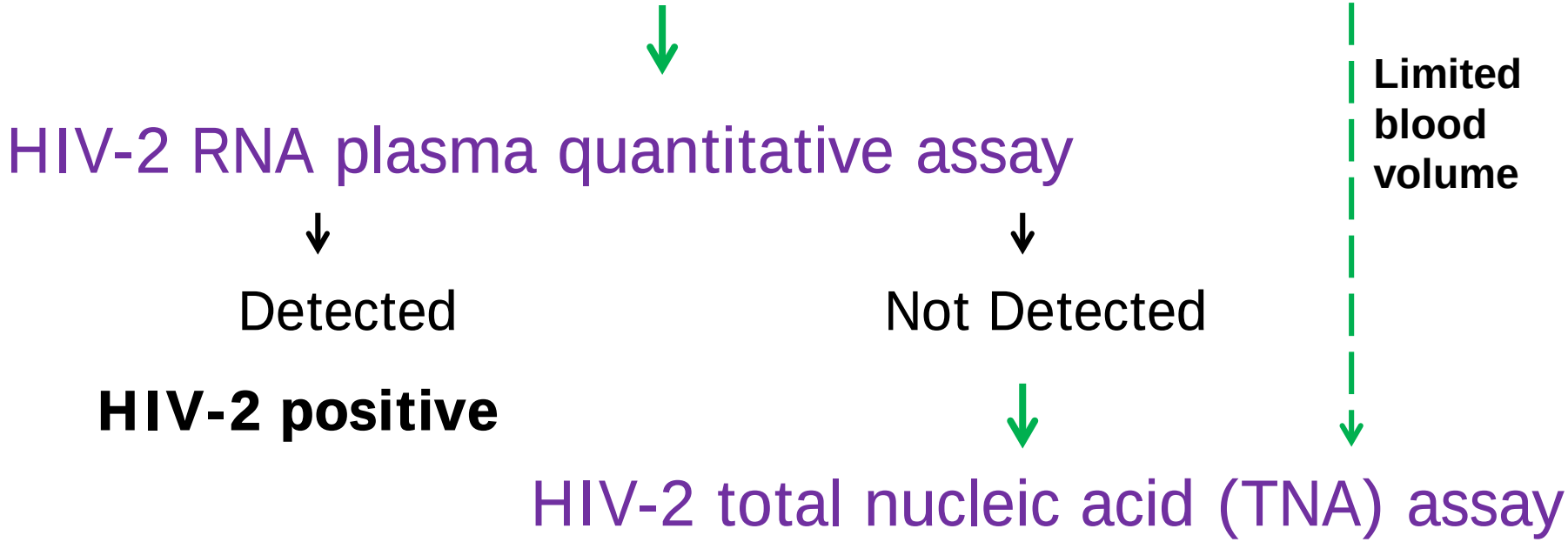


Issues for the diagnosis of HIV-2

- Undetectable plasma RNA viral loads in ~30% of ART-naïve patients
- Unaddressed issues in the CDC 4th Generation HIV testing algorithm
  - HIV-2 Ab indeterminate by differentiation immunoassays
  - Undifferentiated HIV infection (dual HIV-1/2 infection vs. cross-reactive serology)
  - HIV-2 acute infection

Objective

- HIV-2 sero-indeterminate, undifferentiated HIV-1/HIV-2 or “false positive Combo” cases
- Infants born to HIV-2 infected mothers



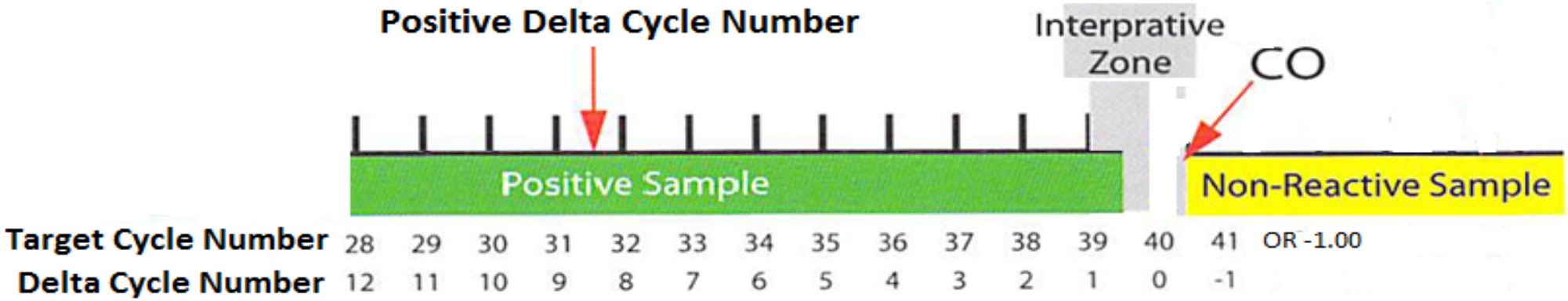
Assay overview

- Adapted from the current UW clinical HIV-2 plasma RNA quantitative assay since Dec. 2011 (Chang et al. JCV 2012)
- HIV-2 specific primers/probe, without cross-reactivity with HIV-1
- Sample: ca. 1-million peripheral white blood cells (WBC) prepared from EDTA whole blood

Assay overview

- Abbott m2000 platform: a DNA & RNA extraction protocol and a laboratory-defined qualitative program
- Endogenous (human Ribonuclease P gene) and exogenous (in-house RNA transcript) controls were used to assess sample quality and assay inhibition

Abbott laboratory-defined qualitative program



Cut-off (CO) cycle number: determined by evaluating cycle numbers of low-positive controls

Delta Cycle (DC): calculated for each sample as CO minus the target cycle number.

Assay precision

- Positive Control: ca. 180 copies of linear HIV-2 pROD plasmid spiked in 1 million human WBC
- Negative control: 1 million WBC
- 24 replicas of Positive and Negative controls were carried out in two days
  - An additional 4 replicas of controls were tested each day for 8 days

Assay precision

- ca. 180 copies of linear pROD plasmid spiked in 1 million WBC

Mean	5.76 DC
Total Standard Deviation	0.40 DC
Total CV	6.94%

- All negative controls were tested negative for the assay

Sensitivity (limit of detection)

Copies of HIV-2_{pROD9} plasmid/million cells	Number tested	Number Detected	Percent detected
46	8	8	100
23	8	7	88
12	8	5	63
6	8	5	63
3	8	0	0

The LOD is estimated to 25 copies/million cells (95% CI, 13-37 copies/million cells).

Assay sensitivity for HIV-2 infected patient samples

- 30 HIV-2 seropositive patients
  - 23 with non-detectable (<8 copies/mL) plasma HIV-2 RNA
  - 7 with detectable plasma HIV-2 RNA up to 2.06 $\log_{10}$ copies/mL plasma
  - All 30 WBC samples tested positive by HIV-2 TNA assay (DC median= 6.34; DC range, 1.07-9.39)

Diagnostic performance with CDC 4th generation algorithm

- Comparing patients' WBC tested by HIV-2 TNA assay to the corresponding plasma tested by Abbott HIV Ag/Ab Combo assay and Bio-Rad Multispot HIV-1/-2 Rapid Test
- Tested samples from 25 HIV-negative donors, 30 HIV-2-infected and 25 HIV-1-infected patients

Diagnostic performance

	Abbott HIV Ag/Ab Combo assay and Bio-Rad Multispot HIV-1/-2 Rapid Test	
HIV-2 TNA assay	HIV-2 Ab reactive	HIV-2 Ab non-reactive
Detected	29	1*
Not-detected	0	50

- *Sample contained the lowest signal-to-cutoff ratio, 5.89, and was non-reactive by Multispot
- Agreement between two methods: 98.75%

Multispot-undifferentiated HIV-1/HIV-2 cases

ID	Plasma		WBC	
	HIV-1 RNA c/mL	HIV-2 RNA c/mL	HIV-1 TNA	HIV-2 TNA
HD01	TND#	TND	Detected	1.38 DC
HD02	85120	2592	Detected	11.60 DC
HD06	510596	311	Detected	5.62 DC
HD09	TND	TND	Detected	8.08 DC
HD12	TND	TND	Detected	Detected*
H2A036	TND	136	Detected	7.96 DC
HD13	<40	28726	TND	9.50 DC
HD10	13730	TND	Detected	TND

#Target Not detected

*Detected by a second HIV-2 probe

Limitations

- When serology and TNA detection are discordant, need to consider Poisson distribution and compartmentalization of HIV-infected cells
- As with any diagnostic test, results from the UW HIV-2 TNA qualitative assay should be interpreted in conjunction with other clinical and laboratory findings

Conclusions

- Diagnostic testing application for 4th generation HIV algorithm
  - For cases such as HIV sero-indeterminate or undifferentiated orthogonal results by HIV-1/HIV-2 differentiation immunoassays
- Future plans
  - Test white blood cells from infants born to HIV-2 infected mothers and provide the assay for the clinical service
- Assay feasibility
  - Reliable Abbott m2000 platform, requiring minimal 0.5mL EDTA whole blood, completing one run in 8 hours

Acknowledgements

- Co-Authors: A. J. Wong, D. N. Raugi, R. A. Smith, A. M. Seilie, J. P. Ortega, K. Bogusz, K. Faye, F. Sall, S. Ba, I. Sall, A.Niang, M. Seydi, G. S. Gottlieb, and R. W. Coombs
- UW Retrovirology Laboratory
- UW–Dakar HIV-2 Study Group
- UW CFAR repository program
- Abbott Molecular, Inc.
- FUNDING. Supported by grants to: GSG from the National Institutes of Health/National Institute of Allergy and Infectious Diseases (2R01-AI060466); RWC from the University of Washington Center for AIDS Research (AI- 827757) and AIDS Clinical Trials Group Laboratory Center (AI-068636).