Use of Multispot HIV-1/HIV-2 Rapid Test to confirm HIV-1/HIV-2 Plus O Enzyme Immunoassay results, shorten reporting time for HIV testing and identify cryptic HIV-2 infection

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Objective

- To test an alternative HIV testing strategy that provides timely and accurate results for clinicians

Hypothesis

- A combination of routine HIV-1/2 EIA and simple, rapid antibody testing would improve reporting turn-around-time in an academic clinical diagnostic laboratory setting
  - Evaluate an algorithm similar to the APHL/CDC **HIV testing Laboratory Algorithm 3:** "Dual HIV-1/HIV-2 Immunoassay," using routine EIA and a simple, rapid antibody test
  - Test the accuracy of reporting "presumptive HIV infection"

Rationale

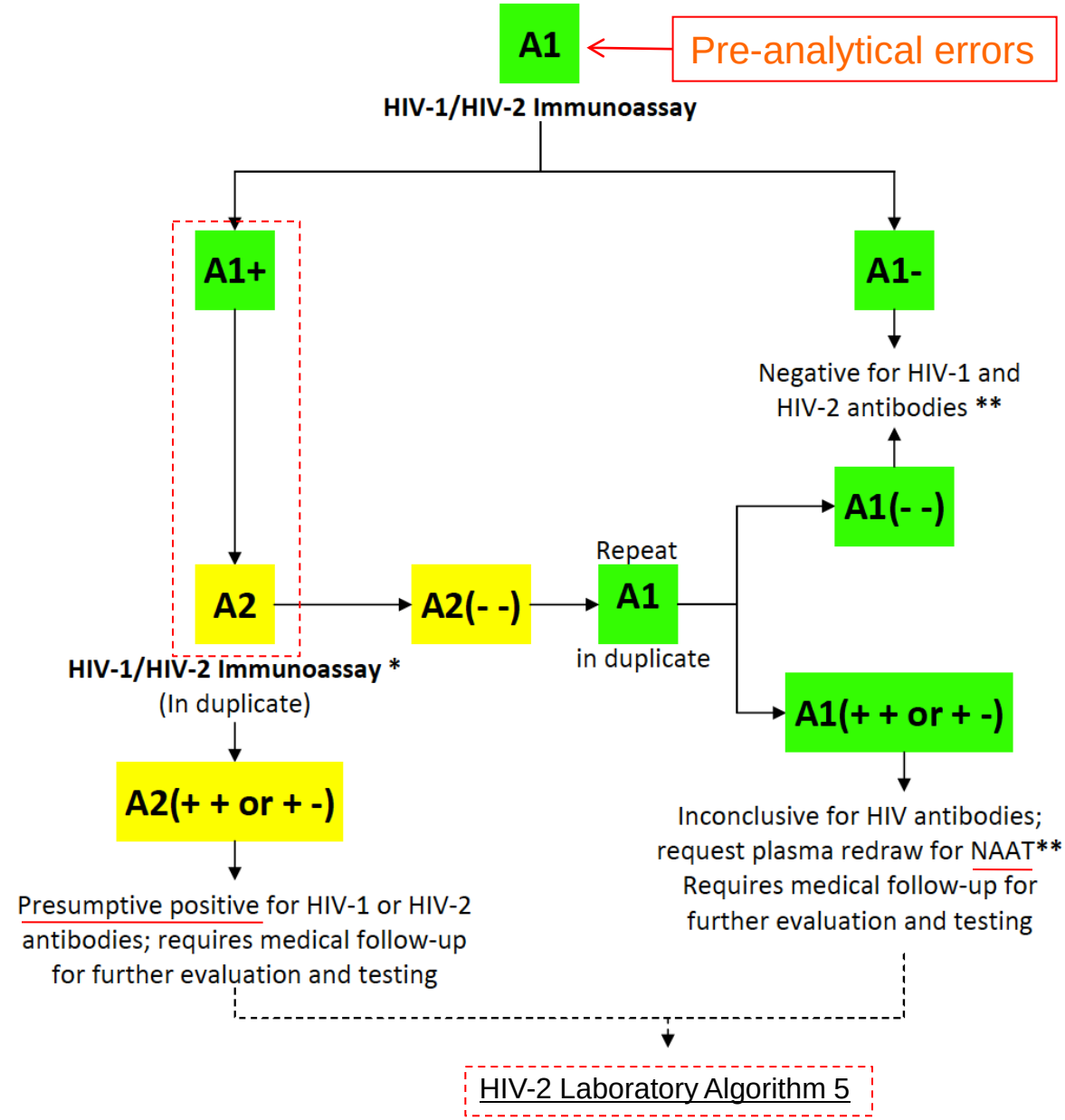
- Traditional laboratory diagnostic testing
  - Screening with EIA (or CIA) and reactive results are confirmed by supplemental testing before releasing test results (WB or IFA): MMWR 1989: 38(S-7):1-7
- Point-of-Care
  - Reporting of a single test result as “preliminary positive” for HIV-1 and/or HIV-2 antibodies is accepted as standard of care: MMWR 2001; 52(RR19): 1-58 and *ibid* 2004; 53(10): 221-222
- Marriage of a 3rd generation EIA (or CIA) and simple/rapid antibody test is the logical next step

Laboratory Algorithm 3: Dual HIV-1/HIV-2 Immunoassay†

- *__A2__ must ust be a different EIA, CIA or rapid test from __A1__: i.e., “orthogonality”

- **If a seronegative-window period infection is suspected (based on risk assessment or discordant testing) refer to Acute HIV Infection Testing Laboratory Algorithm 4

- †Adapted from – *APHL/CDC HIV Testing Algorithm: A status Report, April 2009*



Methods

- Routine clinical specimens submitted for HIV testing
- Bio-Rad HIV-1/HIV-2 Plus O EIA (EIA)
- Bio-Rad Multispot HIV-1/HIV-2 (MS)
- Bio-Rad HIV-1 Western blot (WB)
- HIV-1 RNA PCR (in-house; Abbott Real*T*ime HIV-1); HIV-2 WB (Focus) and HIV-2 RNA (in-house)

UW Algorithm

- If EIA negative: report *"Not HIV infected"*
- If EIA reactive
  - Forward for MS testing & repeat EIA in duplicate
    - Negative MS: forward for HIV-1 WB & initial report *"presumptive not HIV infected"*
    - Reactive MS: initial report *"presumptive HIV infected"* and forward for HIV-1 WB confirmation (or HIV-2 WB if appropriate)
- Confirmed HIV-1 WB: final report as *"HIV-1 infection"*
- Negative HIV-1 WB: final report as *"No HIV-1 infection"*
- Indeterminate WB and/or reactive MS forward for HIV-1 RNA (or further HIV-2 testing, if appropriate)

Results

- Between July 31, 2008 and Oct 8, 2009 a total of 13,943 HIV-1/-2 EI assays were performed
  - 242 specimens were EIA reactive of which
    - 203 (1.5%) were HIV-1 WB confirmed
      - 201 were MS HIV-1 + and 2 were MS HIV-2 +
    - 26 EIA+--/WB-/MS NEG
    - 7 EIA+++/WB-/MS NEG;
    - 6 EIA+++/WB IND/MS NEG
      - Of 13 EIA+++, 6 were available for HIV-1 RNA and 1/6 was RNA positive

Results (cont'd)

- 203 EIA+/HIV-1 WB+
  - 201/203 were MS HIV-1 POS
  - 2/203 were MS HIV-1 NEG/HIV-2 POS
    - WB #1 [gp160, p55, p31, p24] and WB #2 [gp160, p31, p24] both of which were initially signed-off as "HIV-1 infection confirmed" before the MS was done (old protocol); HIV-1 RNA negative
    - Confirmed with HIV-2 WB and HIV-2 RNA

Immunoblot

HIV-1

- ENV
  - gp160/gp120; gp41
- GAG
  - p55, p40, p24, p17
- POL
  - p66, p51, p31

HIV-2

- ENV
  - gp140/gp125; gp36/41
- GAG
  - p56, p26, p16
- POL
  - p68, p53, p34

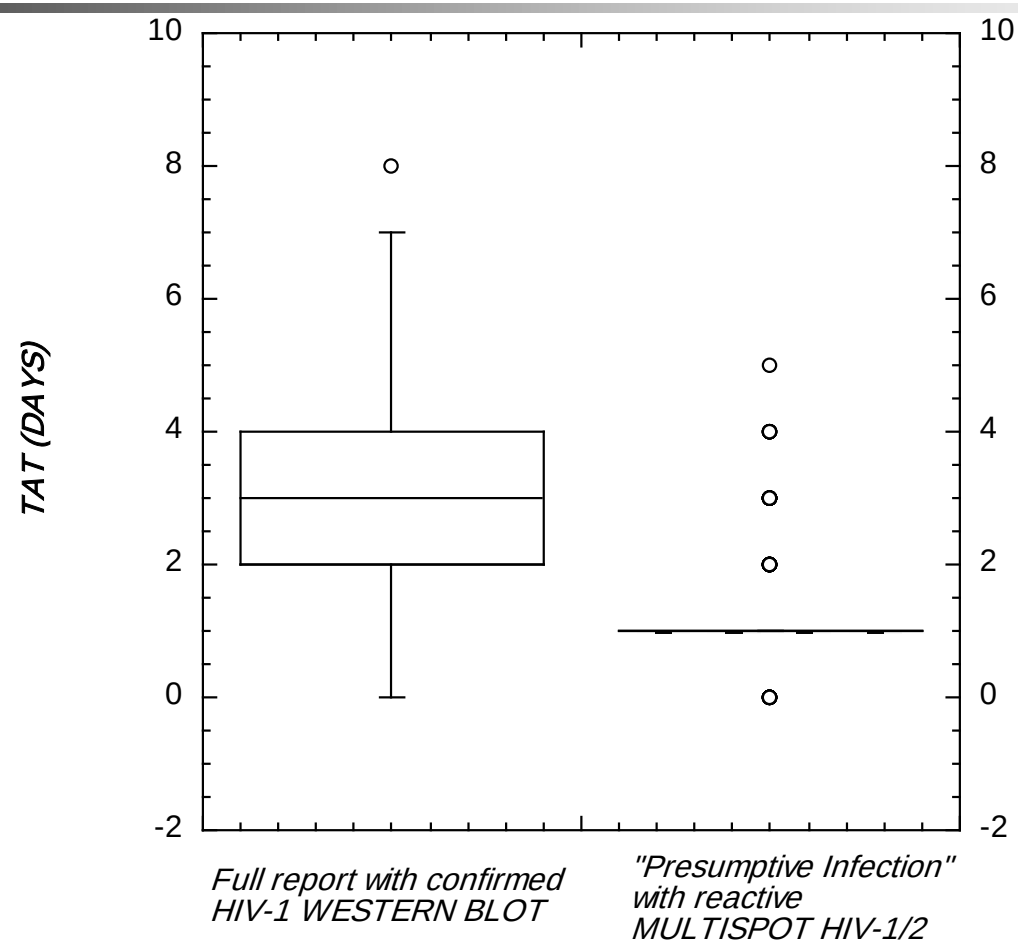
WHO Wkly Epidem Rec 1990; 65(37): 281-88

Multispot assay performance for detection of HIV-1 antibodies

	HIV-1 Infection status† (N=240) YES	NO		
Multispot HIV-1 +	201	0	100%	Positive PV
Multispot HIV-1 -	1	38	97.4%	Negative PV
	99.5% Sensitivity	100% Specificity		

†Determined by HIV-1 antibody or RNA and excludes two HIV-2 infected patients

Turn Around Time (TAT) in days to report a reactive EIA and WB or MS (N=242)



Summary

- Adjunctive MS testing allowed us to confirm reactive EIA results immediately and report “presumptive HIV infection,” pending further confirmation;
- Identified all false-positive EIA results;
- Shortened reporting time by a median of two days;
- Identified two (1%) of 203 EIA-reactive/HIV-1 WB “positive” results as cryptic HIV-2 infection

Conclusions

- These findings have important implications for the clinical management of HIV-infected persons, including timely clinical laboratory diagnosis of HIV infection, treatment of HIV-related conditions and use of appropriate antiretroviral therapy in HIV-2–infected patients

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