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Evaluation of the proposed US CDC algorithm for detection of Acute HIV infection in Serial Samples

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BACKGROUND

A new HIV diagnostic algorithm has been proposed by the US CDC to improve acute HIV infection (AHI) detection and type differentiation of anti-HIV-1 from HIV-2 antibodies. The algorithm employs a 4th generation HIV-1/2 Immunoassay (4th gen) followed by a HIV-1/2 type differentiation immunoassay, and nucleic acid testing, if indicated. We evaluated the proposed algorithm performance on well-characterized panels of serial samples collected from AHI cases identified by the Early Capture HIV Cohort (ECHO) Study, RV217.

METHODS

Individuals at high risk of HIV infection from Tanzania, Uganda, Kenya and Thailand were screened twice-weekly by Aptima HIV-1 Qualitative RNA assay (Gen-Probe, Inc. San Diego, CA). Following a reactive (R) Aptima result, serial samples collected twice weekly for the first 30 days and then subsequently at wider intervals. Serial specimen panels were assayed according to manufacturer's instructions from consenting participants with the following assays:

- GS Combo Ag/Ab EIA (4th Gen),
- GS HIV-1/2 Plus O EIA (3th Gen),
- HIV1/2 Multispot (Bio-Rad Laboratories, Redmond, Washington),
- HIV-1 p24 Antigen (Ag) Test (Bio-Rad, France)
- Abbott m2000 real-time quantitative HIV RNA assay (Des Plaines, Illinois).

Calculation of time (day[s]) to reactive test was determined using day 0 representing the first reactive Aptima test. Simulated algorithm performance was assessed by Combo EIA and Multispot results, when appropriate.

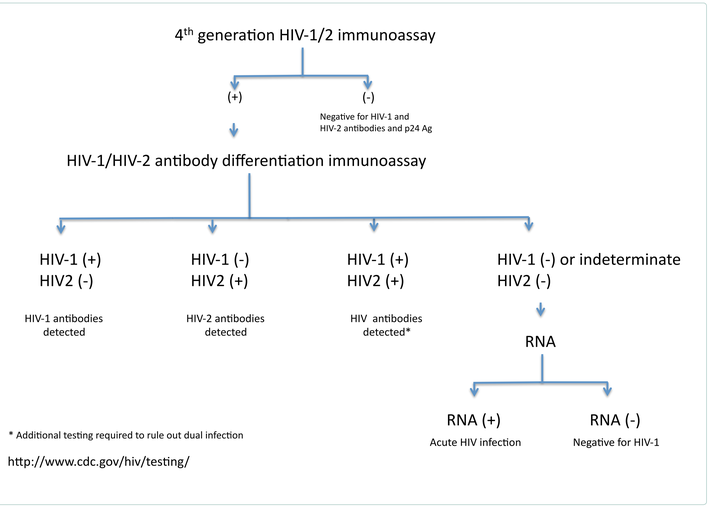


Figure 1. CDC Proposed HIV testing algorithm

In the proposed CDC algorithm, an individual is considered HIV uninfected if the 4th gen immunoassay (IA) is non-reactive (NR), and positive for HIV infection if a repeat reactive (RR) 4th gen IA is confirmed by positive (POS) Multispot result. An individual is considered to be an AHI case, if the 4th gen IA is RR, Multispot is negative, and HIV nucleic acid is detected.

DISCLAIMER

The opinions or assertions contained herein are the private views of the authors and do not reflect the views of the U.S. Army or the Department of Defense.

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RESULTS

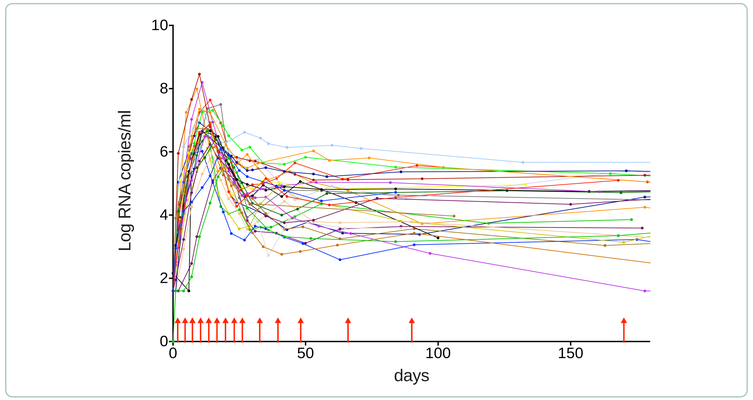


Figure 2. Aggregate Viral Load Profiles

29 acute infection cases were tested for viral load at serial timepoints. The red arrows represent approximate blood draw timepoints. In this sample set, the median number of samples collected before peak viral load was 3 (range 2-5) and the interval between last negative and first reactive Aptima test was 4 days (range 2-14). The median peak Viral Load was log10 6.66 copies/ml.

	Days to Peak VL	Days to p24+	Days to 4th gen +	Days to 3rd gen EIA +	Days to Multispot +
10220	18	15	15	18	23
10428	10	2	2	10	22
10435	14	6	9	14	21
10463	13	7	7	13	16
20225	9	5	5	14	17
20263	11	7	7	15	23
20314	13	5	5	13	18
20337	14	6	2	18	18
20335	11	2	7	11	27
20368	13	9	9	33	40
20442	11	6	6	16	20
20509	14	6	6	18	22
20511	14	2	6	14	26
30112	18	4	7	18	18
30124	14	4	7	14	18
30190	11	2	4	14	18
40007	11/15	4	8	15	19
40061	14	7	11	18	21
40094	16	5	5	16	16
40100	10	7	7	10	14
40123	14	7	10	14	21
40168	11	4	8	22	22
40231	13	6	6	13	15
40250	14		6	17	17
40257	10	7	4	14	20
40265	12	5	7	19	21
40353	17	10	14	17	21
40363	14	7	7	21	17
40436	18	4	11	14	18
MEDIAN	**13**	**6**	**7**	**15**	**20**

Table 1. Summary of Days to Reactive on Diagnostic Tests

The number of days until assay reactivity/positivity is indicated for each subject. Combo reactivity was detected at a median 7 days (range 2-15) after the first reactive RNA test, 6 days before peak viral load, and 8 days before 3rd gen EIA reactivity. Multispot reactivity was detected on median day 20. No HIV-2 infections were observed.

	# cases (%) 4th gen +	# cases (%) 4th gen -
Earliest Viral Load positive timepoint (median 2 days past R Aptima) (n=29)	7 (24%)	22 (76%)
1st timepoint with VL over 100,000 copies/ml (n=29)	27 (93%)	2 (7%)
timepoint with VL between 10,000-100,000 copies/ml (n=15)	1 (7%)	14 (93%)
timepoint with VL between 1,000-10,000 copies/ml (n=9)	1 (11%)	8 (89%)
p24 concentration between 1-50 pg/ml (n=17)	5 (29%)	12 (71%)
p24 concentration between 50-100 pg/ml (n=10)	10 (100%)	0 (0%)

Table 2. Summary of Combo (4th gen IA) reactivity

Of 29 Acute HIV infection cases, only 7 (24%) were R (Reactive) by Combo at the earliest timepoint (median 2 days past R Aptima) with a detectable viral load. HIV-1 viral loads in the very early NR Combo samples ranged from 2.16 to 5.95 log10 copies/ml and were on the early viral upslope before peak viremia. In the 22 4th gen NR cases, p24 concentrations were lower than the package insert sensitivity of 50pg/ml. Five of seven (5/7) cases demonstrated R Combo results with p24 levels less than 50pg/ml (range 25-49 pg/ml). Samples with viral loads at the first timepoint greater than 100,000 copies/ml were R in Combo in 93% of cases (n=29). In contrast, with viral loads between 10,000-100,000 copies/ml, Combo was reactive in only 7% of cases (n=15). Samples with p24 concentrations between 50-100pg/ml were R with Combo in 100% cases (n=10).

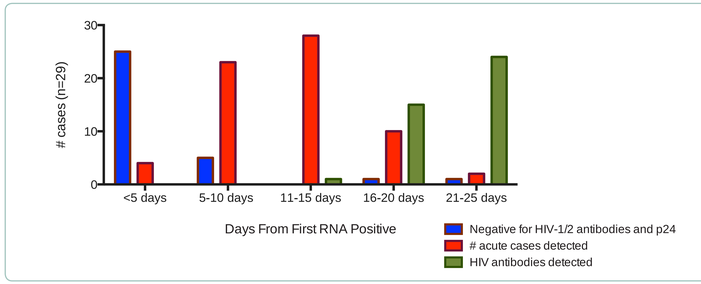


Figure 3. Performance of Proposed CDC algorithm in acute HIV cases

Using the simulated proposed algorithm, maximum AHI detection (reactive Combo and negative Mulispot) occurred 5-15 days from the first reactive RNA result. The algorithm detected 29/29 acute infections in at least one timepoint within the first 15 days post detection of RNA in specimens exhibiting p24 levels above 50pg/ml. After 20 days, most samples were Multispot positive resulting in a "HIV antibodies detected" classification. One sample was non-reactive by Combo between days 16-25, exhibiting a second detection window due to a delayed antibody response.

CONCLUSION

- The proposed US CDC algorithm identified 29/29 individuals with HIV-1 acute infection.
- Only 7/29 AHI cases were detected at the first visit when HIV-1 viremia and p24 concentration were low (<50pg/ml).
- The 4th gen IA reduces the detection window from 3rd gen EIA by 8 days.
- This algorithm, with the addition of a 4th gen IA enhances AHI detection sensitivity.
- Robust point of care HIV viral load detection would further reduce the diagnostic window, especially when screening most-at -risk cohorts.