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The fourth generation Alere™ HIV Combo rapid test improves detection of acute infection in MTN-003 (VOICE) samples

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ABSTRACT

Background: Early and accurate detection of HIV is crucial when using pre-exposure prophylaxis (PrEP) for HIV prevention to avoid PrEP initiation in acutely infected individuals and to minimize the risk of drug resistance in individuals with breakthrough infection.

Objective: To determine if fourth-generation antigen/antibody (Ag/Ab) rapid diagnostic tests (RDT) would have detected HIV infection earlier than the third-generation RDT used in MTN-003 (VOICE).

Study design: 5029 VOICE participants were evaluated with third-generation Alere Determine™ HIV-1/2, OraQuick ADVANCE® Rapid HIV-1/2, Uni-Gold™ Recombigen® HIV-1/2 and Bio-Rad GS HIV-1/2 + O EIA; and fourth-generation Alere Determine™ HIV-1/2 Ag/Ab Combo, Conformité Européene (CE)-Marked Alere™ HIV Combo and Bio-Rad HIV Combo Ag/Ab EIA. Multispot®, GS HIV-1 Western Blot (WB) and Geenius™ (Bio-Rad) were also evaluated.

Results: Of 57 antibody-negative pre-seroconversion plasma samples with HIV RNA > 20 copies/mL identified, 16 (28%) were reactive by CE-Marked Alere™ HIV Combo (1 Ab; 9 Ag; 6 Ag/Ab reactive) and 4 (7%) by Alere Determine™ HIV-1/2 Ag/Ab Combo (2 Ab; 2 Ag; 0 Ag/Ab reactive) ($p = 0.0005$). Multispot® confirmed only 1 of 16 acute infections while WB and Geenius™ confirmed none. GS HIV Combo Ag/Ab EIA identified 27 of 57 (47%) pre-seroconversion RNA-positive samples.

Conclusion: In VOICE, 28% of infections missed by current third-generation RDT would have been identified with the use of CE-Marked Alere™ HIV Combo. Geenius™, Multispot® and WB were all insensitive (< 10%) in confirming infections detected by fourth-generation assays. An improved diagnostic algorithm that includes a fourth-generation RDT with HIV RNA testing will be essential for efficiently identifying seroconverters on PrEP.

1. Background

Early and accurate detection of HIV is crucial when using pre-exposure prophylaxis (PrEP) for HIV prevention to avoid PrEP initiation in acutely infected individuals and to minimize the risk of drug resistance in individuals with breakthrough infection [1]. The World Health Organization recommends two sequential rapid tests for HIV diagnosis [2] however third generation rapid tests detect only HIV antibodies and may miss up to 75% of early acute HIV infection cases [3].

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Fourth generation HIV rapid tests use a lateral flow cassette to separately assay for both anti-HIV antibodies and p24 antigen, however numerous studies have demonstrated the United States Food and Drug Administration (FDA)-approved Alere Determine™ HIV-1/2 Ag/Ab Combo to be insensitive for detection of acute infection. In a total of 54 acute seroconversion samples from 3 studies of acute infection from Malawi, Swaziland, Rwanda and Zambia, only 1 sample (1.9%) was positive for the p24 antigen component [4–6]. Reported sensitivity for antigen detection was higher in samples from the United Kingdom (50%), the United States (45.5% and 75.8%), Italy (88.2%) and in mixed-subtype seroconversion panels (86.6%) suggesting clade differences [3,7–10]. Evaluation of Gag-expressing virus-like particles (VLP) showed that 6 of 7 subtype B VLPs were detected by Alere Determine™ HIV-1/2 Ag/Ab Combo, compared to only 1 of 5 subtype C, and 3 of 31 non B/non-C-subtypes [11].

In February 2015, Alere released a re-formulated fourth generation rapid test kit, the Conformité Européene (CE)-Marked Alere™ HIV Combo. Only one study to date has evaluated this new assay, reporting an 88% sensitivity compared to the Abbott Architect HIV Ag/Ab Combo assay using stored plasma or serum samples from the United Kingdom [12]. Also unknown is the performance of HIV confirmatory tests such as Bio-Rad Multispot® HIV-1/HIV-2 Rapid Test, Geenius™ HIV-1/2 Supplemental Assay and GS HIV-1 Western Blot in the context of an algorithm that includes fourth generation rapids. This is the first evaluation of the new CE-Marked Alere™ HIV Combo in pre-seroconversion samples collected in South Africa, Uganda and Zimbabwe.

2. Objectives

The objective of this study was to evaluate the performance of third and fourth generation HIV rapid diagnostic tests and confirmatory assays to detect acute infection in a large cohort of participants from the MTN-003 (VOICE) HIV prevention trial.

3. Study design

3.1. Study population and specimen collection

VOICE (ClinicalTrials.gov NCT00705679) was a randomized, Phase 2 B placebo-controlled trial to evaluate the safety and effectiveness of oral tenofovir disoproxil fumarate (TDF), oral TDF-emtricitabine (FTC) and vaginal tenofovir 1% gel for the prevention of HIV infection in 5029 HIV-uninfected women from 15 clinical sites in South Africa, Uganda and Zimbabwe. Population characteristics and trial results have been described previously [13].

In VOICE, participants were monitored monthly for seroconversion with one (Uganda) or two (South Africa and Zimbabwe) third generation HIV rapid diagnostic tests (RDT) performed point-of-care using venous or fingerstick-drawn whole blood at clinical research sites. RDTs used included Alere Determine™ HIV-1/2 (Alere Medical Co. Ltd., Matsudo, Japan), OraQuick ADVANCE® Rapid HIV-1/2 Antibody Test (OraSure Technologies, Inc., Bethlehem, PA) and/or Uni-Gold™ Recombigen® HIV-1/2 rapid test (Trinity Biotech™ Wicklow, Ireland). To diagnose HIV infection, clinical research sites followed an HIV algorithm during the study (Fig. 1). Positive or discordant RDT results were confirmed by GS HIV-1 Western Blot (Bio-Rad Laboratories, Redmond, WA) using plasma from a separate draw and HIV-1 RNA PCR using Abbott RealTime HIV-1 (Abbott Molecular, Inc., Des Plaines, IL) was performed on plasma following an indeterminate or negative Western blot result to assess acute infection (Fig. 1). Assay interpretation was defined by the manufacturer's package insert for each test. In the VOICE study, plasma from the visit at which seroconversion was detected and stored plasma from the seroconverting participant's enrollment visit were shipped to the University of Pittsburgh and re-tested to verify HIV infection using GS HIV-1/2 +O EIA (Bio-Rad), GS HIV-1 Western Blot and HIV-1 RNA PCR (Abbott).

3.2. Identification of specimens from acutely infected VOICE participants

After completion of the VOICE study, stored plasma specimens collected up to 91 days prior to detection of seroconversion were shipped to the University of Pittsburgh. Pre-seroconversion plasma specimens were tested for the presence of HIV-1 RNA by the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 with a limit of quantitation of 20 copies/mL (Roche Diagnostics, Indianapolis, IN). Specimens with detectable HIV-1 RNA (including results of < 20 copies/mL, detected) were re-tested with OraQuick ADVANCE® and Uni-Gold™ Recombigen® and if antibody negative, were considered to be from an acutely infected participant. Only those specimens identified as "pre-seroconversion" defined as having detectable HIV-1 RNA and negative antibody reactivity were used for the evaluation of fourth generation RDTs.

3.3. Third and fourth generation diagnostic test evaluations

Third generation RDT-negative samples were tested with the fourth generation FDA-approved Alere™ Determine™ HIV-1/2 Ag/Ab Combo rapid test (Alere North America, Waltham, MA) and the CE-Marked Alere™ HIV Combo Rapid Test (Alere Medical Co. Ltd., Matsudo-chi, Japan) (Supplementary Table S1). The Determine™ Combo and Alere™ HIV Combo RDTs are named similarly but have different formulations, different places of manufacture, and different performance specifications. Samples were also tested in duplicate by the third generation Bio-Rad GS HIV-1/2 + O Enzyme Immunoassay (EIA) and the fourth generation Bio-Rad GS HIV Combo Ag/Ab EIA, both FDA-Approved (Bio-Rad Laboratories).

3.4. Confirmatory test evaluations

Pre-seroconversion samples found to be positive by at least one of the above fourth generation tests were further tested for the presence of antibodies to HIV-1 and/or HIV-2 by Multispot® HIV-1/HIV-2 Rapid Test, Geenius™ HIV-1/2 Supplemental Assay and GS HIV-1 Western Blot (Bio-Rad).

3.5. Statistical analysis

McNemar's test was used to compare the proportion of positive/reactive test results between assays. For calculating sensitivity, specificity, positive and negative predictive value of the results of the third generation HIV RDTs performed in VOICE, the gold standard was defined by the results of the study algorithm (Fig. 1) which included additional WB and/or HIV-1 RNA testing by a local and/or central laboratory during the study. Samples that tested negative or indeterminate for RDT and/or WB but had detectable HIV-1 RNA were considered endpoints in the VOICE study. The gold standard for the same calculations on the results of the HIV tests evaluated in this study was detectable or undetectable HIV-1 RNA by Roche TaqMan. All confidence intervals are exact (Clopper-Pearson) 95% confidence intervals.

4. Results

4.1. Third generation rapid test performance in VOICE

Between 2009 and 2011, 5029 enrolled VOICE participants had 77,915 follow-up visits to screen for HIV infection. Of the 72,169 visits where two rapid tests were used, 55,346 (77%) were conducted with Uni-Gold™ Recombigen® and Determine™ HIV-1/2 while 16,823 (23%) were conducted using OraQuick ADVANCE® and Determine™ HIV-1/2. The rate of discordancy was similar for both rapid test combinations (0.15%). Ultimately, rapid testing correctly identified HIV infection status for 312 seroconverters and 4717 non-seroconverters at 72,055 of