# Alternative Confirmatory Testing Strategy Using Rapid HIV Assays

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## Abstract

Note: Updated to November 2007

**Objectives**: Because alternative HIV testing strategies can offer advantages, particularly for point of care testing, there is a need (1) to determine the confirmatory rate of samples testing positive by two rapid tests, and (2) to determine the concordance of results and ability of a 3-test rapid testing algorithm to confirm results.

**Methods:** To determine the confirmatory rate for samples testing positive by both the Determine and Stat-Pak rapid HIV tests used in a parallel algorithm, 151 samples from the Recruiting Acute Cases of HIV study (REACH) in Nigeria were further tested by the Genetic Systems HIV-1/HIV-2 Plus EIA and the BioRad HIV-1 Western blot (WB). For evaluating the concordance of results using a 3 rapid test strategy, 10,059 samples from the REACH study were tested by the Determine (D) and Stat-Pak (S), but if discordant results were obtained, the Genie II (G) rapid HIV test was used (a small percent were tested by a different 3-rapid test algorithm); all samples producing discordant results between the tests were tested by WB for resolution. Concordance of the results was calculated using percent agreement between paired results.

**Results**: For the confirmatory rate determination of the 2 test algorithm, all 151 samples that were positive by both rapid tests were positive by the EIA and were confirmed as positive by WB, yielding a confirmatory rate of 100%. In the 3-test strategy, 1,693 samples were positive by the first two tests, yielding a concordant prevalence rate of 16.8%. The percent agreement between the Determine and Stat-Pak was 96.1%. There were 391 (3.9%) samples that produced discordant results (387 D+/S- and 4 D-/S+), with 12 of 155 (that could be tested by Western Blot) being positive by the 3rd rapid test (G). Testing by Western blot indicated a false positive rate of 4.6%and 0.05% for the Determine and Stat-Pak, respectively. Of the 12 discordant samples that were positive by the 3rd rapid test (positive by 2/3 rapid tests), 8 (66.7%) were confirmed by WB.

**Conclusions**: The Determine and Stat-Pak HIV parallel rapid test alternative algorithm produced no false positives when both tests produced positive results, indicating a specificity of 100%. In a 3-test alternative testing strategy, there was a high concordance of results with the first two tests, and when results were discordant, a positive result by the third test was most often found to be confirmed positive.

## Background

Alternative HIV Confirmatory Testing Strategies have been shown to be effective as a means to decrease cost, address difficult testing procedures, and are applicable in resource-limited testing venues where stable electricity and limited infrastructure exist. Recently, there has been interest in adopting alternative methods in industrialized countries. More specifically, these strategies usually consist of using two or more HIV screening assays (ELISAs and/or Rapid Tests) in parallel or sequentially to yield a final result that is equivalent to the use of a screening assay followed by a confirmatory test.

Recent studies in Nigeria have shown that when using 2 rapid HIV tests sequentially, there was a 4.2% non-confirmed rate (as compared with Western blot); i.e., nearly 96% of samples that were positive by both rapid tests were from persons confirmed to be infected with HIV (Imade et al., HIV Conference, Toronto, MOPE0157, 2006).

We sought to determine the effectiveness of using a 3-test algorithm in Nigeria to: (1) evaluate what percent of samples that were positive by 2 rapid HIV tests would confirm by Western blot, and (2) assess the concordance, percent agreement, and false positive rate of rapid tests in a 3-test algorithm and evaluate the specifics for samples that yielded discordant results.

## Objectives

1. to determine the confirmatory rate of samples testing positive by two rapid tests, and
2. to determine the concordance of results and ability of a 3-test rapid testing algorithm to confirm results.

## Methods

BRIEF DESCRIPTION of HIV TEST DEVICES USED

**Abbott's Determine HIV-1/2**
- Finger stick, whole blood, plasma, and serum
- Storage 2-30°C
- Simple lateral flow test
- 1-15 minute incubation
- 5 steps for plasma & serum (6 steps for finger stick & whole blood)
- Contains recombinant & synthetic gp41
- Conjugate is selenium colloid
- No reagents unless whole blood or finger stick sample

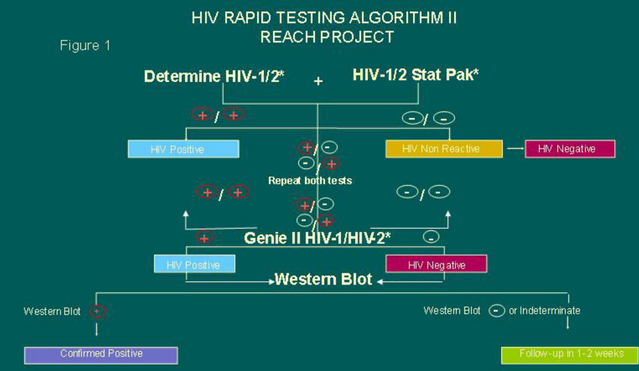
**Chembio's HIV 1/2 Stat-Pak**
- Finger stick, whole blood, plasma, and serum
- Storage 8-30°C
- Simple lateral flow test
- 1-10 minute incubation
- 6 steps for all sample types
- Contains synthetic gp41, gp120, and gp36
- Conjugate is colloidal gold
- 1 reagent (wash buffer)

Bio-Rad's **Genie II HIV-1/HIV-2**
- Plasma and Serum (requires sample dilution)
- Storage 2-8°C
- Lateral flow dual recognition EIA
- Total incubations 10 minutes
- 7 steps
- Contains recombinant gp41 & p24, and gp41 and gp36 peptides
- Conjugate is Streptavidin/AP
- 5 reagents (associated w/ enzymatic reaction)
- Distinguishes HIV-1 from HIV-2

## Methods

- 10,059 people were tested Aug 2006-Nov 2007
- Determine, Stat-Pak, and Genie II rapid assays were used (A small percentage were tested using UniGold and Capillus)
- The HIV 1 Western Blot assay used is manufactured by Bio-Rad.
- Participants were from clinical and community based populations including Commercial Sex Workers, Motorcycle Taxi Drivers, Blood Donors, and attendees of Antenatal and STI clinics.

Figure 1




## Results

**Figure 2: Confirmatory Rate of a Subset of Samples Positive by Both Rapid Tests**

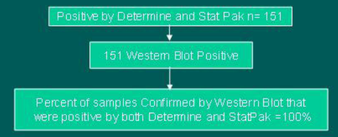
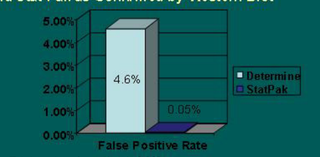



**Figure 3: False Positive Rate for Determine and Stat-Pak as Confirmed by Western Blot**




- Total number of negatives is 8,366
- 41 persons were followed up with no evidence of seroconversion (i.e., presumed false positive)
- Of all false positive results, the Determine was responsible for >98%.

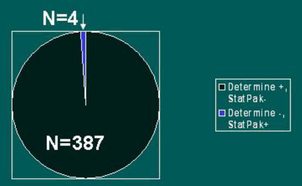
**Table 1: Percent Agreement of Rapid Tests:**

| Determine \ StatPak | Negative | Positive |
|---|---|---|
| Negative | 7975 | 4 |
| Positive | 387 | 1693 |

Total: 10,059

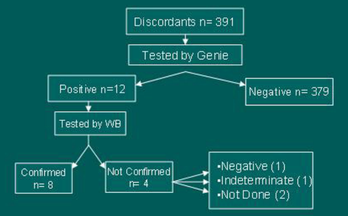
Calculated by taking the # of concurrent negative results + the # of concurrent positive results/Total # of tests

**Figure 4: Samples with Discordant Results (Determine and StatPak)**




391 samples had discordant Determine and StatPak results. Of the155 samples that could be tested by Western Blot, 12 were positive using the third (tie-breaker) Genie II test and 8 of these (5.1%) were confirmed.

**Figure 5**




Concordance by both tests:

Positives = 81.2% (1693/2084)

Negatives = 95.3% (7975/8366)

All Samples = 96.1%

Discordant Rate= 3.9%

-Determine Positive/StatPak Negative = 387

-Determine Negative/ StatPak Positive = 4

## Discussion and Conclusions

The use of an HIV alternative testing strategy incorporating 3 rapid tests has shown utility in a resource-limited country. The concordance of the two initial rapid test results was high (>96%). Greater than 98% of discordant results were attributed to (presumably) false positive results by the Determine test (as compared with <.05% for the Stat-Pak). In addition, some samples that showed discordant results were from confirmed positive persons. When discordant results occurred between the first 2 rapid tests, the 3rd test did not always correctly resolve results. Depending on which test is used as the first test, a parallel testing strategy may be necessary. These results suggest that the rapid tests must be carefully chosen for maximum test indices. Importantly, the confirmatory rate on a subset of samples that were positive by both initial rapid tests simultaneously was 100%, suggesting that positive results from 2 carefully chosen rapid tests have utility as an alternative confirmatory strategy. Further studies are needed to evaluate the cause of the high number of false positive results by some rapid tests, to evaluate the antibody profiles in samples yielding false positive results, and to assess the need for a parallel testing strategy.