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Sensitivity of the Multispot HIV-1/HIV-2 Rapid Test Using Samples from Human Immunodeficiency Virus Type 1-Positive Individuals with Various Levels of Exposure to Highly Active Antiretroviral Therapy

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The Multispot HIV-1/HIV-2 rapid test detects human immunodeficiency virus type 1 (HIV-1) gp41 antibodies, which can wane over time in some HIV-1-infected populations, resulting in false-negative screening results. Multispot sensitivity was 100% using 248 sera from one such population, and it correctly identified serostatus in individuals who previously tested false negative with rapid testing.

The Multispot HIV-1/HIV-2 rapid test received FDA clearance on 12 November 2004 for use in the "detection and differentiation of circulating antibodies associated with HIV-1 [human immunodeficiency virus type 1] and HIV-2 in human plasma and serum, as an aid in the diagnosis of infection with HIV-1 and/or HIV-2" (http://www.fda.gov/cber/pmaltr/P040046L.htm) (1). Both fresh and frozen specimens may be used. It joins three other rapid HIV diagnostic tests as FDA-approved devices currently in production, the OraQuick ADVANCE Rapid HIV-1/2 antibody test (OQ) (8), the Reveal G2 Rapid HIV-1 antibody test (5), and the Uni-Gold Recombigen HIV immunoassay (11). Multispot, a flowthrough device, employs the immunoconcentration method (12) to detect antibodies that bind to immobilized microparticles coated with antigen representing portions of transmembrane proteins of HIV-1, HIV-2, and a control immunoglobulin G (IgG), as shown in Table 1.

Multispot performance has been characterized in a variety of settings, including high- and low-risk U.S. populations (1), pregnant women (13), non-B-subtype panels (1, 3, 9), seroconversion panels (1), and the U.S. military (2). Overall, sensitivity has been 100% in the vast majority of these evaluations, with the only previously published exception being one false negative out of 11 serotype group O specimens tested (1). Specificity is only slightly lower, ranging from 99.91 to 100%.

These data are promising but remain incomplete, since they do not include an assessment of a patient population such as the Triservice AIDS Clinical Consortium (TACC) Natural History Study (NHS) (http://www.hivforum.org/cohorts/TACC%200305.pdf). This prospective continuous enrollment cohort of current and former U.S. military beneficiaries contains a small minority of patients whose serum anti-gp41 titers waned significantly over time, as measured by quantitative enzyme immunoassay (EIA) as well as by Western blotting (WB). In a previous study, antibody levels became sufficiently low to yield false-negative OQ results in 4% of subjects analyzed (7). This phenomenon appears to be restricted to patients undergoing effective highly active antiretroviral therapy (HAART). Others have previously reported decreases in anti-gp41 antibody levels (4) and IgG-secreting cells (6) among HAART-treated patients, possibly due to a decline in HIV-1-specific $CD4^+$ T lymphocytes (10) from a lack of persistent antigen exposure during suppressive therapy. Since patients may undergo HIV testing under circumstances where they are unwilling or unable to disclose their serostatus, testing devices should perform well even on samples from HAART-experienced HIV-positive individuals. This study examined whether the Multispot gp41 antibody detection approach would render the test less sensitive in such a population, where rapid testing was previously less sensitive (7).

This study was approved by institutional review boards at each institution. All Multispot testing was done according to the package insert (1) in the Immunology Research Laboratory at Wilford Hall USAF Medical Center by a single technician. Multispot results were scored using an intensity scale of 0 to 4, where each spot was graded from 0 (no reaction) to 4 (strongest reaction), and are expressed according to the spot order given in Table 1.

Study samples were derived from repository sera maintained at −70°C. Specimens were identified by starting on the study protocol approval date and retrospectively selecting the 248 most recently collected unique-subject specimens associated

TABLE 1. Identification of microparticle types used in the Multispot device[a]

Spot	Antigen	Purpose
1	Anti-human IgG (goat)	Procedural control
2	Peptide, HIV-2 gp36 immunodominant epitope	HIV-2 detection
3	Recombinant protein, HIV-1 gp41	HIV-1 detection
4	Peptide, HIV-1 gp41 immunodominant epitope	HIV-1 detection

[a] See reference 1 for further details on use of the Multispot device.

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TABLE 2. Study subject characteristics[a]

Characteristic	Value
Male[b]	95.2
Age (yr)[c]	40.3 ± 8.45 (22–65)
Race, self-identified[b]	
Caucasian	51
African American	37
Hispanic	7
Asian	2
Other	3
Seroconverters[b]	83.5
Time since HIV Dx (yr)[c]	8.7 ± 5.7 (0.6–20)
HAART	
Ever received[d]	195 (78.6)
Receiving at time of sample[d]	153 (61.7)
Duration (yr)[c]	4.5 ± 2.6 (0.05–8.85)
Duration/time since HIV Dx ratio[c]	0.55 ± 0.29 (0.01–1.0)
Time elapsed from Dx to HAART (yr)[c]	4.1 ± 4.13 (0.02–16.9)
CD4 (no. of cells/μl)[c]	637.0 ± 308.5 (16–2,125)
CD4 groups[d]	
0–199 cells/μl	13 (5.2)
200–349 cells/μl	25 (10.1)
≥350 cells/μl	210 (84.7)
CD4 nadir (no. of cells/μl)[c]	333.6 ± 178.3 (4–1,125)
CD4 nadir groups[d]	
0–199 cells/μl	62 (25.0)
200–349 cells/μl	71 (28.6)
≥350 cells/μl	115 (46.4)
HIV viral load (no. of copies/μl)[e]	469.0 (<50–>750,000)

[a] $n = 248$. Dx, diagnosis.
[b] Value is a percentage.
[c] Value is the mean ± SD. The range is given in parentheses.
[d] Value is the number of patients. The value in parentheses is a percentage.
[e] Viral load is expressed as a geometric mean. The range is given in parentheses.

with a TACC NHS visit with sufficient volume for testing. An additional serum repository sample was tested from each of the four subjects with false-negative results identified in a previous TACC NHS OQ study (7) with specimens obtained on the same date as previously studied. An aliquot from each sample subjected to Multispot testing was frozen at −70°C until shipment on dry ice to the Department of Molecular Diagnostics and Pathogenesis, Walter Reed Army Institute of Research, where HIV-1 Bio-Rad rLAV EIA (Bio-Rad Laboratories, Redmond, WA) and HIV-1 Bio-Rad WB were performed and reported according to the package inserts.

The study was powered (248 samples) to have an 80% likelihood of determining a significant difference between 96% sensitivity and the lower 95% confidence interval (CI) of the Multispot sensitivity stated in the package insert (99.94%) based on the 96% sensitivity of OQ in this population (7). Multispot sensitivity was expressed as a percentage with 95% binomial confidence intervals, descriptive statistics and Mann-Whitney U comparisons were performed using SPSS 13.0 (SPSS, Inc., Chicago, IL), and proportion comparison was accomplished by the Fisher exact test (EpiInfo 2000 version 1.1.2).

Samples included in the main study had been collected at the Wilford Hall USAF Medical Center clinical site from June 2004 through March 2005. Study subjects exhibited a mean time since HIV diagnosis of 8.7 years and a mean CD4 count of 637 cells/μl, with only 13 (5.2%) being below 200 cells/μl. HAART was being given to 153 (61.7%) subjects for a mean of 4.5 years (Table 2). All 248 specimens were reactive for HIV-1 using Multispot as well as the EIA/WB algorithm, yielding a Multispot sensitivity of 100% (95% CI, 98.80 to 100.00%) (Table 3). Spots 1, 3, and 4 were all universally reactive, whereas spot 2 was universally nonreactive. Two subjects failed to produce gp41 WB bands but were nevertheless reactive to both Multispot HIV-1 spots. By chance, 65 subjects contributed samples to both OQ (7) and Multispot evaluations, including three of four subjects that had false-negative results by OQ.

The additional four archived specimens acquired from subjects that tested false negative by OQ were all HIV-1 reactive using Multispot and EIA/WB (Table 3). Compared to the 248 TACC NHS specimens, these sera demonstrated weaker Multispot semiquantitative reactivity at spot 3 (3.64 versus 2.25; $P < 0.001$) and spot 4 (3.94 versus 2.00; $P < 0.001$), in spite of

TABLE 3. Comparative reactivities between the current Multispot study and OQ HIV-1 false-negative specimens

Reactivity	Result for specimen type			
	TACC NHS, 2004–2005 ($n = 248$)		OQ FN, 2000[a] ($n = 4$)	
	No. reactive (%)	Mean ± SD	No. reactive (%)	Mean ± SD[b]
Multispot reactivity				
Spot 1	248 (100)	3.13 ± 0.33	4 (100)	3.75 ± 0.5
Spot 2	0 (0)	0.0 ± 0.0	0 (0)	0.0 ± 0.0
Spot 3	248 (100)	3.64 ± 0.68	4 (100)	2.25 ± 0.96
Spot 4	248 (100)	3.94 ± 0.24	4 (100)	2.00 ± 0.82
Western blot reactivity				
p24	248 (100)	1.94 ± 0.23	4 (100)	1.00 ± 0.00
gp41	246 (99.2)	1.90 ± 0.32	4 (100)	0.88 ± 0.25
gp120	248 (100)	1.93 ± 0.25	4 (100)	1.25 ± 0.50
gp160	248 (100)	2.00 ± 0.00	4 (100)	2.00 ± 0.00
EIA reactivity for HIV-1	248 (100)		4 (100)	
No. (%) of specimens HIV-1 positive[c]	248 (100)		4 (100)	

[a] OQ FN, OQ HIV-1 false-negative subjects (7).
[b] For comparisons between OQ false negatives and TACC NHS semiquantitative Multispot and Western blot reactivity, P was <0.0001 for all comparisons except for spot 2 and gp160, where the P value was 1.000 (Mann-Whitney U).
[c] HIV-1 positivity was determined by a comparison of EIA and WB results.

demonstrating stronger reactivity at spot 1 (3.13 versus 3.75; $P < 0.001$). WB semiquantitative reactivity was also lower in the samples that tested false negative by OQ at p24, gp41, and gp120 ($P < 0.001$ for all comparisons) but not gp160.

This study supports the use of Multispot in settings where test subjects might be HAART exposed with attendant low or undetectable anti-gp41 antibody titers. Low titers were suggested by weak semiquantitative reactions in some individuals, but reactivity remained above the level of Multispot detection. Because a single specialized immunology technologist performed the Multispot testing, the performance characteristics of Multispot in this evaluation may not be generalizable to settings where testing is performed by laboratory generalists.

Multispot sensitivity (100%; 95% CI, 98.80 to 100.00) was superior to OQ sensitivity (7) in this population (96%; 95% CI, 90.17 to 98.91; $P = 0.00672$). The explanation for this difference may involve the flowthrough immunoconcentration design of Multispot, which affords the detection of lower antibody titers than the lateral-flow approach employed by OQ. Differences in antigen structure and preparation may also explain these findings, but the exact composition of antigens is not available for either device (1, 8). It is unlikely that our observations are due to subject selection bias, as similar subjects were drawn from the same population using the same approach in both studies and because Multispot correctly identified samples from individuals who had a false-negative result by OQ. While we did not perform live, side-by-side comparisons between OQ and Multispot for this study, having done so would have been unlikely to yield different results, since the two studies drew from the same patient population, used serum samples from the same repository, included a substantial number of the same individuals, and identified similar decrements in WB gp41 bands in the OQ false-negative specimens.

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REFERENCES

1. **Bio-Rad Laboratories.** 2004. Multispot HIV-1/HIV-2 Rapid Test package insert. Bio-Rad Laboratories, Redmond, Wash.
2. **Calero, E., J. Malia, R. Sawyer, L. Brown, D. Birx, B. Branson, and N. Michael.** 2002. Rapid HIV-1 diagnostic algorithms for use in HIV infection screening. Presented at the XIV International AIDS Conference, Barcelona, Spain.
3. **Holguin, A.** 2004. Evaluation of three rapid tests for detection of antibodies to HIV-1 non-B subtypes. J. Virol. Methods **115:**105–107.
4. **Kim, J. H., J. R. Mascola, S. Ratto-Kim, T. C. VanCott, L. Loomis-Price, J. H. Cox, N. L. Michael, L. Jagodzinski, C. Hawkes, D. Mayers, B. L. Gilliam, D. C. Birx, and M. L. Robb.** 2001. Selective increases in HIV-specific neutralizing antibody and partial reconstitution of cellular immune responses during prolonged, successful drug therapy of HIV infection. AIDS Res. Hum. Retrovir. **17:**1021–1034.
5. **MedMira Laboratories, Inc.** 2004. Reveal G2 Rapid HIV-1 Antibody Test. MedMira Laboratories, Inc., Halifax, Nova Scotia, Canada.
6. **Morris, L., J. M. Binley, B. A. Clas, S. Bonhoeffer, T. P. Astill, R. Kost, A. Hurley, Y. Cao, M. Markowitz, D. D. Ho, and J. P. Moore.** 1998. HIV-1 antigen-specific and -nonspecific B cell responses are sensitive to combination antiretroviral therapy. J. Exp. Med. **188:**233–245.
7. **O'Connell, R. J., T. M. Merritt, J. A. Malia, T. C. VanCott, M. J. Dolan, H. Zahwa, W. P. Bradley, B. M. Branson, N. L. Michael, and C. C. DeWitt.** 2003. Performance of the OraQuick Rapid Antibody Test for diagnosis of human immunodeficiency virus type 1 infection in patients with various levels of exposure to highly active antiretroviral therapy. J. Clin. Microbiol. **41:**2153–2155.
8. **OraSure Technologies.** 2004. OraQuick ADVANCE Rapid HIV-1/2 Antibody Test. OraSure Technologies, Bethlehem, Pa.
9. **Phillips, S., T. C. Granade, C. P. Pau, D. Candal, D. J. Hu, and B. S. Parekh.** 2000. Diagnosis of human immunodeficiency virus type 1 infection with different subtypes using rapid tests. Clin. Diagn. Lab. Immunol. **7:**698–699.
10. **Pitcher, C. J., C. Quittner, D. M. Peterson, M. Connors, R. A. Koup, V. C. Maino, and L. J. Picker.** 1999. HIV-1-specific CD4+ T cells are detectable in most individuals with active HIV-1 infection, but decline with prolonged viral suppression. Nat. Med. **5:**518–525.
11. **Trinity Biotech USA.** 2004. Uni-Gold Recombigen HIV. Trinity Biotech USA, Bray, County Wicklow, Ireland.
12. **Valkirs, G. E., and R. Barton.** 1985. ImmunoConcentration—a new format for solid-phase immunoassays. Clin. Chem. **31:**1427–1431.
13. **Webber, M. P., P. Demas, E. Enriquez, R. Shanker, W. Oleszko, S. T. Beatrice, and E. E. Schoenbaum.** 2001. Pilot study of expedited HIV-1 testing of women in labor at an inner-city hospital in New York City. Am. J. Perinatol. **18:**49–57.