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Analytical and clinical performance evaluation of the cobas TaqScreen MPX Test for use on the cobas s 201 system

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abstract

Clinical specificity, analytical and clinical sensitivities, reproducibility, and subtype/genotype coverage of the cobas TaqScreen MPX Test, a multiplex nucleic acid test with expanded coverage of HIV variants, were determined. A total of 72,281 blood donations were evaluated. The 95% limit of detection (LOD) for the MPX Test inclusive viruses was determined by testing six dilutions of WHO or Roche standards. Over 3000 high-risk and confirmed seropositive specimens were tested with the MPX and COBAS AmpliScreen Tests. Ten subtypes of HIV-1 Group M, HIV-1 Group O, HIV-2 A and B, HBV genotypes A-H, and HCV genotypes 1–6 were tested with the MPX Test. Reproducibility panels were evaluated at three testing sites across three lots. Clinical specificity in pools was 99.99%. There was one HBV yield case. The LODs for HIV-1 Group M, HCV, and HBV were 49, 11, and 3.8IU/mL, respectively, and 89 and 59.3copies/mL for HIV-1 Group O and HIV-2, respectively. Concordance between the MPX and the AmpliScreen Tests was 94.9%. Clinical sensitivity based on AmpliScreen comparison was 97.8–99.5%. All genotype/subtype replicates were detected at three times the LOD. Reproducibility was 98.3–100%. In conclusion, the MPX Test is robust and covers HIV-1 Group O and HIV-2.

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1. Introduction

The high volume burden of modern blood screening along with the appearance of new HIV variants has spurred the drive both to improve nucleic acid testing (NAT) and to develop additional tests that would detect the less common HIV variants (McCutchan, 2006; Schochteman et al., 2005). However, the development of the more efficient multiplex screening tests has highlighted the challenge of balancing and maintaining sensitivity and specificity, regardless of whether the tests are run on individual specimens or in pools and of keeping that balance as multiple viral markers are incorporated into single assays.

To improve the efficiency and coverage of its original individual COBAS® AmpliScreen Tests (AmpliScreen Tests) for HIV-1 Group M, HCV, and HBV, Roche Molecular Systems, Inc. (Roche) developed the qualitative cobas® TaqScreen MPX Test for use on the cobas s 201 system (MPX Test), a single assay, multiplex blood screening NAT that may be performed on individual specimens, but is designedtoruninpoolsofsix.Inadditiontodirectdetectionof HBV, HCV, and the more common HIV-1 Group M subtypes, the MPX Test is the first U.S. Food and Drug Administration (FDA)-licensed test designed to detect additionally two variants most commonly seen in Africa, HIV-1 Group O (outlier group), recently also observed in Europe and North America, and HIV-2, now documented in North and South America, Europe, Australia and Asia (Barin et al., 2007; Cazien et al., 1996; CDC MMWR, 1996; Chen et al., 2002; cobas TaqScreen MPX package insert, 2009; Delwart et al., 2003; Downie et al., 1992; FDA Press Release, 2008; Jonassen et al., 1997; Libonatti et al., 1998; O'Brien et al., 1992; Ramaligam et al., 2005; Soriano et al., 1996, 2000; Weindel et al., 2004).

This report covers key studies in the clinical trials conducted for U.S. FDA licensure. The studies were designed to determine: (1) the analytical sensitivity of the MPX Test using standard dilution panels, (2) its concordance with the original COBAS® AmpliScreen Tests in high-risk and clinical sensitivity in seropositive groups, (3) its clinical specificity, (4) its genotype inclusivity, and (5) its reproducibility.

2. Materials and methods

2.1. Overview of cobas TaqScreen MPX technology

The MPX test is a qualitative, multiplex in vitro test for simultaneous detection of human immunodeficiency virus (HIV) type 1 (HIV-1) Group M RNA, HIV-1 Group O RNA, HIV-2 RNA, hepatitis C virus (HCV) RNA, and hepatitis B virus (HBV) DNA in human plasma (Assal et al., 2009; cobas TaqScreen MPX Test package insert, 2009; Jarvis et al., 2008; Li et al., 2008; Margaritis et al., 2007).

2.2. Licensed AmpliScreen Tests used for comparative evaluation

Three separate COBAS AmpliScreen Tests were used in the evaluation: COBAS AmpliScreen HIV-1 v. 1.5, COBAS AmpliScreen HCV v. 2.0, and COBAS AmpliScreen HBV. Their respective 95% limit of detections (LODs) are: 78.4IU/mL (68.4–94.4), 41.9IU/mL (28–111.8), and 4.41IU/mL (3.56–6.13) (COBAS AmpliScreen HCV Test package insert, 2006, and HBV and HIV Test package inserts, 2009).

2.3. Clinical specificity: reactivity in whole blood donor population

Clinical specificity of the MPX Test was evaluated by testing 72,281 whole blood donations, including 10,090 pools of six, at three independent U.S. blood centers. Plasma was tested from individual blood donations and pooled specimens prepared from equal plasma aliquots from individual donations.

2.4. Analytical sensitivity

The LODs for the MPX Test target viruses were determined using the following standards: the WHO International Standard for HBV DNA (97/746), the WHO Second International Standard for HCV RNA (96/798), and the Roche standards for HIV-1 Group M, Group O and HIV-2 RNA which are commercially available cultured virus stocks, the former two traceable to the WHO HIV-1 standard (cobas MPX TaqScreen Test package insert, 2009). For the WHO and Roche standards, three independent dilution series of each viral standard were prepared with normal, virus-negative human plasma. Each dilution series was tested at Roche using three different lots of the MPX Test kit with 22 replicates per lot, for a total of 198 replicates perconcentration.Thefollowingconcentrationsweretestedforthe target viruses: HIV-1 Group M: 6, 20, 40, 50, 60, 190IU/mL; HIV1 Group O: 9.4, 29.3, 93.8, 117, 147, 293copies/mL; HIV-2: 10.5, 34.2, 60.5, 81.5, 105, 316copies/mL; HCV: 1.0, 3.0, 10.0, 11.3, 15.4, 30.0IU/mL; and HBV: 0.4, 1.3, 3.6, 4.0, 5.0, 12.0IU/mL.

Probit analysis on the combined run data from all replicates tested undiluted, as is routine, for each virus was used to determine the 95% LOD and the two-sided fiducial confidence intervals.

2.5. Studies in high-risk populations

Between October 2005 and September 2006 plasma specimens were collected prospectively from populations determined by the Centers for Disease Control and Prevention (CDC) to be at high risk for HIV, HCV, and/or HBV infection. Certificates of Analysis, which included classification into four viral risk categories along with enzyme immunoassay results, were provided for each specimen.

Institutional Review Board (IRB) approval was obtained to use and evaluate further the specimens with the MPX Test. Of these specimens,1526weretestedundilutedand,tosimulatepools,1571 were tested diluted 1:6 using two kit lots of reagents at four testing sites. A total of 1256 of the 1526 undiluted high-risk specimens and 1284 of the 1571 diluted high-risk specimens were tested at an external site and at Roche also with the HIV-1, HCV, and HBV AmpliScreen Tests. Specimens were distributed between three kit lots. The 1256 specimens were tested undiluted with both the MPX Test and, using the Standard Processing Procedure, with the AmpliScreen Tests. The 1284 specimens were tested diluted 1:6 with the MPX Test and, using the Multiprep Specimen Processing Procedure,diluted1:24withtheAmpliScreenTests.MPXTestundiluted results were compared to AmpliScreen Test undiluted results. MPX Test 1:6 diluted results were compared to AmpliScreen Test 1:24 diluted results.

To characterize further the 44 discordant high-risk specimens that were MPX Test non-reactive and positive with one or more AmpliScreen Tests, specimens with adequate residual volume had viral load quantified by the COBAS® TaqMan® Tests.

2.6. Studies in seropositive populations

Plasma specimens were procured from seropositive donors in the continental U.S. and Asia between October 2005 and September 2006. Certificates of Analysis assured the specimens were collected with informed consent, under IRB approval, and were serologically reactive for HBsAg, anti-HCV, and/or anti-HIV1/2 by an FDA-approved test. Some were also tested with one or more NATs. Their seropositive status was confirmed by HIV-1 Western Blot, HCV RIBA, or HBsAg neutralization, and following explicit IRB approval, these specimens were tested with AmpliScreen, the licensed comparator for determination of clinical sensitivity. Specimens from donors on therapy (e.g., antiretroviral treatment for HIV) were not excluded from the study.

A total of 2387 confirmed seropositive specimens were tested undiluted and, to simulate pools, a total of 2441 were tested diluted 1:6 with the MPX Test distributed across six testing sites and two kit lots of reagents. Of the 2387 undiluted and the 2441 diluted specimens, 2042 and 2090, respectively, were tested also with the AmpliScreen Tests at an external testing site or at Roche. These specimens included: 713 undiluted and 737 diluted for HIV-1, 950 undiluted and 961 diluted for HCV, and 379 undiluted and 392 diluted for HBV. Specimens tested undiluted with the MPX Test were tested undiluted, using the Standard Specimen Processing Procedure, with the AmpliScreen Tests, while those that were tested1:6dilutedwiththeMPXTestweretested1:24diluted,using the Multiprep Specimen Processing Procedure, with the AmpliScreen Tests.

Twenty-two HIV-1 Group O seropositive specimens, characterized by serology and/or DNA sequencing, and 200 HIV-2 seropositive specimens (not confirmed by a second serological test) were also tested with the MPX Test at Roche. Of the HIV-1 Group O specimens, five were tested both undiluted and 1:6 diluted, and 17 were tested only diluted 1:6. All 200 HIV-2 specimens were tested both undiluted and diluted 1:6 with the MPX Test and undiluted specimens also with a quantitative research use only NAT (Damond Assay) with an LOQ of 100copies/mL (Damond et al., 2005), the latter serving as a reference standard in the determination of clinical sensitivity.

2.7. Genotype and subtype inclusivity

The ability of the MPX Test to detect various HIV-1 and HIV-2 subtypes, as well as HCV and HBV genotypes was determined.

2.7.1. HIV-1 Group M

Fifty HIV-1 Group M clinical specimens and 25 cultured isolates with known subtypes (seven subtype A, 15 subtype AE recombinants, ten subtype AG recombinants, 12 subtype B, nine subtype C, seven subtype D, four subtype F, eight subtype G, two subtype H and one subtype J) were quantified by the COBAS® AMPLICOR HIV-1 Monitor Test v. 1.5 and diluted with normal, virus-negative human plasma to concentrations at one and three times the MPX Test's HIV-1 Group M LOD.

2.7.2. HIV-1 Group O and HIV-1 Group N

Eight HIV-1 Group O cultured isolates and one HIV-1 Group N cultured isolate were similarly tested. Since no molecular method was available to quantify the HIV-1 Group N isolate, half log dilutions of the cultured stock were prepared in normal, virus-negative human plasma and each dilution was tested with the MPX Test.

2.7.3. HIV-2

Nine HIV-2 cultured isolates of the following subtypes: five A, one A/B, one B and two unknown and 11 HIV-2 clinical specimens of unknown subtype and viral load and unconfirmed serology were tested. (Of eight subtypes, A is most pathogenic, A and B are most clinically relevant (Schim van der Loeff and Aaby, 1999; Soriano et al., 2000).)

2.7.4. HCV

Seventy-four HCV clinical specimens with the following genotype distribution: eight of 1a, 13 of 1b, one of 2, seven of 2a, one of 2a/c, nine of 2b, eight of 3a, one of 3a/b, six of 4, one of 4a, one of 4b/c, one of 4h, two of 5, nine of 5a, two of 6, and four of 6a, were quantified by the COBAS® AMPLICOR® HCV Monitor Test v. 2.0 and diluted with normal, virus-negative plasma to one and three times the MPX Test's HCV LOD and tested with the MPX Test.

2.7.5. HBV

Sixty-four HBV clinical specimens and one molecular clone with known genotype with the following genotype distribution: 13 of A, nine of B, ten of C, nine of D, 14 of E, six of F, two of G, two of H were quantified by the COBAS® AMPLICOR® Monitor Test and diluted with normal, virus-negative plasma to one and three times the MPX Test's HBV LOD and tested with the MPX Test.

2.8. Reproducibility

Eighteen member randomized, blinded panels including three negative plasma specimens and three positive plasma specimens each for HIV-1 Group M, HIV-1 Group O, HIV-2, HCV, and HBV were tested at concentrations of approximately 0.54, one and three times the LOD of the MPX Test of each virus. One operator evaluated three kit lots of the MPX Test on one cobas s 201 system at each of the three testing sites. At each site, four panels and four control sets weretestedeachdayforfivedayswitheachofthethreekitlots,fora totalof2160tests–180testsofeachpanelmember.Thepercentage of reactive test results for each panel member was calculated for all valid reproducibility data. Data were analyzed by kit lot and site/operator.

3. Results

3.1. Clinical specificity: reactivity in whole blood donor population

Of the 72,281 blood donations tested, 9678 were tested individually, and 62,603 were tested in pools of six. The clinical specificity for samples tested individually was 99.90% (9668/9678: 95% CI 99.81–99.95), for samples tested in pools it was 99.99% (62,598/62,603: 95% CI 99.81–100.00). The combined specificity was 99.98% (72,266/72,281: 95% CI 99.97–99.99). The invalid rates for pooled and individual specimen results were 5.9 and 4.4%, respectively. Invalid rates of complex tests usually decline with increased use. Reactive pools were resolved to a single reactive donation using the MPX test and then discriminated to specific viral reactivity by further testing their individual components with the AmpliScreen Tests.

3.2. Clinical specificity: pool reactivity

A total of 10,090 pools were tested of which 36 (0.36%) were reactive initially. Twenty-three of these 36 were associated with a positive donor status, assigned based on serological and AmpliScreen Test results, and occasionally also by the results of an alternative NAT. Twelve (0.12%) initially reactive pools were associated with a negative donor status and were considered false positives. One reactive pool was secondary to a yield case.

3.3. Yield case from specificity study

The index donation and follow-up results of the yield case are shown in Table 1. This case was associated with a first-time male donor who was not immunized against HBV. His index donation was seronegative for all three viruses and screen negative by all three AmpliScreen Tests when tested in pools of 24, negative by HBV SuperQuant™ (National Genetics Institute, Los Angeles, CA) (limit of quantification [LOQ] 100copies/mL or ~31.4IU/mL), but reactivewhentestedundilutedbytheMPXTestandbyUltraQual™ HBV (National Genetics Institute, Los Angeles, CA) (LOD 0.9IU/mL) (National Genetics Institute web site).

3.4. Analytical sensitivity

Results of the Probit analysis to determine the 95% LOD for the individual viruses included in the MPX Test are shown in Table 2.

3.5. High-risk population study

Data analysis was conducted on the complete dataset. Analysis beyondthatincludedinthecommercialpackageinsertispresented here.ValidAmpliScreenTestresultswereobtainedfor1251of1256 undiluted specimens with MPX Test results. Of the 1251, a total of 406 were positive undiluted with one or more of the AmpliScreen Tests and 410 were reactive undiluted with the MPX Test (Table 3). Of the 30 that were positive in one or more AmpliScreen Tests and MPX Test non-reactive, four were positive for HIV-1, five for HCV, 20 for HBV, and one for both HCV and HBV. Fourteen of these 30 AmpliScreen Test-positive specimens had sufficient volume for viral load testing with the COBAS® TaqMan® Tests: two HIV-positive specimens, of which one was negative and one had a viral load of 104copies/mL; 11 HBV-positive specimens of which five were negative and six had detectable viral loads <6IU/mL. One HCV/HBV double-positive specimen tested HCV negative and had an HBV titer of 358IU/mL.

Valid AmpliScreen Test results (specimens diluted 1:24) were obtained for 1283 of 1284 1:6 diluted specimens with MPX Test results. Of these 1283 specimens, 406 were positive with one or more of the AmpliScreen Tests when diluted 1:24 and 418 were reactive with the MPX Test when diluted 1:6 (Table 3). Of the 42 that were positive with one or more of the AmpliScreen Tests and negative with the MPX Test, 12 were positive for HIV-1, 14 were positive for HCV, 14 were positive for HBV, and two were positive for both HIV-1/HCV. Twenty-three of the 42 had sufficient volume for viral load testing with the COBAS® TaqMan® Tests: eight HIVpositive specimens, of which seven were negative and one had a viral load of 5600copies/mL, nine HCV specimens of which eight were negative and one had an HCV viral load of 212IU/mL, and five HBV specimens, of which three were negative and two had viral loads of 37 and 133IU/mL, respectively. One AmpliScreen HIV1/HCV double-positive specimen with sufficient volume for only one HIV viral load assay tested negative.

A total of 7.4% (30/406) of undiluted specimens that were AmpliScreen Test-positive were non-reactive with the MPX Test and of those that were AmpliScreen Test-negative, 4.0% (34/845) were reactive with the MPX Test. Among diluted specimens, 10.3% (42/406) of AmpliScreen Test-positive specimens were nonreactive with the MPX Test, while of those that were AmpliScreen Test-negative, 6.2% (54/877) were reactive with the MPX Test.

Table 1
Index and follow-up test results for yield donor.

	Day of test				
	Day 0	Day 19	Day 23	Day 43	Day 59
MPX Test (undiluted)	Reactive	Reactive	Reactive	Non-reactive	Reactive
AmpliScreen HBV Test (undiluted)	Positive	Negative	Negative	Negative	Negative
Modified AmpliScreen HBV Test (Multiprep procedure×3)	Not tested	3/3	1/3	1/3	1/3
HBsAg (Ortho HBsAg 3.0)	Non-reactive	Non-reactive	Non-reactive	Non-reactive	Non-reactive
Anti-HBc (Ortho)	Non-reactive	Non-reactive	Non-reactive	Non-reactive	Non-reactive
Anti-HBs (Bio-Rad qualitative test)	Reactive	Reactive	Reactive	Reactive	Reactive

Table 2
Limits of detection for the cobas TaqScreen MPX Test target viruses.

Virus	Standard	Units	Average 95% LOD	95% CI
HIV-1 Group M	Roche Secondary Standard	IU/mL[a]	49	42.4–58.1
HIV-1 Group O	Roche Primary Standard	copies/mL	89	56–217
HIV-2	Roche Primary Standard	copies/mL	59.3	51.9–69.7
HCV	WHO Second International Standard	IU/mL[b]	11	7.0–21.7
HBV	WHO International Standard	IU/mL[c]	3.8	3.3–4.4

[a] Conversion factor 0.6 copies/IU. [b] Conversion factor 2.7 copies/IU. [c] Conversion factor 5.0 copies/IU.

Comparative results for the MPX Test and the combined AmpliScreen Tests in a high-risk population are shown in Table 3. The concordance was 94.9% for undiluted results and 92.5% for diluted results.

3.6. HIV-1-, HCV-, and HBV-seropositive population study: clinical sensitivity

Data analysis was conducted on the complete dataset. Analysis beyond that included in the commercial package insert is presented here. Of the 713 HIV-1 specimens tested undiluted with the AmpliScreen HIV-1 Test, a total of 582 (81.6%) were HIV-1 positive. The 18.4% of seropositive specimens that tested negative by the AmpliScreen HIV-1 Test may be explained by the inclusion of specimensfrompatientsonanti-viraltherapy.Ofthe950HCVspecimens tested undiluted with the AmpliScreen HCV Test, 818 (86.1%) wereHCVpositive.Ofthe379HBVspecimenstestedundilutedwith the AmpliScreen HBV Test, 367 (96.8%) were HBV DNA positive. Of the 737 HIV-1 specimens tested 1:24 diluted with the AmpliScreen HIV-1 Test, a total of 461 (62.6%) were HIV-1 positive. Of the 961 HCV specimens tested 1:24 diluted with the AmpliScreen HCV Test,
797 (82.9%) were HCV positive. Of the 392 HBV specimens tested 1:24 diluted with the AmpliScreen HBV Test, 360 (91.8%) were HBV DNA positive.

Comparative results for the MPX Test and the AmpliScreen Tests forthosespecimenswithdemonstrableHIV-1andHCVandHBVare shown in Table 4. In undiluted testing, the MPX Test showed high clinical sensitivity (98.7%), which decreased 2.1% for HIV-1 (from 97.8to95.7%),butlessthan1%forallviruses(to97.9%)whentesting was performed on specimens diluted 1:6.

Table 3
Concordance of cobas TaqScreen MPX Test and AmpliScreen Test results in a high-risk population.

Test	Sample	Total tested	COBAS AmpliScreen Test[a] Positive		COBAS AmpliScreen Test[b] negative		Concordance (%)
			MPX reactive	MPX non-reactive	MPX reactive	MPX non-reactive	
Combined COBAS AmpliScreen Tests	Undiluted	1251[c]	376	30	34	811	94.9 92.5
	Diluted[d]	1283[e]	364	42	54	823	

[a] Positive with one or more of the COBAS AmpliScreen Tests. [b] Negative with all 3 COBAS AmpliScreen Tests. [c] This group included the following co-infected specimens: 11 HIV-1/HCV/HBV, 37 HIV-1/HCV, 20 HCV/HBV, and 7 HIV-1/HBV.
[d] Tested 1:6 diluted with the MPX Test and 1:24 with the AmpliScreen Tests. [e] This group included the following co-infected specimens: 2 HIV-1/HBV/HCV, 33 HIV-1/HCV, 12HCV/HBV, and 8 HIV-1/HBV.

3.7. HIV-1 Group O-seropositive population

The MPX Test results for HIV-1 Group O seropositive specimens are shown in Table 5a. Of the five specimens tested both undiluted and diluted 1:6, four (80%) were reactive with the MPX Test and one was invalid with insufficient volume for repeat testing. Of the 22 specimens that were tested diluted 1:6, 19 (86.4%) were reactive with the MPX Test. The three non-reactive specimens were subsequently found to have undetectable viral loads (<60copies/mL) by Abbott Real Time HIV-1 Test (research use only) (AbbottLaboratories,AbbottPark,IL),thecomparatortestforsensitivitydeterminati on,andwerethereforeexcludedfromtheoriginal sensitivity analysis. Either these three unconfirmed seropositive specimens were false positives or they had viral loads below the LOD of both the Abbott and the MPX assays. Among these 22 HIV-1 Group O specimens, there was one HIV-1 Group O/HIV-1 Group M co-infected specimen as shown by positive HIV-1 Group M serology and sequence analysis, one HIV-1/HCV co-infected specimen as shown by positive results with the HCV AmpliScreen Test v. 2.0, and one HIV-1/HBV co-infected specimen as shown by positive results with the HBV AmpliScreen Test. After the removal of the one specimen with an invalid result from the undiluted analysis and the three specimens with undetectable viral loads from the diluted analysis, the clinical sensitivity for the MPX Test was 100% (4/4: 95% CI 82.4–100.0, and 19/19: 95% CI 82.4%–100.0).

3.8. HIV-2 seropositive population

Of the 200 HIV-2 unconfirmed seropositive HIV-2 specimens, 93 (46.5%) were reactive undiluted and 69 (34.5%) were reactive

Table 4
Comparison of clinical sensitivities of the MPX Test and AmpliScreen Tests for HIV-1 Group M, HCV, and HBV in confirmed seropositive specimens.

cobas TaqScreen MPX Test	Sample	Total tested[a]	MPX reactive	Clinical sensitivity (%)	95% CI

Undiluted	All	1767[b]	1744	98.7	98.1–99.2
	HIV-1 Group M	582	569 810	97.8	96.2–98.8
	HCV	818		99.0	98.1–99.6
	HBV	367	365	99.5	98.0–99.9
Diluted[c]	All	1618[d]	1584	97.9	97.1–98.5
	HIV-1 Group M	461	441	95.7	93.4–97.3
	HCV	797	789	99.0	98.0–99.6
	HBV	360	354	98.3	96.4–99.4

[a] Number of specimens with valid MPX Test results and positive with one or more COBAS AmpliScreen Tests.

[b] There were 6 HIV-1/HCV/HBV co-infected specimens, 37 HIV-1/HCV co-infected specimens, 116 HCV/HBV co-infected specimens, and 106 HIV-1/HBV co-infected specimens in this population.

[c] Tested diluted 1:6 with the MPX Test and diluted 1:24 with the COBAS AmpliScreen Tests.

[d] There were 6 HIV-1/HCV/HBV co-infected specimens, 9 HIV-1/HCV co-infected specimens, 101 HCV/HBV co-infected specimens, and 66 HIV-1/HBV co-infected specimens in this population.

Table 5a
Clinical sensitivity of MPX Test for HIV-1 Group O.

	Total	MPX reactive	MPX non-reactive	Sensitivity (%)	95% CI
HIV-1 Group O (undiluted)	5	4[a]	0	100.0	82.4–100.0
HIV-1 Group O (1:6 diluted)	22	19[b]	3[c]	100.0	82.4–100.0

[a] One of the 5 specimens was invalid on initial testing with insufficient volume for a repeat test.

[b] There was one HIV-1 Group O/HIV-1 Group M co-infected specimen (as shown by positive HIV-1 Group M serology and sequence analysis), one HIV-1 Group O/HCV co-infected specimen (as shown by positive results with the COBAS AmpliScreen HCV Test), and one HIV-1 Group O/HBV co-infected specimen (as shown by positive results with the COBAS AmpliScreen HBV Test).

[c] All 3 non-reactive specimens had undetectable HIV-1 viral load (<60copies/mL) using the Abbott Real Time HIV-1 Test (research use only) and were excluded from the sensitivity analysis.

Table 5b
Clinical sensitivity of the cobas TaqScreen MPX Test for HIV-2 specimens that tested positive with the Damond Assay.

	Alternate NAT positive	MPX reactive	MPX non-reactive	Sensitivity (%)	95% CI
HIV-2 (undiluted)	43[a]	43	0	100.0	91.8–100.0
HIV-2 (1:6 diluted)	43[b]	40	3	93.0	80.9–98.5

[a] One HIV-2 specimen had a viral load of 117copies/mL and was reactive in 5 out of 8 replicates when tested undiluted with the cobas TaqScreen MPX Test.

[b] HIV-2 NAT RNA quantification test (research use only) (limit of quantification 100copies/mL) developed by Dr. Florence Damond (Hopital Bichat Claude Bernard, Paris, France) run on undiluted specimens only.

diluted 1:6 with the MPX Test. The undiluted HIV-2 specimens were also tested with the Damond Assay, the alternate NAT used for clinical sensitivity comparison, and MPX results for those that were Damond Assay reactive are shown in Table 5b (Damond et al., 2005). It is not surprising that the MPX Test, with an LOD of 59.3copies/mL for HIV-2, detected RNA in over twice as many (93/200 vs. 43/200) undiluted HIV-2 specimens as did the less sensitive Damond Assay (LOQ=100copies/mL). Most likely the three specimens not detected when tested diluted 1:6 with the MPX Test had viral loads below 356copies/mL (6×59.3).

3.9. Genotype and subtype inclusivity

3.9.1. HIV-1 Group M

All 50 clinical specimens and 25 cultured isolates were reactive at one and three times the LOD of the MPX Test.

3.9.2. HIV-1 Group O and HIV-1 Group N

Seven of eight HIV-1 Group O isolates were detected at dilutions below 100copies/mL. The HIV-1 Group N isolate was detected in all dilutions up to 3.3×10^{-10}.

3.9.3. HIV-2

FiveHIV-2subtypeAculturedisolatesweredetectedbytheMPX Test in all dilutions up to 1×10^{-8} to 3.3×10^{-9}. The HIV-2 subtype A/B isolate was detected in dilutions up to 1×10^{-9}. The subtype B isolate and two isolates of unknown subtype were detected in dilutions up to 3.3×10^{-9}. All 11 HIV-2 clinical specimens were detected by the MPX Test at the following dilutions: up to 1:100 (2 specimens), 1:30 (3 specimens), 1:10 (2 specimens), 1:3 (3 specimens), and undiluted (1 specimen).

3.9.4. HCV

All 74 of the isolates were reactive at three times the LOD of the MPX Test. Seventy-three of the 74 were reactive at the LOD.

3.9.5. HBV

Sixty-four clinical specimens and one molecular clone were reactive at both one and three times the LOD of the MPX Test.

A summary of HIV subtype and HCV and HBV genotype inclusivity by the MPX Test is shown in Table 6.

3.10. Reproducibility

The reproducibility of the MPX Test at the LOD of each of its inclusive viruses across three kit lots and for three instrument/operator pairs performing across five days is shown in

Table 6
HIV-1 Group M subtypes and HBV and HCV genotypes detected at 3 times the targetspecific limit of detection of the cobas TaqScreen MPX Test.

Virus	Subtype/genotype
HIV-1 Group M	Subtypes: A, AE, AG, B, C, D, F, G, H, J
HBV	Genotypes: A, B, C, D, E, F, G, H
HCV	Genotypes: 1a, 1b, 2, 2a, 2a/c, 2b, 3a/b, 4, 4a, 4b/c, 4h, 5, 5a, 6, 6a

Table 7
Reproducibility of the cobas TaqScreen MPX Test at the limit of detection for each target virus.

Virus	Concentration	Kit lot	Reactive results by reagent lot
	0	1	0.0% (0/60)
		2	1.7% (1/60)
		3	0.0% (0/60)
HIV-1 Group M	1 ×	1	100% (60/60)
		2	98.3% (59/60)
		3	98.3% (59/60)
HIV-1 Group O	1 ×	1	100% (60/60)
		2	98.3% (59/60)
		3	100% (60/60)
HIV-2	1 ×	1	100% (60/60)
		2	100% (60/60)
		3	98.3% (59/60)
HCV	1 ×	1	100% (60/60)
		2	100% (60/60)
		3	98.3% (59/60)
	1 ×	1	100% (60/60)
		2	98.3% (59/60)
		3	100% (60/60)

Table 7. At 0.54 times the LOD, reactive results ranged from 61.7% for HCV to 100% for HIV-1 Group O. At three times the LOD, one HIV1 sample was missed by one operator, allowing a range for reactive results of 98.3–100%.

4. Discussion

Laboratory efficiency encourages the combination of tests when feasible. A new test, however, should have comparable performancecharacteristicstoexistingones.Inadditiontodemonstrating the robust performance of the MPX Test, this study evaluated unique populations to assess the assay's comparative concordance and clinical sensitivity for the detection of HIV-1, HCV, and HBV with those of the respective AmpliScreen Tests currently in use.

The study showed good concordance between the two tests and some improvement in the clinical sensitivity of the MPX test compared with AmpliScreen, most notably for HCV. In the high-risk population, concordance was almost 95% for undiluted specimens and fell only 2.4% when specimens were tested diluted 1:6 for the MPX Test and 1:24 for the AmpliScreen Tests. One hundred percent clinical sensitivity is rarely observed and discordance between tests, often due to stochastic sampling errors, is expected when the viral load of samples tested is close to the assays' detection limits.

This study's overall performance findings for the MPX test are largely concordant with those observed by independent investigators in Europe, Asia, and Australia (Assal et al., 2009; Jarvis et al., 2008; Li et al., 2008; Margaritis et al., 2007). The estimated 95% LODs for the hepatitis viruses in this study, 11IU/mL for HCV and 3.8IU/mL for HBV, fall in the respective ranges – 6.9–14.4 and 2.7–8.4IU/mL – of those observed by these independent investigators using the same WHO standards (Assal et al., 2009; Jarvis et al., 2008; Li et al., 2008; Margaritis et al., 2007).

Direct comparison of the HIV-1 LOD is more difficult because this study used the Roche Secondary Standard for HIV-1 Group M and the others used two different WHO HIV-1 standards (cobas TaqScreen MPX Test package insert, 2009). Nevertheless, the 49IU/mL 95% LOD reported here is close to the 47.6, 50.5, 62.53IU/mL 95% LOD reported by Assal, Li, Margaritis and colleagues and falls in the 43.0–79.2IU/mL range observed in the multicenter study (Assal et al., 2009; Jarvis et al., 2008; Li et al., 2008; Margaritis et al., 2007).

Comparison of other performance attributes is possible only to the extent they were evaluated by others. While this study found the MPX Test to be

98.3–100% reproducible, 100% reproducibility was noted in three smaller studies (Assal et al., 2009; Li et al., 2008; Margaritis et al., 2007). Only Assal and colleagues also studied clinical specificity and genotype coverage. This group observed a clinical specificity of 99.93%, very close to this study's combined specificity of 99.98% and detected all 14 genotypes/subtypes studied, all of which, along with additional ones, were detected in this study (Assal et al., 2009).

One HBV yield case was found in both this study and the smaller European multicenter study which evaluated a third the number of donors, but which included Mediterranean study sites where HBV is moderately endemic (National Travel Health Network and Centre, 2006). Unlike the anti-HBc positive yield from the multicenter study, the only HBV marker in this study's yield was anti-HBs. This pattern might be observed in a donor experiencing post-vaccination breakthrough, possible only in this case if the donor were unaware of a previous vaccination. More frequently it is observed in occult cases where low level viral replication persists despiteapparentrecoveryfrominfectioncharacterizedbyanti-HBs or secondary to escape mutants (Allain, 2004).

The detection of HBV DNA in the yield sample at levels between 0.9 and ~31.4IU/mL demonstrates the assay's ability to detect low level HBV infection that can, nevertheless, be responsible for transfusion-transmitted HBV (Allain, 2004; Liu et al., 2006a; Liu et al., 2006b; Satake et al., 2007). In high endemic areas, where occult HBV is not uncommon and anti-HBc screening is impractical, an ultrasensitive NAT will have great value. While the undiluted index specimen was AmpliScreen HBV positive, all four follow-up specimens were AmpliScreen negative. In comparison, the index specimen and at least one of the three replicates of four follow-up specimens were MPX Test reactive. Inconsistent viral detection in replicates with viral levels near the assay's detection limit may be due to stochastic sampling error (Margaritis et al., 2007). However, these results suggest the viral load of the three follow-up samples fell between 3.8 and 4.41IU/mL, the respective estimated LODs of MPX and AmpliScreen Tests. While these results and the LODs suggest the MPX Test may be slightly more sensitive than the HBV AmpliScreen Test, comparison of the LOD values must be undertaken with caution, as they were determined in separate studies with separate panels (COBAS AmpliScreen HBV Test package insert, 2009; cobas TaqScreen MPX Test package insert, 2009).

In addition to the sensitivity of the MPX Test which enables detection of window period infections of all three viruses and late stage occult HBV infections, the expanded test coverage to include HIV-1 Group O and HIV-2 will safeguard further the blood supply and provide information critical to donor management and counseling. These HIV variants, once rarely observed outside of Africa, currently represent an increasing proportion of new infections (Chen et al., 2002; Delwart et al., 2003; Dougan et al., 2005; Downie et al., 1992; Libonatti et al., 1998; Ramaligam et al., 2005; Schochteman et al., 2005; Soriano et al., 1996, 2000). While the FDA has encouraged coverage of all HIV-1 variants and mandated HIV-2 coverage for new serological tests, not all countries employ the expanded tests. Detection of these variants by older serological tests is inconsistent, allowing some infected donations to enter the blood supply (University of California San Francisco web site). Also other commercially available NATs used in blood screening do not cover HIV-2 (Margaritis et al., 2007; McCormick et al., 2006; Procleix Ultrio package insert, 2006).

HIV-1 Group O, once found mainly in Cameroon, represents only a very small fraction of HIV strains worldwide, but has been noted in several European countries and in the U.S. (Centers for Disease Control and Prevention, MMWR, 1996; Jonassen et al., 1997; Paraskevis and Hatzakis, 1999; Soriano et al., 1996; Sullivan et al., 1997; University of California, San Francisco web site). Its detection may not currently have a large impact on blood safety, but this could change in the future.

ThelessvirulentHIV-2variant,onceconfinedtoWestAfrica,has now been documented in five continents (Chen et al., 2002; Downie et al., 1992; Libonatti et al., 1998; O'Brien et al., 1992; Ramaligam et al., 2005; Soriano et al., 2000). It is currently present in over 1% of the general population of 19 West African countries and represents a significant infection in France and Portugal, in countries with past socio-economic ties to Portugal, as well as in southwest India, where it is one of two predominant HIV types (National Institute for PublicHealthSurveillanceAnnualReport,2004;Peetersetal.,1996; Ramaligametal.,2005;SchimvanderLoeffandAaby,1999;Soriano et al., 2000). A decade ago it was reported in the U.S. (Centers for Disease Control and Prevention – Division of HIV/AIDS web site).

The first 15–20 years of HIV-2 infection are characterized by low viral loads, requiring an ultrasensitive assay for its detection (Soriano et al., 2000). In this HIV-2 seropositive population study, the MPX assay detected all clinically relevant subtypes tested undiluted and more than twice as many HIV-2 seropositive specimens than the alternative NAT.

The excellent performance features of the MPX Test along with its expanded HIV subtype coverage should make the assay a significant contributor to the blood safety armamentarium.

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