Sensitivity and specificity of a new automated system for the detection of hepatitis B virus, hepatitis C virus, and human immunodeficiency virus nucleic acid in blood and plasma donations

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BACKGROUND: Use of nucleic acid testing (NAT) in donor infectious disease screening improves transfusion safety. Advances in NAT technology include improvements in assay sensitivity and system automation, and real-time viral target discrimination in multiplex assays. This article describes the sensitivity and specificity of cobas MPX, a multiplex assay for detection of human immunodeficiency virus (HIV)-1 Group M, HIV-2 and HIV-1 Group O RNA, HCV RNA, and HBV DNA, for use on the cobas 6800/8800 Systems.

STUDY DESIGN AND METHODS: The specificity of cobas MPX was evaluated in samples from donors of blood and source plasma in the United States. Analytic sensitivity was determined with reference standards. Infectious window periods (WPs) before NAT detectability were calculated for current donor screening assays.

RESULTS: The specificity of cobas MPX was 99.946% (99.883%-99.980%) in 11,203 blood donor samples tested individually (IDT), 100% (99.994%-100%) in 63,012 donor samples tested in pools of 6, and 99.994% (99.988%-99.998%) in 108,306 source plasma donations tested in pools of 96. Seven HCV NAT-yield donations and one seronegative occult HBV infection were detected. Ninety-five percent and 50% detection limits in plasma (IU/mL) were 25.7 and 3.8 for HIV-1M, 7.0 and 1.3 for HCV, and 1.4 and 0.3 for HBV. The HBV WP was 1 to 4 days shorter than other donor screening assays by IDT.

CONCLUSION: cobas MPX demonstrated high specificity in blood and source plasma donations tested individually and in pools. High sensitivity, in particular for HBV, shortens the WP and may enhance detection of occult HBV.

ABBREVIATIONS: CAS = COBAS AmpliScreen; IC = internal control; CE = Conformité Européene; IDT = individual donation testing; LoD(s) = limit(s) of detection; MP6 = minipool of up to 6 donations; MP96 = minipool of up to 96 donations; MPXv1 = cobas TaqScreen MPX Test; MPXv2 = cobas TaqScreen MPX Test, version 2.0; OBI(s) = occult HBV infection(s); RMS = Roche Molecular Systems, Inc.; RR = repeat reactive; WP(s) = window period(s).
Multiplex tests for the detection of nucleic acids from hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV) are in widespread use for blood and plasma donation screening. These tests supplement serologic testing by detecting early infection before seroconversion, as well as chronic occult HBV infections (OBIs) and have demonstrably improved the safety of the blood supply.\(^1\,^2\) Testing is typically accomplished through the use of automated instrumentation, and reagents and instruments are subject to continuous improvement.

Current technologies for this testing employ nucleic acid amplification, generally by polymerase chain reaction (PCR) or transcription-mediated amplification. A relatively recent advance consists of multidye real-time PCR, which identifies the amplified viral target (HIV, HBV, or HCV) during the primary multiplex screen, eliminating the need for a second round of discriminatory testing.

Assays used for testing blood donations require a high level of sensitivity to assure detection of infectious blood units but at the same time must also have high specificity to avoid wastage of safe donations and unnecessary donor deferral. The cobas MPX test for use on the cobas 6800/8800 Systems (Roche Molecular Systems, Inc. [RMS]) is a qualitative in vitro nucleic acid screening test (NAT) for the direct detection of HIV Type 1 (HIV-1) Group M RNA, HIV-1 Group O RNA, HIV Type 2 (HIV-2) RNA, HCV RNA, and HBV DNA in human plasma and serum. Results are simultaneously detected and discriminated for HIV, HCV, and HBV. This article describes the results of preclinical and clinical performance studies evaluating the sensitivity and specificity of this new donor screening test.

**MATERIALS AND METHODS**

**Assays and systems**

The cobas MPX test is a qualitative multiplex PCR test intended for use on the cobas 6800/8800 Systems. These automated systems incorporate ready-to-use assay reagents that can be stored in an on-board refrigerator, other universal reagents shared across all tests, and automated process control with traceability of all consumables and reagents. Individual donor samples can be loaded directly onto the system or may be pooled before testing using an optional pooling instrument. Pooling for the studies described here was performed using the cobas p680 instrument (Roche Diagnostics GmbH).

The cobas MPX master mix contains virus-specific forward and reverse primers and detection probes for HIV-1 (Groups M and O), HIV-2, HCV, HBV, and internal control (IC) nucleic acid. The assay contains primers and probes for two distinct target regions of HIV-1M and dual probes for HCV. The HIV, HCV, HBV, and IC detection probes are each labeled with one of four unique fluorescent dyes, which acts as a reporter. The four reporter dyes are measured at defined wavelengths, thus permitting simultaneous detection and discrimination of amplified HIV, HCV, and HBV targets and the IC. Results of the cobas MPX screen are reported as reactive (R) or nonreactive (NR) for each of three viruses: HIV, HCV, and HBV.

The cobas TaqScreen MPX Test for use with the cobas s 201 system (MPXv1, RMS) was the donor screening NAT in use by the sites participating in the clinical specificity studies and was used as the comparator for these studies. Where indicated, pooling for MPXv1 was performed using a Microlab STAR (Hamilton). MPXv1 simultaneously screens for HIV, HCV, and HBV but does not differentiate among the analytes. Donations reactive on MPXv1 at the individual sample level were further evaluated by use of individual COBAS AmpliScreen (CAS) tests for HIV-1, HCV, and HBV (RMS) using the standard specimen processing procedure.

**Clinical specificity studies**

**Blood donor specificity**

Blood samples from volunteer whole blood and apheresis donors in the continental United States, aged 18 or older, were tested at four different test sites (Bloodworks Northwest, Creative Testing Solutions, Indiana Blood Center, Mississippi Valley Regional Blood Center). Each donation was tested by both cobas MPX and MPXv1. Donations were tested either by individual donation testing (IDT) or in pools by both tests. Pools were composed of up to six donor samples. Reactive pools were resolved by testing the individual members of the pools. Results for a donation were considered evaluable if a valid result for both cobas MPX and MPXv1 was obtained by the same testing format (i.e., either IDT or pools).

For donations screened on cobas MPX by IDT, approximately 50% of donations were screened using a serum sample type and 50% using EDTA plasma. EDTA plasma samples were used for all screening in pools and for all MPXv1 and CAS testing.

Donations with discordant results between cobas MPX and the combined MPXv1/CAS were further tested by an alternative NAT performed on a sample from the index plasma unit (UltraQual HIV, HCV, and HBV assays, National Genetics Institute [NGI]), and, if reactive, further tested by a quantitative viral load assay (SuperQuant PCR [National Genetics Institute]). Participating sites provided the results of their routine serologic testing for anti-HIV-1/2, anti-HCV, antibody to hepatitis B core antigen (anti-HBc), and hepatitis B surface antigen (HBsAg).

**Source plasma donor specificity**

Sodium citrate plasma samples from donations of source plasma for further manufacture were tested at a single test
site (CSL Plasma Laboratory). The donations were negative for HBsAg and for antibodies to HCV and HIV by routine serologic testing. Each donation was tested by both cobas MPX and MPXv1 in pools of up to 96 donor samples. (Note: Larger pool sizes are used for source plasma donation screening based on risk assessment taking into account virus-reduction steps in manufacturing.) Reactive pools were resolved by testing intermediate pools of 12 samples and, if reactive, by testing individual samples. Samples individually reactive on MPXv1 were further tested by CAS, and donations with discordant results between cobas MPX and MPXv1/CAS were further evaluated as described. Donations reactive for HBV DNA were also tested for anti-HBc.

Follow-up studies
For both the blood donor and the plasma donor studies, donors who were seronegative for any NAT-reactive analyte were invited to participate in a follow-up study. Donors were followed until seroconversion for up to a maximum of 8 weeks (and up to a maximum of four follow-up visits). The study site performed the following tests on blood specimens obtained at each follow-up visit: cobas MPX and MPXv1 (IDT), anti-HIV-1/2, anti-HCV, anti-HBc, and HBsAg. Plasma remaining from each follow-up specimen was frozen and provided to RMS for possible additional qualitative or quantitative NAT.

Specificity analysis
Donations that were reactive by cobas MPX or MPXv1/CAS were considered confirmed positive if any of the following was true:

- cobas MPX and MPXv1/CAS results were reactive for the same analyte(s);
- Index serology was positive for the NAT-reactive analyte;
- Index plasma was positive for the analyte by alternative NAT;
- Donor was seropositive for the analyte on follow-up testing;
- Donor was NAT positive for the analyte on follow-up.

Positive serology that would confirm a reactive NAT was defined as HIV—anti-HIV-1/2 repeat-reactive (RR) and positive by immunofluorescence assay or Western blot; HCV—anti-HCV RR and reactive on a second licensed screening test; HBV—anti-HBc RR or HBsAg RR and confirmed by neutralization. Specificity was calculated as the frequency of cobas MPX nonreactive results among status-negative (uninfected) donations, defined as total donations minus confirmed positive donations.

Pool construction or deconstruction and target identification studies
The ability to screen samples in minipools of up to 6 donations (MP6) or 96 donations (MP96) and correctly identify viral targets in reactive samples was evaluated in studies using two pooling systems, the cobas p 680 and cobas Synergy, which is composed of the Hamilton STAR pooling instrument and cobas Synergy Software (STRATEC Biomedical UK Ltd). For each pooling system, 60 samples containing a single viral target and 18 samples containing two viral targets were distributed among 882 negative samples and tested by cobas MPX in MP6 and/or MP96. The details of the studies are described in the Appendix.

Limits of detection
The limits of detection (LoDs) of cobas MPX were determined using reference standards and methods described in the Appendix.

Estimated window periods
Infectious window periods (WPs) were defined as the time period required for viral concentration to increase from the minimum infectious dose in 20 mL of plasma to the viral concentration representing 50% detection by the donor screening assay, assuming a constant virus doubling time. WPs were calculated for four donor screening NAT assays in current use: cobas MPX, cobas TaqScreen MPX Test, v2.0 (RMS), Procleix Ulitro Plus, and Procleix Ulitro Elite (Grifols) and for pool sizes for each assay that are in common use. Assumptions for the calculations were: 1) minimum infectious dose of one copy in 20 mL of plasma (0.05 copies/mL) for HIV and HCV and 3.16 copies in 20 mL (0.158 copies/mL) for HBV; 2) doubling times of 0.85, 0.45, and 2.56 days and conversion factors of 0.6, 2.7, and 5.26 copies/IU for HIV, HCV, and HBV, respectively. Fifty percent LoDs were determined from package inserts (Ulterior assays) or internal files (RMS assays). The LoDs from the Conformité Européenne (CE) versions of package inserts were used because some claims differ between US and CE versions, and at time of writing the Ulitro Elite assay was only available in the CE market.

RESULTS
Specificity studies
Blood donor specificity
Figure 1 summarizes the results of donor screening in IDT. There were 11,203 evaluable donations. Of these, five donations were concordant reactive on both NAT assays and had positive serology (three HCV NAT with anti-HCV and two HBV NAT with HBsAg). There were three donations with apparent false-reactive results on MPXv1. All three were nonreactive on CAS. Two of these donors completed follow-up and one made one follow-up visit, all
with negative results. There were six donations with apparent false-reactive results on cobas MPX (five HIV and one HBV). The five HIV-reactive donors completed follow-up with negative results, but the HBV-reactive donor did not participate in follow-up. Of the six apparent false-reactive cobas MPX donations, four were screened in plasma and two in serum. The specificity of cobas MPX in IDT is shown in Table 1. The specificity in serum versus plasma did not differ significantly (p = 0.12).

A total of 63,012 evaluable donations were screened in pools of up to 6 donations (MP6; Fig. 2). Twenty-seven donations were concordant reactive on both NAT assays and had positive serology (24 HCV NAT with anti-HCV and three HBV NAT with HBsAg). Two donations were concordant reactive for HCV on both NAT assays but were nonreactive for anti-HCV and thus represent “NAT-yield” donations. One of the NAT-yield donors was anti-HCV positive on the first follow-up visit (Day 17). The other NAT-yield donor remained consistently HCV reactive on both NAT assays but seronegative through the last day of follow-up (Day 50). There were two donations with apparent false-reactive results on MPx1. One was CAS positive for HCV; this donor completed follow-up with nonreactive results. The other donor was CAS negative on the index donation and did not participate in follow-up. There were no donations with false-reactive results on cobas MPX. The specificity of cobas MPX in MP6 was 100% (Table 2).

Table S1 in the Appendix (available as supporting information in the online version of this paper) shows the distribution of positive donations by test site. There was a
significant difference in the distribution of HCV NAT positive, seropositive donations among the sites; 20 of 27 of these were at CTS, located in the southeastern United States. In this study, 10,534 MP6 did not contain true positive donations. Of these, 10,524 were nonreactive on cobas MPX, and 10 were reactive but did not resolve to a confirmed positive donation. Thus the frequency of pool reactivity in uninfected donations screened in MP6 was 0.1%.

Source plasma specificity
A total of 108,306 seronegative evaluable source plasma donations from 24,514 unique donors were screened in pools of up to 96 donations (Fig. 3). Five donations (from four donors) were concordant reactive for HCV on both NAT assays (HCV NAT-yield); three of these donors enrolled in follow-up. All three donors remained seronegative on last follow-up (Day 44). One donation was HBV reactive by cobas MPX and nonreactive by MPXv1 when screened in MP96. This donation was reactive for HBV by alternative qualitative NAT with a 95% LoD of 0.9 IU/mL but nonreactive by an HBV quantitative assay with a lower limit of quantification of 29 IU/mL.

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**TABLE 2. Specificity of cobas MPX for donations screened in MP6 and MP96**

| Pool size                  | Target | Number of status negative donations | Number reactive for target | Number nonreactive for target | Specificity (%) | 95% CI (%)      |
|----------------------------|--------|-------------------------------------|-----------------------------|--------------------------------|----------------|-----------------|
| Pools of 6 (blood donations) | HIV    | 62,982                              | 0                           | 62,982                        | 100.00         | 99.994-100.00  |
|                            | HCV    | 62,982                              | 0                           | 62,982                        | 100.00         | 99.994-100.00  |
|                            | HBV    | 62,982                              | 0                           | 62,982                        | 100.00         | 99.994-100.00  |
|                            | Any target | 62,982                            | 0                           | 62,982                        | 100.00         | 99.994-100.00  |
| Pools of 96 (source plasma donations) | HIV    | 108,297                             | 3                           | 108,294                       | 99.997         | 99.992-99.999  |
|                            | HCV    | 108,297                             | 1                           | 108,296                       | 99.999         | 99.995-100.00  |
|                            | HBV    | 108,297                             | 2                           | 108,295                       | 99.998         | 99.993-100.00  |
|                            | Any target | 108,297                          | 6                           | 108,291                       | 99.994         | 99.988-99.998  |

* Clopper-Pearson exact CI.
This donor gave three subsequent donations that were NAT nonreactive in MP96 before enrollment in follow-up. On follow-up specimens obtained on Days 14 through 43 after the reactive index donation, the donor was reactive for HBV on cobas MPX and was MPXv1 reactive but CAS negative. On Follow-up Days 57 and 89, the donor was reactive for HBV by both cobas MPX and MPXv1 plus CAS. The donor remained nonreactive for HBsAg and anti-HBc throughout follow-up. Anti-HBs testing performed on retained samples from the index donation and a donation 9 days after the index donation showed nonreactive results.

There were six donations with apparent false-reactive results on cobas MPX (three HIV, one HCV, two HBV). Three of these donors participated in follow-up (two HIV and one HCV) with negative results through Days 44, 53, and 59, respectively; three donors did not participate in follow-up. The specificity of cobas MPX in MP96 is shown in Table 2.

In this study, 1097 MP96 samples did not contain true-positive donations. Of these, 1090 were nonreactive on cobas MPX, and seven were reactive but did not resolve to a confirmed positive donation. Thus the frequency of pool reactivity in uninfected source plasma donations screened in MP96 was 0.6%.

**Poooling studies**

The results of the final determination of sample reactivity, after pool deconstruction, are shown in Tables S2 and S3 in the Appendix (available as supporting information in the online version of this paper). Results were available for 1919 samples; one sample had insufficient volume for final testing. All viral targets in the 156 spiked samples were correctly identified and resolved to the correct individual sample. One of 1763 negative samples had a reactive result for HIV.

**LoDs**

The 95 and 50% LoDs for each target when tested by cobas MPX with plasma are shown in Table 3.

**Estimated WPs**

The estimated infectious WPs for HIV, HCV, and HBV for the four donor screening assays in current use and the testing formats commonly used for each assay are shown in Table 4. These WPs represent the time required for viral concentration to increase from the concentration representing the minimum infectious dose of virus in 20 mL of plasma to the concentration representing 50% detection by the donor screening assay.


**TABLE 3. 95% and 50% LoDs for cobas MPX in EDTA plasma by probit analysis, with 95% CIs**

| Target          | Measuring unit | Measuring unit | 95% detection | 50% detection |
|-----------------|----------------|----------------|---------------|---------------|
|                 |                | LoD            | CI            | LoD           | CI            |
| HIV-1 Group M   | IU/mL          | 25.7           | 21.1-32.8     | 3.8           | 3.4-4.3       |
| HIV-1 Group O   | copies/mL      | 8.2            | 7.0-10.0      | 1.7           | 1.5-1.9       |
| HIV-2           | IU/mL          | 4.0            | 3.3-5.2       | 0.9           | 0.8-1.1       |
| HCV             | IU/mL          | 7.0            | 5.9-8.6       | 1.3           | 1.1-1.4       |
| HBV             | IU/mL          | 1.4            | 1.2-1.7       | 0.3           | 0.3-0.3       |

**TABLE 4. Estimated infectious WPs for HIV-1M, HCV, and HBV for four donor screening assays in current use, calculated for the minipool sizes in common use for each assay**

| Target | Assay       | 50% LoD IU/mL | 50% LoD copies/mL | IDT | MP4 | MP6 | MP16 | MP24 |
|--------|-------------|---------------|-------------------|-----|-----|-----|------|------|
| HIV    | cobas MPX   | 3.8           | 2.3               | 4.7 | 6.9 |     |      |      |
|        | MPXv2       | 9.4           | 5.6               | 5.8 | 8.0 |     |      |      |
|        | Ultrio Plus | 6.3           | 3.8               | 5.3 | 7.0 |     |      |      |
|        | Ultrio Elite| 5.4           | 3.2               | 5.1 | 6.8 |     |      |      |
| HCV    | cobas MPX   | 1.3           | 3.5               | 2.8 | 3.9 |     |      |      |
|        | MPXv2       | 1.3           | 3.5               | 2.8 | 3.9 |     |      |      |
|        | Ultrio Plus | 0.8           | 2.2               | 2.5 | 3.4 |     |      |      |
|        | Ultrio Elite| 0.9           | 2.4               | 2.5 | 3.4 |     |      |      |
| HBV    | cobas MPX   | 0.3           | 1.6               | 8.6 | 15.2|     |      |      |
|        | MPXv2       | 0.46          | 2.4               | 10.0| 16.7|     |      |      |
|        | Ultrio Plus | 0.6           | 3.2               | 11.1| 16.2|     |      |      |
|        | Ultrio Elite| 0.9           | 4.7               | 12.5| 17.7|     |      |      |

* 50% LoD from package inserts (Ultrio assays)8,9 or internal files (RMS assays) for Conformité Européene (CE) mark assays.
† Assumptions for the calculations were: 1) minimum infectious dose of 1 copy/20 mL plasma (0.05 copies/mL) for HIV and HCV and 3.16 copies/20 mL (0.158 copies/mL) for HBV; and 2) doubling times of 0.85, 0.45, and 2.56 days and conversion factors of 0.6, 2.7, and 5.26 copies/IU for HIV, HCV, and HBV, respectively.3-7
‡ WPs were calculated for the minipool sizes in common use for each assay.

**DISCUSSION**

This article reports the sensitivity and specificity of cobas MPX for use on the cobas 6800/8800 Systems, a new donor NAT screen for HIV, HCV, and HBV. The specificity of cobas MPX was 99.946% (95% exact confidence interval [CI], 99.883%-99.980%) in samples from donors of blood and blood components tested by IDT and 100% (95% exact CI, 99.994%-100.000%) in samples from these donors tested in MP6. Specificity was similarly high for source plasma donations tested in MP96, 99.994% (95% exact CI, 99.988%-99.998%). The clinical specificity and pool-reactive rates described here for cobas MPX are similar to those reported for other blood screening assays in current use.8,9,11 Because cobas MPX reports results on a target-specific basis, assay specificity can also be calculated for each target. Altogether among the 182,521 donations included in the studies reported here, there were 12 donations with unconfirmed reactive results on cobas MPX including eight reactive for HIV, one for HCV, and three for HBV. Specificity for the HIV target was 99.955% in IDT, 100% in MP6, and 99.997% in MP96. Specificity for each target is reported in Tables 1 and 2.

Clinical specificity is higher when donations are screened in minipools rather than by IDT. This is because screening in MP format requires detection of reactivity at both the pool and the individual sample level to assign reactivity to a donation. Many blood banks outside the United States that screen donations in the IDT format perform repeat testing on initially reactive donations, to improve specificity.12,13 In the United States, donors must be managed on the basis of the initial result at the individual sample level, although donor reinstatement is possible.14,15 The unconfirmed reactive rate in IDT for cobas MPX reported here (0.054%) is similar to other donor screening NAT assays. For example, the false-positive rate of the Ultrio Elite in IDT is reported as 0.1%.9 For all NAT assays, a reactive result that does not confirm can be explained by a low level of viral nucleic acid in the sample (below the 95% detection limit of the assay) or by nonspecific amplification within the assay (i.e., amplification that is not related to viral nucleic acid in the sample). A low level of viral nucleic acid in the sample, in turn, can be caused by preanalytic sample contamination or by a true infection in the donor with a low viral load. The specificity studies described here were performed in a donor...
population that has a low prevalence of HIV, HCV, and HBV infections. In this setting, unconfirmed reactivity is likely to reflect nonspecific amplification. In other settings where donor prevalence of infection is higher, both preanalytic sample contamination and infected donors with low viral loads are more common. When using donor screening NAT assays that do not initially discriminate the viral target, such as the Ultrio assays, it is not possible for the user to determine which set of primers and probes triggered a reactive signal. An initial reactive result on an Ultrio assay potentially indicates amplification of any of the three viral targets. For donations that are reactive on an Ultrio screen but negative by the blood bank’s serologic screening, blood banks have historically gauged the likelihood of infection with each target on the basis of the incidence of WP infections in their donor population and the presence or absence of evidence of other markers of infection, such as anti-HBc. For example, Charlewood and Flanagan 16 studied 499 donations in New Zealand that were initially reactive in Ultrio or Ultrio Plus assays, seronegative by routine screening, and nonreactive on discriminatory testing, representing 0.09% of all donations. Anti-HBc was found in 13% of the Ultrio and 57% of the Ultrio Plus nondiscriminated donations, suggesting that in these cases the initial reactive NAT could have represented OBI. Conversely, the remaining 87 and 43% of the Ultrio- and Ultrio Plus–reactive nondiscriminated donations, respectively, lacked anti-HBc and thus were unlikely to reflect OBI, leaving the Ultrio signal unexplained. Even in the presence of anti-HBc the cause of the reactive Ultrio signal is only an assumption, and in all cases a blood bank cannot exclude the possibility that an initially reactive Ultrio signal reflects amplification of HIV or HCV. Because the cobas MPX test reports reactivity on a target-specific basis, the blood bank is able to focus its additional testing of initially reactive donations and its assessment of donor risk factors on the specific viral target that is reported as reactive. Replicate testing is commonly used to differentiate between true- and false-positive initial-reactive NAT results, 12,13 although samples with very low viral loads may not react in all replicates. The relative likelihood that an initial reactive target-specific result in a seronegative donation represents a true infection in the donor versus nonspecific amplification varies with the prevalence and incidence of that infection in the donor population. Follow-up testing of donors can provide reassurance that an initially reactive result does not represent true infection. 14,15

The poorer studies demonstrated the accurate tracing of reactive samples when screening was conducted in minipools. The studies also demonstrated that cobas MPX accurately identified and reported the specific virus, HIV, HCV, or HBV, when these viruses were present in samples individually or in combination.

NAT-yield (NAT-reactive, seronegative) donations are infrequent among donors of blood and blood components in the United States. 2,17 This reflects the low incidence of new HIV, HCV, and HBV infections in US blood donors and the routine screening for anti-HBC, which interdicts the majority of donations at risk for OBI. In the blood donor specificity study reported here involving a total of 74,215 blood donations, there were two NAT-yield donations (1/37,108 donations), both HCV. These donations were reactive on both the MPXv1 and the cobas MPX NAT screens. Although one donor had seroconverted to anti-HCV by the first follow-up visit, the other remained seronegative through Day 50 of follow-up. Among the 108,306 source plasma donations, the most common NAT-yield virus was also HCV, found in five donations from four donors (1/21,661 donations). One of these donors also remained seronegative through Day 44 of follow-up. These delays to seroconversion are consistent with prior reports of a median of 35 and 42.5 days from NAT detection to seroconversion by third-generation anti-HCV assays. 18,19 These cases highlight the relatively long time to seroconversion for HCV 20 and the value of NAT screening to detect HCV infection in donors before seroconversion.

In these studies, HCV was the most commonly detected virus, accounting for 27 of 32 seropositive and NAT-positive donations and seven of eight NAT-yield donations. The predominant detection of HCV was also observed in a pilot surveillance study of approximately 14.8 million US donations. 21 The surveillance study also observed a higher prevalence of HCV in the southeastern United States as was noted here. Donor demographic data are not available for the participants in the studies reported here so the potential drivers of the differences between sites cannot be assessed.

In contrast to the predominance of HCV detection in the United States, the predominant virus detected by donor screening NAT globally is HBV, including both OBI and WP infections. 1,22 Thus detection of HBV is a critical function of donor screening NAT. The source plasma study detected an interesting HBV-reactive donor with a low level of HBV DNA. The absence of anti-HBs in the index donation and lack of both HBsAg and anti-HBc in follow-up suggest that this NAT-yield donor represents a case of primary OBI. 23,24 The HBV DNA concentration in the index donation was below 29 IU/mL, and it may seem surprising that this was detected in a MP96. The LoD for samples screened in a pool can be estimated by multiplying the LoD of the assay times the dilution factor of the pool size. The 95% LoD for HBV in a pool of 96 tested by cobas MPX could therefore be estimated as 1.4 IU/mL \times 96, or 134 IU/mL. This case illustrates the fact that NAT screening assays may detect donations with viral nucleic acid concentrations well below the 95% LoD of the assay, albeit at a likelihood of less than 95%. The cobas MPX assay is extremely sensitive for HBV; with its 50%
LoD of 0.3 IU/mL, the estimated 50% detection level for a donation tested in a MP96 would be 29 IU/mL, with a lower probability of detecting donations containing lower concentrations of viral nucleic acid.

Window periods (i.e., the period of time in which a donor with a new infection might be infectious but not detected by the donor screening test) were estimated for the current donor screening NAT assays in various pool sizes. As noted previously, the short doubling times for HIV and HCV result in little difference in the duration of WP between IDT and MP screening for these agents. In donor populations with a low incidence of new infections, the likelihood is remote that a donor with a new HIV or HCV infection would donate precisely during the 1- to 4-day period when their infection might be detected by IDT and not by MP. In the case of HBV, the longer virus doubling time results in a longer period when there may be differential detection by IDT versus MP and a greater potential impact of pool size during the WP. Screening in small pool sizes with cobas MPX, which is highly sensitive for HBV, could present a balanced approach that combines a high level of HBV WP coverage with the advantage of the higher specificity afforded by testing in the MP format.

In populations where HBV is prevalent, chronic infection not detected by HBsAg testing (OBI) poses an additional risk to the blood supply. In this setting, HBV NAT sensitivity is particularly important because such infections can be associated with low levels of circulating HBV DNA (<10 IU/mL). A highly sensitive HBV NAT can produce a high detection rate of OBI, even in small minipools. With cobas MPX one would predict a 50% likelihood of detecting a donation with a viral load of 1.8 IU/mL in a MP6 or 0.3 IU/mL by IDT. In some HBV low-prevalence countries, donor screening with anti-HBc is used to interdict donors at risk for OBI. In higher-prevalence settings, exclusion of donations containing anti-HBc would not be feasible. In these settings, the choice between IDT NAT and MP NAT for detection of OBI involves consideration of OBI prevalence, balancing of NAT sensitivity versus specificity, throughput, and cost.

Like the MPXv1 and cobas TaqScreen MPX Test, version 2.0 (MPXv2) assays, the primers and probes of the cobas MPX assay are designed to include detection of the HIV-1 Group O and HIV-2 viruses. Although these strains of HIV are found most commonly in Africa, HIV-1 Group O infections have been documented in Europe and North America and HIV-2 infections in Europe, North America, South America, and Asia. In a recent investigation of a blood donation in the United States that was HIV antibody positive but nonreactive on Ultrio Plus, the presence of HIV-2 was confirmed by sequencing. The donation was demonstrated to be detectable by cobas MPX in 10 of 10 replicates. The donor antibody screening tests for HIV that are in current use are not well characterized as to the time from infection to detection (WP) for HIV-1 Group O or HIV-2 infections, and HIV-1 p24 antigen assays may not detect HIV-2 infections. Thus, NAT is of particular importance for the detection of donors recently exposed to these HIV strains.

In summary, the cobas MPX assay demonstrated excellent specificity and accurate real-time viral target discrimination. The assay is suitable for use to screen donors in either IDT or MP testing format and is performed on a new highly automated system that offers improvements in laboratory efficiency. The sensitivity of cobas MPX for HBV is expected to be of particular value globally for detection of both WP and OBI infection.

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CONFLICT OF INTEREST
SAG, RB, JRD, KM, JV, NH, and LLP are employees of Roche Molecular Systems, Inc. TLS, PCW, JPA, DAW, and YE were site principal investigators for the specificity studies.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article.

Table S1. NAT positive blood donations by site.
Table S2. Results of testing of 10 pools of 96 using the cobas p 680 pooling instrument.
Table S3. Combined results of testing of pools of 6 and pools of 96 using cobas Synergy.