First Zika-positive donations in the continental United States

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BACKGROUND: Zika virus (ZIKV) has spread in the Americas, including parts of the southern United States, and infection can be associated with serious complications, including congenital brain abnormalities. Probable transfusion transmission of ZIKV has been documented in Brazil.

STUDY DESIGN AND METHODS: Preemptive testing of blood donations for ZIKV RNA was implemented in southern US states at risk of local transmission using a test approved under a Food and Drug Administration (FDA) investigational new drug application, cobas Zika. Screening was expanded after issuance of an updated FDA guidance. Donations reactive on initial screening were further tested by nucleic acid and antibody tests to determine the donor status.

RESULTS: Of 358,786 donations from US states screened by individual donation testing, 23 were initially reactive on cobas Zika. Fourteen of these represented probable ZIKV infection based on reactivity on additional nucleic acid testing or anti-Zika immunoglobulin M. Ten of the 14 donors reported travel to an identified ZIKV-active area within 90 days before donation (median time from end of travel to donation, 25 days; range, 6-71 days). Three donors with travel history also had a potential sexual exposure. Only seven of the 14 donations with probable ZIKV infection were detectable upon 1:6 dilution to simulate minipool testing. The estimated specificity of the cobas Zika test was 99.997%.

CONCLUSION: Screening of donations for ZIKV RNA can interdict ZIKV-infected donors. Donor risk factors include travel more than 4 weeks before donation and sexual exposure. Minipool screening would have detected only 50% of the RNA-positive donations.

ABBREVIATIONS: DOH = Department of Health; IND = investigational new drug; RMS = Roche Molecular Systems, Inc.; ZIKV = Zika virus.

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Data presented here about the performance of the cobas Zika test are preliminary and have not been reviewed by FDA.

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Zika virus (ZIKV) is an enveloped single-stranded RNA arbovirus of the Flaviviridae family. Infection is primarily transmitted to humans via Aedes species mosquitoes, but intrauterine, sexual, laboratory-acquired, and probable transfusion-associated transmission have also been reported.

ZIKV infection is asymptomatic in an estimated 80% of infections in adults and, when symptomatic, usually presents as a mild self-limited illness. Intrauterine infection, however, can cause microcephaly and other serious brain and nervous system abnormalities in the fetus. ZIKV infection has also been linked to Guillain-Barré and other neurologic syndromes. Due to the potential severe consequences of infection, ZIKV was deemed to be a significant public health threat and became a nationally notifiable condition in the United States in 2016.

ZIKV appeared in Brazil in 2013 or 2014 and became epidemic there in 2015. By early 2016, locally acquired ZIKV cases had been reported in Mexico and more than 30 countries in South America, Central America, and the Caribbean. The first locally acquired ZIKV cases in the United States were reported in Puerto Rico, an US territory, in December 2015. Travel-related ZIKV cases began to appear in several US states by January 2016, followed by an increasing number of sexually transmitted cases involving returning travelers.

During a 2013 to 2014 French Polynesia ZIKV outbreak, approximately 3% of donations from asymptomatic blood donors were reactive when tested with an in-house ZIKV polymerase chain reaction (PCR) assay. Cases of probable transfusion-transmitted infection have been documented in Brazil. Concern about the risk of ZIKV transmission via transfusion prompted the US Food and Drug Administration (FDA) to issue a guidance on February 16, 2016, recommending that blood components collected in ZIKV-active areas of the United States or its territories could be used only if 1) the donations were screened with a ZIKV nucleic acid test (NAT)—no ZIKV NAT was then available—or 2) the components were treated with an FDA-approved pathogen-reduction technology, which was available for use with platelets or plasma but not red blood cells (RBCs). The impact of the FDA’s February 16th guidance was to halt local whole blood collection in Puerto Rico on March 7, 2016. This necessitated importation of blood products from the US states to Puerto Rico. For areas without local transmission, the guidance recommended deferral of potential donors, if in the prior 4 weeks they had traveled to or resided in ZIKV-active areas or had sexual contact with a man who had been diagnosed with ZIKV or who had been in ZIKV-active areas.

On March 30, 2016, the FDA approved the use of an investigational donor screening test for ZIKV RNA, cobas Zika (Roche Molecular Systems, Inc. [RMS]) under an investigational new drug (IND) application. On April 2, 2016, whole blood donations resumed in Puerto Rico with testing under this IND. The prevalence of Puerto Rico donations reactive for ZIKV RNA was approximately 0.3% initially, increasing to approximately 1.8% by July 2016. From May through August 2016, testing of donations under IND was initiated by some blood banks in southern US states, where Aedes aegypti mosquitoes are present. In August 2016, after detection of some ZIKV RNA–positive donations in US states, the FDA issued a revised guidance requiring initiation of testing of all US blood donations for ZIKV RNA within a specified time frame. With initiation of testing under the August 2016 guidance, donor questioning for ZIKV risk could be discontinued. This report describes the first cases of ZIKV-positive donations in US states.

MATERIALS AND METHODS

Donor screening
Donor screening for ZIKV RNA was conducted under an IND application for the cobas Zika test for use with the cobas 6800/8800 systems (cobas Zika) and with approval of an institutional review board. Donors from participating blood centers were provided with information about the investigational assay and informed consent was obtained. EDTA plasma from a blood sample obtained at the time of donation was tested with cobas Zika at a participating testing laboratory. Each donation was tested using an individual, unpoled, plasma sample.

If a donation was reactive on cobas Zika, the testing laboratory performed two repeat tests with cobas Zika using a plasma sample from a donor tube or plasma bag and also tested a donor plasma sample in a simulated pool of 6 by diluting the sample 1:6 with ZIKV-negative human plasma provided by RMS. In addition, samples from all donations reactive on cobas Zika were shipped to Blood Systems Research Institute for alternate NAT and serology testing.

Alternate NAT and serology testing
Detailed methods for the Zika alternate NAT and serology are provided in Appendix S1 (available as supporting information in the online version of this paper). Zika alternate NAT was performed on plasma samples from index donations and on residual RBC samples, when available. Zika alternate NAT results were interpreted as equivocal or positive if one or both, respectively, of two replicates crossed the fluorescence threshold within 40 cycles. “Viral load” was estimated by comparing sample cycle threshold to a standard curve. The limit of detection of this alternate NAT is approximately 40-fold higher (i.e., less sensitive) than the cobas Zika test by PROBIT analysis of testing performed with each test on the same material (data on file at RMS). Zika serology was performed using Zika MAC-ELISA (Centers for Disease Control and Prevention...
[CDC).19 for anti-Zika immunoglobulin (IgM) detection and by the same assay modified with an anti-IgG capture antibody for anti-flavivirus IgG detection.

Follow-up study
Each donor with an initially reactive cobas Zika result was invited to enroll in a follow-up study that involved retesting with cobas Zika and Zika serology at two additional time points: within 2 weeks and between 2 and 8 weeks after the initial cobas Zika-reactive donation.

Definition of probable ZIKV infection
For the purpose of this article, donors reactive on the initial screen by cobas Zika were considered to represent probable ZIKV infection if they were reactive on at least one replicate of repeat testing by cobas Zika on the index donation or a follow-up sample, reactive on the alternate NAT on plasma from the index donation or positive for anti-Zika IgM on the index donation or a follow-up sample.

Assessment of donor risk factors
Donors with reactive results on cobas Zika were contacted by the blood centers and/or the state health departments and assessed for potential exposure to, or risk factors for, ZIKV infection. In Florida, notification to the state Department of Health (DOH) by blood centers or testing facilities generally occurred within 24 to 48 hours of the initial reactive test result. Blood centers immediately provided the state DOH officials donor contact information. Testing facilities forwarded a portion of the reactive sample to the state public health laboratory for public health confirmatory testing. County health department epidemiologists were provided donor information and instituted standard protocols for ZIKV infection case finding. This included interviewing the donor to identify the donor’s potential exposure location and potential route of transmission and identifying locations where an infected donor could have introduced ZIKV to mosquito vectors. The donor was also requested to provide additional samples including serum, urine, and whole blood. Local mosquito control districts were promptly notified of any locations mosquito abatement activities should occur. Standard risk factor information collected by the Florida DOH included travel history for the 6 months before blood donation and contact with potentially infected sexual partners during the same period of time. A travel destination was considered to have active ZIKV transmission according to the CDC designation. This included travel to three small areas of Miami-Dade County, Florida, during times these areas were temporarily determined by CDC and state health officials to have active ZIKV transmission. A donor was designated as having risk for sexual transmission of ZIKV if, in the 6 months before donation, the donor reported sexual contact with a male or female partner who had traveled to a ZIKV-active area within the 6 months before the contact. When possible, samples for ZIKV testing were obtained by the DOH from sexual contacts. Donors were also asked whether they had experienced any of the four common symptoms of ZIKV infection (rash, fever, conjunctivitis, and arthralgia) in the 2 weeks before or after donation, as well as during and up to 2 weeks after reported travel to a ZIKV-active area.

RESULTS
From May 23 through October 9, 2016, a total of 358,786 donations from US states were screened by cobas Zika (Fig. 1). The majority of donations were from the southern United States. Blood centers from additional states joined after the FDA issued its August 2016 guidance. Twenty-three donations were reactive on the initial screen. Nine of these donations were nonreactive on all additional testing performed on the index donation. Of these, four donors completed follow-up and were negative for Zika RNA, anti-Zika IgM, and anti-flavivirus IgG on two follow-up visits. Five donors did not complete follow-up. Of the nine donations that were nonreactive on all of the additional testing performed on the index donation as part of the IND protocol, three also had residual RBCs tested by alternate NAT, and all three were negative.

Fourteen donations met criteria for probable ZIKV infection based on reactivity on additional NAT on the index donation or follow-up or positive anti-Zika IgM on index or follow-up (Fig. 1 and Table 1). Nine of these donations were repeat reactive on cobas Zika; of these, seven donations were positive on the alternate NAT performed on plasma, with estimated viral loads ranging from \(1 \times 10^3\) to \(8 \times 10^6\) copies/mL. Of the nine repeat-reactive donations, four were positive for anti-Zika IgM.

Five of the initially reactive donations were nonreactive on repeat cobas Zika and alternate NAT performed on plasma, but were positive for anti-Zika IgM (Table 1, Donors 9, 11, 14, 18, and 19). Residual RBCs were available for testing by alternate NAT from the index donation from four of these donors and from a follow-up sample from the remaining donor (Donor 18). The alternative NAT was positive on all of these RBC samples, with estimated viral loads in the RBC ranging from \(1.7 \times 10^3\) to \(6.5 \times 10^4\) copies/mL (Table 1). Simulated minipool testing (testing at a 1:6 dilution) was reactive only on the seven donations that were positive by alternate NAT on plasma.

The specificity of the cobas Zika test was calculated as the percentage of donations without ZIKV infection that were nonreactive on cobas Zika. For this calculation, donations were assumed to be without ZIKV infection if they were nonreactive on the initial screen with cobas Zika or were initially reactive on cobas Zika but did not satisfy the criteria for probable ZIKV infection. The
estimated specificity of the cobas Zika test is 358,763/358,772 or 99.997% (95% exact confidence interval [CI], 99.995%-99.999%).

Risk factors for ZIKV for the 14 donors with probable ZIKV infection are summarized in Table 2. Donors ranged in age from 17 to 70 years and included nine males and five females. Donor residence included seven counties in south Florida as well as counties in the central and northern parts of the state. Ten of 14 donors were found to have travel or sexual exposure risks, including three donors who had risk factors for both. Risk factor information could not be obtained for one donor. Of the 10 donors with travel risk, the median time from date last in a ZIKV-active area to donation date was 25 days (range, 6-71 days). Travel locations are shown in Table 2. Three donors had no travel to identified ZIKV-active areas and were attributed as sporadic individual infections acquired in Miami-Dade County. Three donors had potential sexual exposure to ZIKV in addition to a travel risk: two had traveled with their sexual partner to the ZIKV-active area, and one reported sexual contact with a visitor from a ZIKV-active area. Two of the sexual partners were female and one was male. Sexual contact was within 2 months of donation for all three donors. One donor (Donor 4) had sexual contact with his partner while she had symptoms compatible with ZIKV infection; the partner was tested and had positive ZIKV laboratory results. The other two partners reportedly had no symptoms and were not available for testing.

Six (43%) of 14 donors with probable ZIKV infection reported one or more of the four major symptoms associated with ZIKV infection; seven donors reported no ZIKV symptoms, and symptom information was not available for one donor. Three donors reported symptom onset 0 to 6 days after donation and, in all of these cases, the index donation plasma was confirmed as positive by repeat cobas Zika testing and alternate NAT and was negative for anti-Zika IgM. Three additional donors reported symptoms consistent with ZIKV infection 21 to 62 days before donation. Each of these three donors had traveled to a
| US donor no. | Collection date | cobas Zika repeat tests | Simulated pool (1:6) | Alternate NAT, plasma, Estimated viral load (copies/mL) | Anti-Zika IgM | Anti-flavivirus IgG | Follow-up serology and cobas Zika |
|-------------|-----------------|-------------------------|----------------------|-------------------------------------------------------|--------------|-------------------|----------------------------------|
| 3           | Aug 13, 2016    | R/R                     | R                    | Positive 1.17 x 10^3                                    | Positive     | Positive          | NT                               |
| 4           | Aug 19, 2016    | R/NR                    | NR                   | Negative                                             | Positive     | Equivocal        | Positive 6.5 x 10^4              |
| 5           | Aug 22, 2016    | R/NR                    | R                    | Positive 1.89 x 10^3                                    | Positive     | Positive          | NT                               |
| 7           | Sep 1, 2016     | R/R                     | R                    | Positive 8.00 x 10^6                                    | Negative     | Negative          | NT                               |
| 9           | Sep 8, 2016     | NR/NR                   | NR                   | Negative                                             | Positive     | Positive          | Positive 2.9 x 10^4              |
| 11          | Sep 13, 2016    | NR/NR                   | NR                   | Negative                                             | Positive     | Positive          | Positive 9.1 x 10^3              |
| 14          | Sep 22, 2016    | NR/NR                   | NR                   | Negative                                             | Positive     | Equivocal        | Positive 2.1 x 10^3              |
| 16          | Sep 25, 2016    | NT*                     | R                    | Positive 4.05 x 10^4                                   | Negative     | Negative          | NT                               |
| 17          | Sep 26, 2016    | NR/NR                   | NR                   | Negative                                             | Positive     | Positive          | NT                               |
| 18          | Sep 29, 2016    | NT*                     | NR                   | Negative                                             | Positive     | Positive          | Positive 1.7 x 10^3              |
| 19          | Sep 29, 2016    | NR/NR                   | NR                   | Negative                                             | Positive     | Positive          | Positive 2.6 x 10^4              |
| 21          | Oct 5, 2016     | R/R                     | R                    | Positive 2.81 x 10^4                                   | Equivocal    | Equivocal        | NT                               |
| 22          | Oct 7, 2016     | R/R                     | R                    | Positive 6.14 x 10^5                                   | Negative     | Negative          | NT                               |
| 23          | Oct 8, 2016     | R/R                     | R                    | Positive 8.39 x 10^3                                   | Negative     | Negative          | Negative                          |

* Sample not available for repeat NAT.
† The sample used for the alternate NAT on RBC was the index donation in all cases shown except Donor 18 where the second follow-up sample was used.
flu = follow-up; NR = nonreactive; NT = not tested; R = reactive.
ZIKV-active area within 2 weeks before symptom onset and, in all three, the index donation was negative by alternate NAT on plasma but positive for anti-Zika IgM. In one case (Donor 9), the donor had been diagnosed with ZIKV infection just over 1 month before donation. This donor’s physician had ordered clinical testing for ZIKV because of symptoms arising after her travel to Mexico; a serum sample collected 35 days before blood donation was reported as ZIKV PCR positive by a commercial laboratory.

**DISCUSSION**

This article reports the first blood donations in US states found to be positive for ZIKV infection. As of October 9, 2016, a total of 23 donations were identified that had initially reactive results on the cobas Zika test. Of these, 14 represent probable ZIKV infection based on the presence of reactivity on additional NAT or anti-Zika IgM testing. Seven of these 14 donations were reactive on the less sensitive alternate NAT assay performed on plasma samples. Seven donations were nonreactive on the alternate NAT on plasma but were IgM positive; RBCs were available from six of these for testing by alternate NAT, and all were positive. This suggests that donations initially reactive by cobas Zika and containing anti-Zika IgM represent true positive infections.

We cannot rule out the possibility that some of the nine initially reactive donors with negative results on additional testing of the index donation may also represent true positive infections. Only four of these donors completed follow-up, and we cannot determine whether the others may have seroconverted. Assuming, however, that these nine represent false-positive initial reactivity, the estimated specificity of the cobas Zika assay was 99.997% (95% exact CI, 99.995%-99.999%).

All of the probable ZIKV infections were collected in Florida. Local mosquito-related transmission has been identified in some areas of southern Florida; however, eight of the probable ZIKV donors reported risk factors related to travel to a Zika-active area outside of the 50 US states and/or sexual contact with an individual who had been in a Zika-active area outside of the US states. Three donors (Donors 3, 4, and 5) donated when questioning about travel to ZIKV active areas was included in donor screening but did not reveal the travel risk at the time of donation. Four donors (Donors 9, 14, 17, and 18) had a travel risk that ended more than 4 weeks before the donation, including one donor who returned from travel 39 days before donation and tested ZIKV PCR positive at a commercial laboratory 35 days before donation. This suggests that a 4-week travel deferral may not be sufficient to exclude all RNA-positive donors. In the donors with remote travel risk, ZIKV RNA was not consistently detectable by individual-donation NAT and not detectable in plasma by the less sensitive alternate NAT; these donations were IgM positive with detectable ZIKV RNA in RBC samples.

### TABLE 2. Risk factors for donors with additional evidence of Zika infection

| US donor No. | Collection date | Donor's state of residence | Donor sex | Was donor in identified Zika risk area within 90 days before donation? | Location of Zika risk area | Days between return from Zika risk area and donation | Sexual transmission risk within 2 months before donation? | Location of partner's risk |
|--------------|-----------------|---------------------------|-----------|---------------------------------------------------------------|-----------------------------|------------------------------------------------------|--------------------------------|---------------------------|
| 3            | Aug 14, 2016    | Florida                   | Female    | Yes                                                          | Trinidad                    | 7                                                    | No                                            |                           |
| 4            | Aug 19, 2016    | Florida                   | Male      | Yes                                                          | Puerto Rico                 | 25                                                   | Yes                                           | Puerto Rico               |
| 5            | Aug 22, 2016    | Florida                   | Male      | Yes                                                          | Miami ZIKV-active area      | 7                                                    | Yes                                           | Colombia                  |
| 7            | Sep 1, 2016     | Florida                   | Male      | Yes                                                          | No information available    | 39                                                   | Yes                                           | Mexico                    |
| 9            | Sep 8, 2016     | Florida                   | Female    | Yes                                                          | Mexico                      | 60                                                   | No                                            |                           |
| 11           | Sep 13, 2016    | Florida                   | Male      | No                                                            | Jamaica                     | 71                                                   | No                                            |                           |
| 14           | Sep 22, 2016    | Florida                   | Female    | Yes                                                          | Miami ZIKV-active area      | 69                                                   | No                                            |                           |
| 16           | Sep 25, 2016    | Florida                   | Male      | No                                                            | Dominican Republic          | 24                                                   | No                                            |                           |
| 17           | Sep 26, 2016    | Florida                   | Female    | Yes                                                          | Puerto Rico                 | 6                                                    | No                                            |                           |
| 18           | Sep 29, 2016    | Florida                   | Male      | Yes                                                          | Puerto Rico                 | 25                                                   | No                                            |                           |
| 19           | Sep 29, 2016    | Florida                   | Female    | Yes                                                          | Cuba                        | —                                                    | No                                            |                           |
| 21           | Oct 5, 2016     | Florida                   | Male      | Yes                                                          | Puerto Rico                 | —                                                    | No                                            |                           |
| 22           | Oct 7, 2016     | Florida                   | Male      | Yes                                                          | Cuba                        | —                                                    | No                                            |                           |
| 23           | Oct 8, 2016     | Florida                   | Male      | No                                                            |                             | —                                                    | No                                            |                           |
Two male donors reported sexual contact with females with ZIKV risk factors; this contact had not been identified as a potential risk in the initial FDA guidance but has more recently been reported. A third donor with potential sexual exposure was a female who had sexual contact with a male partner. All three of these donors with potential sexual exposure had also been in ZIKV-active areas, so it is not possible to determine the route by which they acquired their ZIKV infection.

Only seven of 14 probable ZIKV-infected donations were detectable when retested at 1:6 dilution to simulate minipool testing. The donations nonreactive in simulated minipool testing were all IgM positive. These findings are consistent with recent findings in Puerto Rico where approximately 70% of the cobas Zika–reactive donations have been detectable in a simulated minipool (data on file at RMS).

Transfusion transmission of ZIKV has been documented outside of the United States. Although symptomatic infection in recipients has not been documented in the few cases reported to date, the potential exists for serious consequences not only to transfusion recipients themselves, but also to fetuses or pregnant contacts of recipients. The minimum infectious dose for transmission of ZIKV is not known, nor is it known how the presence of antibody, found in nine of 14 probable ZIKV donations, may impact the infectivity of these donations. In the absence of this information, and with evidence that a significant proportion of infected donations might be missed by screening using a minipool format, the FDA has mandated that ZIKV donor screening be performed in an individual-donation testing format.

As of November 16, 2016, more than 4000 ZIKV-infected travelers had been reported in 49 US states and the District of Columbia. Of these, 39% were reported in Florida and New York. There were 35 additional cases linked to sexual transmission from travelers. This article highlights the fact that donors may acquire ZIKV infection by travel or sexual contact, and therefore, screening donations only in areas identified as having active mosquito-related transmission may fail to fully protect the blood supply. Donors who have traveled to ZIKV-active areas may not always report this at the time of donation. Furthermore, it appears that a 4-week travel deferral may not be sufficient to exclude all RNA-positive donors.

Consistent with recent reports, Zika RNA was detectable in the RBC of some anti-Zika IgM–positive samples in which RNA was not reproducibly demonstrable in plasma. Persistent detection of viral RNA in the RBC compartment, compared to plasma, has also been previously reported for West Nile virus; however, transfusion transmission of West Nile virus by products with RNA only in RBCs has not been documented. Thus, the blood safety implication of the detection of ZIKV RNA in RBC samples after appearance of IgM antibody and clearance of ZIKV RNA from plasma is not clear. Thus far, virus has not been cultured from such samples. Additional information regarding infectivity may be gained from inoculation studies in nonhuman primates and recipient lookback studies.

Donor screening enhanced public health surveillance in Florida by resulting in the direct identification of 14 ZIKV infections. Prompt reporting of reactive donors resulted in rapid notification to local mosquito control officials of suspected ZIKV cases. Timely mosquito control response can reduce the probability of ZIKV introduction and facilitate a rapid response to potential ZIKV introductions.

Preemptive implementation of donor screening in US states has identified asymptomatic infections that would have otherwise been unrecognized, including donors with RNA circulating more than 4 weeks after the end of travel to ZIKV-active areas. In addition to improving blood safety, donor screening can remove the need for regional deferrals, thereby maintaining the adequacy of the local blood supply. Given that most ZIKV infections are asymptomatic, donor screening also enhances public health surveillance and understanding of the epidemiology of infection.

In conclusion, screening of blood donations by individual donation testing for ZIKV RNA has intercepted ZIKV-positive donations. Risk factors for donors of these ZIKV-positive donations included travel and sexual contact, indicating the potential shortcoming of donor testing only in areas of active mosquito transmission. Donor testing for ZIKV RNA has now been mandated throughout the United States.

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CONFLICT OF INTEREST

SAG, LLP, ACB, RB, NH, JRD, CC, MS, JTT, JN, and NHCD are employees or contractors of Roche Molecular Systems, Inc. PCW, SJ, SNR, RR, and LB are site principal investigators for the Roche cobas Zika IND. MPB is an employee of Blood Systems, Inc., and Blood Systems Research Institute receives funding from Roche Molecular Systems, Inc., for performing supplemental testing of ZIKV-reactive samples reported in this article. He is the principal investigator on NHLBI- and CDC-funded studies about ZIKV that includes enrollment and follow-up of confirmed infected donors reported in this article. The remaining authors have disclosed no conflicts of interest.
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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher’s website: Appendix S1. Alternate NAT and serology testing.