Feasibility of routine individual donation testing for West Nile virus RNA during epidemic season using the investigational Roche cobas TaqScreen West Nile virus test and cobas s 201 system prototype

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BACKGROUND: Minipool (MP) screening for West Nile virus (WNV) RNA may fail to detect presumptive viremic donations (PVDs) detectable by individual donation screening (IDS). Most blood centers switch collection regions to IDS when PVD detection by MP screening reaches a certain frequency. Use of IDS for all donations during WNV season was assessed during a clinical trial of the Roche cobas TaqScreen WNV test. Also evaluated was whether PVD detection reliably identifies regions that should be targeted for IDS.

STUDY DESIGN AND METHODS: Test results, deviation reports, and service records were reviewed for 13.5 weeks of IDS in 2006 and 11.5 weeks of IDS in 2007. Numbers of PVDs and clinical WNV cases were obtained from public health and AABB Web sites and regional donor centers.

RESULTS: Approximately 1000 donations were tested per week divided in six test runs. Each run required 1.2 shifts of technologists plus volunteers. A total of 7.2 percent of samples were initially unreportable in 2006 and 4.8 percent in 2007. Of 26,952 donations screened by IDS, none were reactive for WNV. A comparison of PVD and clinical case reports indicates that PVD detection in areas with intermediate or high clinical case prevalence may not reach commonly used criteria for triggering testing to IDS.

CONCLUSION: Seasonal IDS was feasible using the cobas TaqScreen WNV test on the s 201, although staffing was impacted and a relatively high number of samples required retesting because of error messages. Seasonal IDS utilizing this highly specific assay may be a reasonable alternative to IDS triggered by regional PVD detection.

West Nile virus (WNV) was first recognized in the northeastern United States in 1999. Since that time, disease activity has spread through North America in annual epidemics each summer and autumn. Transmission of WNV by transfusion was first documented in 2002.[1] Implicated donations were found to contain WNV RNA but not WNV antibody, indicating that donor screening for WNV nucleic acid would be required to intercept potentially infectious units. By the summer of 2003, screening of blood donors for WNV RNA was widely implemented throughout the United States using investigational nucleic acid amplification tests (NATs) applied to minipools (MPs) of donor samples (6-16 donors/pool). Any pools testing positive were resolved to the individual donation (ID).

During the 2003 WNV epidemic season, approximately 800 presumptive viremic donors (PVDs) were detected by these investigational tests.[2] Six cases of probable transfusion-transmitted WNV were also identified,

ABBREVIATIONS: AP(s) = cobas AmpliPrep instrument(s); ARC = American Red Cross; BSL = Blood Systems Laboratories; ID(s) = individual donation(s); IDS = individual donation screening; MP(s) = minipool(s); PVD(s) = presumptive viremic donation(s); RMS = Roche Molecular Systems, Inc.; S-tube = sample tube; TM = cobas TaqMan analyzer; WNV = West Nile virus.

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however. These breakthrough transmissions were found to be associated with very low levels of RNA that had apparently escaped detection by screening MPs of donor samples. Further studies demonstrated that MP donor screening failed to detect approximately 25 percent of viremic donations that could have been detected by ID NAT.[3-5] To enhance detection of PVDs in regions with high WNV activity, while considering limitations in NAT resources, US blood banking organizations recommended in 2004 that testing laboratories "trigger" particular regions to ID NAT when detection of positive donors in that region via MP screening reached a certain frequency.[6,7] The initial triggers for American Red Cross (ARC) and Blood Systems Laboratories (BSL), together responsible for testing the majority of US blood donations, were reported to be 2 or 4 positive donors in a particular region in association with a WNV prevalence of at least 1 per 1000 donations in that region during 1 week. Regions were switched back to MP screening after 7 days with no positive donors detected.[4,5] The size of a geographic region was not specified in these recommendations.

During the 2004 and 2005 epidemics, California accounted for approximately 30 percent of the human cases of WNV in the United States.[8] Although few of these cases occurred in Santa Clara County, the county in which our main site is located,[9] our donor population includes residents of many other counties who commute to our region to work, as well as individuals who travel broadly for work and recreation. We were concerned that individuals exposed to WNV could contribute to our blood supply during the WNV season and that MP screening would miss approximately 25 percent of these donations. Furthermore, our total donation volume of approximately 1000 per week was too small for us to discern a WNV prevalence of 1 per 1000 among residents of any particular region. With the support of Roche Molecular Systems, Inc. (RMS), we decided to perform ID WNV testing of all donations through the 2006 and 2007 California WNV seasons using Roche's investigational cobas TaqScreen WNV test and the automated cobas s 201 system platform. In this article, we report our experience with seasonal ID testing on this investigational platform and analyze seasonal versus triggered approaches to WNV donor screening in Northern California in a retrospective review of the 2006 epidemic season.

MATERIALS AND METHODS

Cobas TaqScreen WNV test

The cobas TaqScreen WNV test is a qualitative test for screening and detecting WNV RNA in donated blood, manufactured by RMS (Pleasanton, CA). This automated polymerase chain reaction (PCR) assay uses the cobas s 201 system, an integrated system for pooling and screening blood donations. The system includes a pipettor (Microlab STAR IVD pipettor, Hamilton Co., Reno, NV), an instrument for sample preparation (cobas AmpliPrep [AP], RMS), an analyzer for PCR amplification and detection (cobas TaqMan [TM], RMS), and software for automated result compilation and reporting.[10,11] In 2006, the controlling software for the s 201 system, used under an IND, was Configuration B (AmpliLink v3.1.0) and in 2007 it was Configuration B Maintenance Release 1 (B-MR1; AmpliLink v3.1.2).

The STAR pipettor delivers control samples and ethylenediaminetetraacetate donor plasma into sample (S)-tubes. Donor samples can be transferred individually or pipetted as pools of 6. The S-tubes are processed in batches of up to 24 tubes, each batch containing 2 control samples and either 22 ID samples or 22 pools of donor samples. After manually capping the S-tubes, the operator places the batches into the AP instrument for RNA extraction and then manually transfers them to the TM for amplification and detection. Each AP can hold three batches at once, and the TM can hold four batches. The s 201 system is configurable for variable numbers of component instruments; in our center we had two systems, each containing one of each instrument.

This trial evaluated a prototype system. An FDA-approved version of the test and system are now commercially available. The sensitivity and specificity of the FDA-approved test are reported in the package insert. The estimated 95 percent limit of detection using the Health Canada Standard—Lineage 1 (on individual samples) for the licensed cobas TaqScreen WNV test is reported to be approximately 40 copies per mL; the clinical sensitivity is reported to be 100 percent with neat specimens and 97.5 percent with 1:6 diluted specimens.[12]

Test performance

Our testing was performed as part of a clinical trial of the cobas TaqScreen WNV test and cobas s 201 system, under approval of the Stanford University Institutional Review Board. Deviation reports were completed for all error messages from instruments or on result reports and included a list of samples affected. These reports, along with service records for each instrument, were maintained in study binders. Numbers of donations tested were tracked on daily logs and reported monthly to RMS. In this article, we review system performance during the 13.5 weeks of ID WNV testing from July to October 2006 using Software Configuration B and during the first 11.5 weeks of ID testing in 2007 (July-September) utilizing upgraded system software (Software Configuration B-MR1).

Regional WNV activity

Human clinical cases of WNV in California are reported to local health departments and summary tables of cases per county are posted by the California Department of Public

Health at http://www.westnile.ca.gov/. The estimated population of each county was obtained from the US Census Bureau Web site at http://quickfacts.census.gov/qfd/states/06000.html. For our analysis, the distance of each county from our blood center was estimated to be the distance from the administrative office of the county to our headquarters.

Numbers of PVDs, dates of donation, and zip codes of PVDs were obtained from the AABB biovigilance Web site.[13] The AABB biovigilance site defines a PVD as a donor sample that meets one of the following criteria: 1) a reactive sample that is repeatable by ID NAT or 2) reactivity in the Procleix WNV assay (Chiron Corp., Emeryville, CA) that has a signal-to-cutoff ratio exceeding or equal to 17. A confirmed PVD is defined by the AABB Web site as NAT reactivity confirmed on an independent sample, on the same sample by alternative NAT, or demonstration of donor WNV antibody. The county corresponding to each PVD zip code was determined from http://www.zipinfo.com/. We contacted the blood centers in Northern California to verify that the PVDs from their centers were accounted for on the AABB Web site. The blood center in Kern County indicated that four PVDs from their center had not been reported to the AABB Web site; information about these donors was provided to us by the blood center and its testing lab. For our analysis of "viremic donors," we included all PVDs that had a signal-to-cutoff ratio of greater than or equal to 17 and/or were listed as "confirmed" in the final interpretation field of the AABB Web site.

All blood centers in Northern California indicated that they had determined the need to trigger to ID NAT on the basis of PVD detection in specific collection regions. Most used triggering criteria similar to BSL or ARC, although the blood center headquartered closest to the highest prevalence counties indicated that they had triggered their smaller collection sites to ID NAT on the basis of one PVD detected, regardless of collection volume, and untriggered based on 1 week with no reactivity. The number of blood donations collected from residents of individual counties could not be determined because this was not tracked by the blood centers.

RESULTS

System performance during ID testing

Our donations were tested in six test shifts per week. For ID WNV testing, a volunteer prepared empty S-tubes for loading onto the Hamilton STARs by uncapping them and placing them into S-clips. Pipetting of samples on the STARs was begun 1 to 2 hours before the testing shift. Technologists capped the S-tubes and processed the samples through the AP and TM. During the 8-hour testing shift, one technologist was fully occupied with WNV testing, using the two s 201 systems in parallel. Occasionally another 1 to 2 hours of technologist time was required in the following shift to complete testing and review.

In 2006, a total of 15,383 donations were tested individually, from mid-July to mid-October, averaging approximately 1,100 donations per week and 190 donations per test shift. During the first 11.5 weeks of ID testing during 2007, from July to mid-September, 11,569 donations were tested, averaging 1,000 donations per week and 170 donations per test shift.

In 2006, a total of 7.2 percent of results were not reportable on first processing. All donor samples that were initially unreportable had valid results on retesting. Error codes associated with 0.3 percent or more unreportable samples are shown in Table 1. In 2006, AP hardware and consumable handling errors accounted for almost half of the unreportable results. Although the two s 201 systems were used in parallel to process samples, one of the two AP instruments (AP-1) was noted to be associated with a disproportionate share of the AP hardware and consumable handling error messages as shown in Table 1. In 2007, with upgraded software, the overall rate of unreportable results decreased to 4.8 percent. AP-1 continued to account for a disproportionate share of the AP hardware and consumable handling errors. In 2007, we noted an increase in

TABLE 1. Error categories responsible for 0.3 percent or more unreportable results*

	2006 (13.5 weeks)			2007 (11.5 weeks)		
Error category	Events	Number of samples affected	Percentage of total samples	Events	Number of samples affected	Percentage of total samples
TM hardware or consumable handling	9	318	2.1	8	157	1.4
AP hardware or consumable handling						
AP-1	17	349		5	140	
AP-2	11	190		3	55	
Total	28	539	3.5	8	195	1.7
AP tube missing message	13	88	0.6	35	143	1.2
AP dispense error	10	53	0.3	6	6	0.1

* Analysis includes 15,383 donations tested during 13.5 weeks of IDS in 2006 and 11,569 donations tested during the first 11.5 weeks of IDS in 2007. Of these, 1106 (7.2%) were initially unreportable in 2006 and 561 (4.8%) were initially unreportable in 2007. All of these were reportable after retesting. The table shows error categories accounting for 0.3 percent or more unreportable results. AP-1 and AP-2 denote the two AmpliPrep instruments. AP-1 was noted to have more error messages than AP-2.

TABLE 2. Total numbers of viremic donors and clinical WNV cases reported for the 2006 season in counties within 250 miles of our blood center

County	Distance*	Viremic donors	Clinical cases	Population (thousands)	Cases/100,000 population
Santa Clara	16	0	5	1699	0.3
Alameda	27	0	1	1449	0.1
Contra Costa	40	1	8	1017	0.8
Marin	45	1	1	247	0.4
Solano	58	1	8	412	1.9
Napa	60	0	1	133	0.8
San Joaquin	61	0	8	664	1.2
Stanislaus	67	1	11	506	2.2
Sacramento	88	2	15	1363	1.1
Yolo†	89	4	27	185	14.6
Merced	94	0	4	246	1.6
El Dorado	117	0	2	177	1.1
Placer	118	1	8	317	2.5
Sutter†	121	4	12	89	13.5
Yuba†	123	2	5	67	7.5
Colusa†	123	0	4	21	19.0
Fresno	140	1	11	878	1.3
Nevada	142	0	1	99	1.0
Glenn†	145	0	12	28	42.9
Butte†	149	3	31	214	14.5
Kings	159	1	1	143	0.7
San Luis Obispo	170	0	1	255	0.4
Tulare	174	1	6	411	1.5
Tehama†	190	1	6	61	9.8
Shasta	217	0	4	180	2.2
Kern†	225	4	49	757	6.5

* Distance between our blood center headquarters and the administrative office of the county.
† Counties with case prevalence above 5 per 100,000.

"tube missing" error messages from both APs. These were found to be related to a defect in S-tube manufacturing that impacted the ability of the AP to access the tube.

During the 13.5 weeks of ID testing in 2006, there were 13 service calls initiated: 9 for the AP, 4 for TM, and none for the Hamilton STAR. During the first 11.5 weeks of ID testing in 2007, there were 8 service calls initiated: 6 for the AP, 1 for TM, 1 for the Hamilton STAR. In both years, AP-1 was associated with twice as many service calls as AP-2 (6 vs. 3 calls in 2006 and 4 vs. 2 calls in 2007). No donations to our center were reactive on the cobas TaqScreen WNV test.

PVD detection in Northern California compared to clinical case prevalence in 2006

The total number of PVDs and clinical WNV cases reported for Northern California during the 2006 season are shown in Table 2 along with the estimated prevalence of clinical cases by population. The location of each county is shown in Fig. 1. There were 2 PVDs and 15 cases reported within 50 miles of our main site and 21 PVDs and 175 clinical cases reported within 150 miles of our main site. A total of 16 of 21 PVDs were detected during triggered ID NAT at one blood center whose headquarters is 90 miles away from our main site.

Five counties reported high clinical case prevalence rates (i.e., greater than 10 cases per 100,000 population); these five counties are clustered together northwest of Sacramento County. There were no PVDs detected in the two counties with the highest clinical case prevalence, Glenn and Colusa. These counties have small total populations (28,000 and 21,000, respectively). The other three counties with clinical case prevalence rates above 10 cases per 100,000 (Yolo, Sutter, and Butte) each had 3 to 4 PVDs detected over the course of the 2006 season. The median elapsed time between PVDs *within* each of these counties was 9.5 days (Table 3). If these three counties are considered together, however, 2 to 3 PVDs were detected each week over a 5-week period by one blood center in this region, which triggered its smaller collection sites to individual donation screening (IDS) on the basis of any PVDs detected in a week. In Kern County, a large populous county with the highest total number of clinical cases and an intermediate clinical case prevalence rate (6.5 cases per 100,000 population), there were a total of 4 PVDs detected during the season, with 9 to 20 days between PVDs; IDS was not triggered.

DISCUSSION

We have successfully utilized the Roche cobas TaqScreen WNV test and cobas s 201 automated platform to test IDs for WNV RNA throughout the WNV season in California. The TaqScreen WNV test is highly specific, with no false-positive results in almost 27,000 donations tested.

Fig. 1. Northern California counties within 250 miles of our blood center headquarters. The location of our headquarters in northern Santa Clara County is identified with a star. Map is modified from the US Census Bureau 2000 map at http://ftp2.census.gov/geo/maps/general_ref/stco_outline/cen2k_pgsz/stco_CA.pdf.

TABLE 3. Days between viremic donors in counties with clinical case prevalence above 5 per 100,000

County	Clinical case prevalence (per 100,000 population)	Days between viremic donors
Yolo	14.6	12, 9, 10
Sutter	13.5	10, 6, 15
Butte	14.5	7, 7
Yuba	7.5	24
Kern	6.5	9, 20, 13

Although the system is automated, a significant amount of manual preparation and manipulation of consumables is required, including uncapping of empty S-tubes, placing the uncapped tubes in S-clips, loading S-clips onto racks, and recapping individual S-tubes after the donor samples are pipetted into them by the STAR. During ID testing, these manual steps not only consume staff time but also pose a potential risk of repetitive motion injury to staff. Staff haste in recapping the tubes can increase the likelihood that the cap or the tube will be improperly seated, which in turn can cause the AP to be unable to access the tube or to mishandle it.

In 2006, a high rate of samples required retesting because of unreportable results on first processing. With improved software in 2007, the unreportable rate decreased substantially. In both years, AP hardware or consumable handling errors accounted for a large proportion of the unreportable results. A more detailed analysis indicated that one of our AP instruments had about twice the number of hardware and consumable handling events and twice the number of service calls compared to the other AP. Subsequent analysis by RMS indicates that this instrument has service call rates about twice as high as

other APs in the field. Thus, our overall experience may have been skewed by one instrument with below-average reliability. In 2007, the overall performance was impacted by a new manufacturing defect in the plastic S-tubes, which caused an increase in "tube missing" errors on the AP; this consumable defect should be correctable.

Because of hardware error messages, we initiated an average of one service call per week in 2006. This rate of service calls would suggest that back-up instruments or access to immediate service would be required to ensure daily donor testing. The upgraded software used in 2007 reduced the rate of hardware errors and improved the overall performance of the s 201 system. It is possible that a more detailed analysis of error messages by the manufacturer could identify other improvements to hardware, software, service schedules, consumable quality, or operator training that could further improve overall system performance.

When our center is carrying out MP screening testing for WNV, one technologist can complete both WNV testing and other tasks during an 8-hour testing shift. During our seasonal IDS experience, WNV testing required the full dedication of a technologist over approximately 1.2 shifts, as well as volunteers to prepare consumables (uncapping empty S-tubes and loading them into S-clips). The throughput we observed on the cobas s 201 system does not reflect the best potential of the system. For example, the AP is able to overlap processing of sequential batches, but our staff did not consistently take advantage of this feature. In the TM, the processing time of a batch is fixed regardless of the number of samples it contains. Our staff reported that they sometimes loaded partial batches, which would result in lower throughput in the TM. Upgraded software implemented in 2007 appears to have improved the reliability of the instruments, improving our confidence in processing full loads and reducing the number of samples requiring retesting.

Widespread screening of the US blood supply for WNV RNA was first initiated in 2003 by testing of 6- to 16-member MPs of donor specimens utilizing investigational WNV NAT assays. The majority of the US blood supply was screened in 16-member pools by the semiautomated Procleix WNV assay from Gen-Probe/Chiron, while some laboratories tested donors in 6-member pools utilizing a fully automated prototype system from RMS (TaqScreen WNV test).[4,5,14] Public health surveillance identified six cases of transfusion-transmitted WNV in the United States during the 2003 WNV season. These cases were attributed to low-level viremia in donors that was missed by MP donor screening but that could have been detected by IDS.[2] Further studies indicated that MP testing would fail to detect approximately 25 percent of viremic donations detectable by ID screening.[3-5] The majority of the low-level viremic donations detectable only by ID screening were found to also contain WNV antibody. The infectivity of donations containing both RNA and antibody has been questioned, as transmissions thus far have been traced only to donations from donors without antibody.[4,5] In vitro studies, however, suggest that the presence of WNV antibody in a blood component does not necessarily preclude infectivity.[15]

Since the reports of low-level viremia missed by MP screening, US blood bank organizations have struggled to define the most appropriate method of screening the blood supply for WNV given the perception that testing resources (equipment, reagents, and technologists) were insufficient to test all donations individually.[6,7,16,17] In 2004, Custer and colleagues[7] analyzed 2003 data from BSL to determine the most effective strategy for screening this organization's customers, based on an assumption that BSL had the capacity to test no more than 25 percent of its specimens by ID screening. These authors found that a strategy targeting ID screening to centers that had at least 2 MP-reactive donations *and* a weekly PVD detection rate of at least 1 per 1000 donations would include approximately 81 percent of MP-reactive donations in the targeted ID testing and yet keep BSL's testing burden within its operating constraints. BSL adopted this strategy for 2004, triggering to ID NAT based on 2 reactive MPs and a PVD prevalence of 1 in 1000 in the region for the week, and ARC reportedly adopted a similar strategy. Regions were switched back to MP screening after 7 days with no positive donors detected.[4,5] The size of a region was not specified. During the 2004 season, only one case of transfusion-transmitted WNV was identified in the United States.[18] The implicated donation was collected before the implementation of regional triggering and was negative by MP testing. Retrospective testing of the donor plasma showed reactivity by ID WNV RNA testing in 9 of 10 replicates. There were no documented cases of transfusion-transmitted WNV in the United States in 2005.

In 2006, one blood donation negative by MP screening transmitted WNV to two transfusion recipients.[19] Samples from the original unit were not available for retesting by ID NAT. This case engendered controversy because the laboratory that tested the implicated donation had not reached its regional prevalence trigger for ID NAT, although some other donations from the same state were reportedly screened by ID NAT in a different laboratory that had triggered to ID NAT. The Centers for Disease Control report of this case described the donor as living in a region "where substantial WNV activity in birds, mosquitoes, and humans occurred during the 2006 transmission season." South Dakota WNV public health service reported a total of 10 human clinical cases for this county (Minnehaha) in 2006, representing a prevalence of 5.9 clinical cases per 100,000 population based on a census population estimate of 169,284. The AABB biovigilance

site shows only two PVDs detected in this county in 2006, separated by more than 1 month in time. Only 6 additional PVDs were reported to the AABB Web site for the entire remainder of the state of South Dakota in 2006, each from a different county of residence. The prevalence of clinical cases in the South Dakota county in which the transfusion transmissions occurred (5.9 per 100,000) is similar to that in Kern County, California (6.5 per 100,000), a large county with the largest number of clinical WNV cases in Northern California (49 cases). There were 4 PVDs detected in Kern County during the 2006 season, but these were separated in time by 9 to 20 days; there was no week in which more than 1 PVD was detected in the county.

In response to the 2006 South Dakota transmission report, the AABB issued in 2007 a recommendation that blood centers with adjacent or overlapping collection regions establish a process to communicate with regard to PVD detection and decisions to trigger to ID screening.[20] The AABB recommended that the minimum triggering criteria for ID NAT should be 2 PVDs in a region during a 1-week period, together with a prevalence of more than 1 PVD per 1000 donations. The AABB acknowledged the difficulty in defining regions and recommended that blood centers collecting fewer than 1000 donations per week not subdivide their donations further. The AABB indicated that MP screening could be resumed after 1 week with no PVDs detected in a region. Blood centers in California adopted varying triggers for 2007 and defined regions of greatly varying geographic sizes, with some centers triggering either their whole center or a region to ID testing based on one PVD detected and switching back to MP screening after 7 days with no further PVDs detected. Centers throughout the state have triggered on and off through a domino effect after receiving electronic communications from their neighbors.

Although counties represent arbitrarily defined geographic regions, our analysis of data by county permits a comparison of PVD detection with clinical case prevalence. It is clear from this analysis that PVD detection is a relatively insensitive indicator of human WNV activity. PVD detection appears to be infrequent in areas with intermediate clinical case prevalence (5-10 cases per 100,000) such as Minnehaha County, South Dakota, where the transfusion transmission of WNV occurred in 2006, and Kern County, California, where there were a large number of clinical WNV cases. Even areas with very high clinical case prevalence (more than 10 per 100,000) may generate an insufficient number or frequency of PVDs to trigger ID testing. Clinical case reports are not suitable for use as triggers for ID NAT because of the typical delay of weeks between the onset of symptoms and official public health reporting of these cases.[9]

At present, in most areas of the United States, a donation is screened by IDS only if it is collected in a region that has been "triggered" to ID screening. Blood centers and blood bank organizations continue to struggle to define the appropriate size of a region and whether it should be a PVD number or prevalence that should trigger ID screening within that region. If a blood center uses a trigger that is based on PVD prevalence, the grouping of several collection locations together into one region may cause failure to recognize a need for IDS in one area by increasing the denominator over which the PVD prevalence rate is calculated. Alternatively, grouping several collection areas together as one region could expand the areas triggered to IDS if the blood center trigger is based on one PVD detected anywhere in the region. Regardless of the strategy chosen, all of the geographically targeted strategies determine the need for IDS according to PVD detection in a collection region in a particular week, not on an individual donor's exposure history. If a region is not triggered to IDS, no donation collected in that region is screened by IDS, regardless of whether a donor resides in, or has recently vacationed in, a region with high WNV activity.

Our total blood center collection volume is only about 1000 donations per week. Although most of our donors reside in Northern California, they live in many different counties; many travel widely within and outside of California for work and recreation. We would be unable, through our own collection volume, to discern a PVD prevalence above 1 per 1000 in any particular geographic region. Although we did not detect any PVDs by seasonal ID screening, we believe that a seasonal ID testing approach may be reasonable for our center given our proximity to regions with intermediate or high WNV activity.

We have demonstrated here that ID testing throughout the WNV season was feasible at our blood center utilizing the Roche cobas TaqScreen WNV test on the automated cobas s 201 system prototype. The throughput of two s 201 systems each containing a STAR, AP, and TM was sufficient for our testing volume and throughput would be further improved by minor work flow adjustments as well as recent software enhancements. The Roche cobas TaqScreen WNV test and cobas s 201 system (with upgraded software) were recently approved by the FDA for use in blood donor screening.

Another automated WNV assay, Gen-Probe/Chiron's Procleix WNV test on the automated Tigris instrument, was also recently approved by FDA for donor screening. Although the Tigris has a higher throughput than the s 201 system and requires less manual manipulation of consumables, the Procleix WNV test has lower specificity than the Roche cobas TaqScreen WNV test. The Procleix WNV package insert reports a false-positive rate of approximately 1 per 1000,[21] compared to our experience of zero false-positive samples in nearly 27,000 specimens tested with the Roche cobas TaqScreen WNV test on the s 201 system platform. IDS using an assay that has a false-

positive rate of 1 per 1,000 could lead to confusion as to the status of the epidemic and the need to continue ID screening; furthermore, donors reactive on an individual sample are deferred from all donations for 120 days according to current FDA guidelines.[22]

In summary, we found that seasonal ID NAT for WNV was feasible utilizing the investigational Roche cobas TaqScreen WNV test and cobas s 201 system prototype. Assay specificity was excellent, but there were a significant number of samples unreportable on first processing and staffing requirements were increased compared to MP screening. Although neither automated WNV platform appears perfect for ID testing, it is also clear that geographically targeted ID screening based on PVD detection is imperfect. PVD detection can be infrequent or even absent in areas with intermediate or high clinical case prevalence. Given the availability of automated testing platforms, some laboratories now have a capacity for ID WNV testing that is higher than when the geographic targeting strategies were first developed. We believe that seasonal ID screening may be considered a reasonable alternative to regional prevalence-triggered ID screening for WNV RNA.

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