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研究報告の概要	使用上の注意記載状況・その他参考事項等
西ナイルウイルス(WNV) RNA の有無について調べるための 16 人サンプルの「ミニプール」の核酸増幅テスト(NAT)は 2003 年 7 月に開始された。 2003 年 7 月 1 日～10 月 31 日迄の 4 ヵ月間に 677,603 の供血者がミニプールテストによってプロスペクティブにスクリーニングされたところ、183 人が陽性で、検出率は 0.027% (1/3703)であった。 高罹患地域でのミニプールテスト陰性供血者 23,088 検体のレトロスペクティブな個別検査によって、さらに低レベルの WNV 血症 30 検体が検出された。 ウイルス血症ユニットの 5%は IgM 抗体陰性で個別テストによってのみ検出され、29%は IgM 抗体陽転後の個別テストによって発見され、66%はミニプールテストによって検出された。 WNV 感染は、個別テスト陽性で IgM 陰性供血で認められ、抗体陽性で個別テスト陽性の受血者では認められなかった。 供血者のミニプールの核酸増幅テストは 2003 年において、WNV 感染の何百もの症例を防止したが、低レベルウイルス血症のユニットを見逃し、そのいくつかは抗体陰性で、感染性があった。 これらのデータは、2004 年の高い罹患率の地域における個別 NAT 実施の後押しをする。	

報告企業の意見	今後の対応
血漿分画製剤での WNV 感染伝播の報告はなく、製造工程中に WNV と同じフラビウイルスであるウシ下痢症ウイルス(BVDV)の不活化除去が確認された工程を設けているが、今後とも関連情報に注意していく。	今後とも WNV に関連する情報の収集に努めていく。

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ORIGINAL ARTICLE

Screening the Blood Supply for West Nile Virus RNA by Nucleic Acid Amplification Testing

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ABSTRACT

BACKGROUND

The use of nucleic acid amplification tests of "minipools" of 16 samples to screen blood donors for West Nile virus RNA began in July 2003. We report the yield and characteristics of positive donations and the incremental yield and safety of nucleic acid amplification tests of individual donations.

METHODS

Reactive minipools were analyzed to identify the individual reactive donations. For the regions with the highest yield on minipool testing, retrospective nucleic acid amplification testing was performed on individual donations that were negative on minipool testing. Reactive donations were confirmed by alternative nucleic acid amplification tests and IgM and IgG tests, and donors were followed to document seroconversion.

RESULTS

From July 1 through October 31, 2003, 677,603 donations were prospectively screened for West Nile virus by minipool testing, yielding 183 confirmed viremic donations (0.027 percent, or 1 in 3703 donations). Retrospective individual testing of 23,088 donations from high-prevalence regions that were negative on minipool testing yielded 30 additional units with a low level of viremia, with 14 additional viremic units detected by prospective testing of individual donations late in the 2003 transmission season. Of all the viremic units detected, 5 percent were detected only by individual testing and were negative for IgM antibody, 29 percent were detected by individual testing after IgM seroconversion, and 66 percent were detected by minipool testing. West Nile virus infection was confirmed in both recipients of IgM-negative units that were reactive on individual testing, whereas neither recipient of antibody-positive blood components that were reactive on individual testing was infected. In 2004, prospective testing of individual donations in regions that yielded donations that were reactive on minipool testing resulted in a 32 percent incremental yield of units with a low level of viremia that would have been missed by minipool testing.

CONCLUSIONS

Although nucleic acid amplification testing of minipools of blood donations prevented hundreds of cases of West Nile virus infection in 2003, it failed to detect units with a low level of viremia, some of which were antibody-negative and infectious. These data support the use of targeted nucleic acid amplification testing of individual donations in high-prevalence regions, a strategy that was implemented successfully in 2004.

WEST NILE VIRUS, A MOSQUITO-borne flavivirus, emerged as a cause of meningoencephalitis in the United States in 1999, and infections reached epidemic proportions in 2002.[1,2] In 2002, West Nile virus was shown to be transmissible by transfusion, when 23 cases were documented.[3,4] In late 2002, the Food and Drug Administration (FDA), U.S. blood-collecting organizations, and test-kit manufacturers accelerated programs to develop nucleic acid amplification tests to screen blood donors for West Nile viremia in an effort to implement such programs before the 2003 transmission season.[4-7] The resulting assays involved the testing of pools of 6 to 24 samples, or "minipools," an approach that is now also routinely used to screen blood donors for human immunodeficiency virus (HIV) and hepatitis C virus (HCV).[8]

We report the results of a large, multicenter testing program conducted during the summer and fall of 2003 and of studies that compared minipool results with those obtained by nucleic acid amplification tests of individual (undiluted) donations. Our results allow an estimation of the number of infections averted in 2003 by the implementation of screening with minipool nucleic acid amplification tests and an estimation of the additional benefit of screening individual donations in regions with seasonal epidemics of West Nile virus infections. We also summarize experience with implementation of a targeted screening strategy involving nucleic acid amplification testing of individual donations in 2004, a strategy that successfully identified units with a low level of viremia that would have been missed by minipool testing.

METHODS

Blood Systems Laboratories screens blood donations in two FDA-licensed laboratories in Tempe, Arizona, and Bedford, Texas. Clients include 18 blood-collection facilities owned by Blood Systems (Scottsdale, Ariz.) and 59 other community-based and hospital-based blood-collection programs. The geographic areas covered by this client base include much of the Southwest, the South, the Central Plains, and parts of California. The annual testing volume is approximately 2.2 million donations, which represents approximately 20 percent of the U.S. blood supply.

The West Nile virus Transcription-Mediated Amplification system (Procleix WNV Assay, Gen-Probe and Chiron) was used for nucleic acid amplification tests. This technique involves lysis of viral particles in plasma, either from individual donations or from a minipool of plasma specimens from 16 donations and the isolation of West Nile virus RNA with the use of probes bound to magnetic beads, amplification with the use of RNA transcription, and subsequent detection by a chemiluminescent probe.[9,10] All samples within a reactive minipool are then tested individually. The assay has an analytical sensitivity of approximately 4 RNA copies per milliliter when used for individual donations (50 percent limit of detection by probit analysis of dilutions of West Nile virus standards) and a sensitivity of approximately 45 copies per milliliter when used for minipool testing.[10]

Individual specimens identified as reactive were evaluated by means of a confirmatory algorithm with the use of multiple assays for West Nile virus.[10-12] These tests included an alternative nucleic acid amplification test (either a modification of a TaqMan polymerase-chain-reaction [PCR] assay or another primer-based transcription-mediated amplification assay, performed by Bayer Reference Laboratory) and assays of plasma for viral IgM and IgG antibody (Focus Diagnostics).[10,13] All donors with reactive tests were promptly asked to enroll in a follow-up study, involving return visits approximately every week. Follow-up specimens were tested for West Nile virus RNA and for IgM and IgG antibodies against West Nile virus. A confirmed positive result was defined by the detection of viral IgM in either the index specimen or a follow-up specimen, the detection of viral RNA in the index specimen by means of the alternative nucleic acid amplification test, or the detection of viral RNA in a follow-up specimen by means of a transcription-mediated amplification assay.[11] Since the actual times at which donors returned for follow-up varied (resulting in intermittent blood collection) and since seroconversion would have occurred in the interval between the last seronegative and the first seropositive result, median times (and interquartile ranges) to IgM and IgG seroconversion were estimated with the use of an analysis in which data were censored in the intervals between visits.[14] The viral load in confirmed positive index donations for which frozen plasma components were available for analysis was evaluated by a kinetic PCR assay based on target-capture TaqMan techniques (Chiron).[10]

Blood Systems Laboratories also conducted geo-

graphically and temporally targeted nucleic acid amplification testing of individual donations in 2003 according to two protocols. The first protocol involved testing of individual frozen specimens from donations previously found to be negative on nucleic acid amplification testing of minipools of specimens from regions with a high prevalence of West Nile virus infections. Donors whose specimens were retrospectively determined to be reactive on individual testing were asked to enroll in the follow-up study. In addition, in-stock blood products from these donors were retrieved, and in collaboration with the Centers for Disease Control and Prevention (CDC), recipients of blood components from donors confirmed to be positive for West Nile virus on nucleic acid amplification testing of individual donations were evaluated to ascertain whether transmission of West Nile virus had occurred.[15] The second protocol was conducted in selected blood-collection regions that had had a high number of reactive minipools on nucleic acid amplification testing in the previous weeks. Individual donations were prospectively screened exclusively by nucleic acid amplification testing (i.e., minipool testing was not performed). Reactive units were subsequently diluted 1:16 and retested individually to determine what the results of minipool testing would have been. No blood components that were reactive on nucleic acid amplification testing of individual donations were transfused during this prospective study.

In June 2004 Blood Systems Laboratories implemented a targeted screening strategy involving nucleic acid amplification testing of individual donations (described elsewhere in detail[16]) and real-time tracking of the results of minipool testing. Prospective nucleic acid amplification testing of individual donations was implemented in geographically defined zones if there were two or more reactive donations on minipool testing and a rate of more than 1 reactive minipool per 1000 tested. Testing reverted to the minipool format when regions had had no individual donations with reactive tests for at least seven consecutive days and had a weekly rate of reactivity of fewer than 1 per 1000 donations.

All studies were approved by the FDA and the relevant institutional review boards. All donors and recipients gave written informed consent to undergo screening and follow-up testing for West Nile virus. Drs. Busch, Tomasulo, and Kleinman and Ms. Caglioti designed the studies; supervised data collection, management, and analyses; and drafted and revised the manuscript. Drs. Robertson, Tobler, Linnen, and Shyamala and Ms. McAuley supervised testing. Dr. Kamel supervised donor follow-up and participated in "look-back" activities involving recipients of blood components. All authors approved the manuscript, which was written primarily by Drs. Busch and Kleinman.

RESULTS

The results of minipool nucleic acid amplification testing during the active West Nile virus season in 2003 are shown in Figure 1. Of 677,603 donations tested between July 1 and October 31, 2003, 183 were confirmed to be positive, for an aggregate rate of 0.027 percent (1 in 3703 donations). The highest rates occurred during a six-week period from mid-July through mid-August.

Forty-seven additional viremic units were detected on nucleic acid amplification testing of individual donations. Retrospective testing of 23,088 individual donations that had been negative on minipool testing, collected from donor centers in Texas, North Dakota, and South Dakota (i.e., centers with high rates of reactivity on minipool testing during the summer of 2003), identified 30 additional confirmed positive specimens. Prospective testing of individual donations was subsequently performed on 3964 donations collected in North Dakota and South Dakota in September 2003 and identified 17 confirmed positive donations, of which 14 tested negative for West Nile virus with the use of the transcription-mediated amplification system at a 1:16 dilution, indicating they would have been missed by minipool testing. Thus, 186 units were detectable by minipool testing and 44 units were detectable only by nucleic acid amplification testing of individual donations.

West Nile virus antibody status was determined for 41 of the 44 donations that were negative on minipool testing and confirmed positive by individual testing (3 samples had insufficient volume for testing). Thirty-one specimens (76 percent) had detectable West Nile virus antibody: 10 were IgM-positive, and 21 were positive for both IgM and IgG. In contrast, only 16 of the 183 confirmed positive donations detected by minipool testing (9 percent) were positive for IgM antibody at the time of donation ($P<0.001$ by the chi-square test). Among 145 viremic donors who were initially seronegative and who enrolled in the follow-up assessment, West