Role of nucleic acid testing in cadaver organ donor screening: detection of hepatitis C virus RNA in seropositive and seronegative donors

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SUMMARY. Hepatitis C virus (HCV) transmission by both seropositive and seronegative cadaver organ donors has been documented, yet nucleic acid testing is not routinely used to identify active infection in these donors prior to transplantation. Between November 2001 and February 2004, we screened 1445 cadaver organ donors for anti-HCV antibodies with either HCV EIA-2.0 (Abbott Diagnostics, Chicago, IL, USA) and/or Ortho® HCV Version 3.0 ELISA (Ortho-Clinical Diagnostics, Raritan, NJ, USA) and confirmed seropositive samples with Chiron RIBA®3.0 SIA (Chiron Corporation, Emeryville, CA, USA). Samples with sufficient volume ($n = 726$) were tested by the VERSANT® HCV [transcription-mediated amplification (TMA)] Qualitative assay (Bayer Healthcare LLC, Tarrytown, NY, USA) which can be performed in approximately 5 h. Those with detectable HCV RNA and sufficient volume were quantified by the VERSANT® HCV 3.0 (bDNA) Assay (Bayer Healthcare LLC) and/or the HCV RNA TMA Quantitative Assay ($n = 23$) and genotyped ($n = 57$). Seventy-seven of 1445 (5.3%) donors were seropositive, reactive by either one or both anti-HCV assays. Fifty-two of 63 (82.5%) of the seropositive samples had detectable HCV RNA and were genotyped. Seventeen of these samples had quantifications ranging from 128,123 to >7,692,307 IU/mL. Six of 663 (0.9%) seronegative samples had detectable HCV RNA. Their quantifications ranged from <9.3 to 1,464,799 IU/mL, and five of these six were successfully genotyped. As HCV RNA was demonstrated in samples from both our seropositive and seronegative cadaver organ donors, we are now incorporating nucleic acid testing into our donor screening/diagnostic algorithm.

Keywords: cadaver organ donors, nucleic acid tests, screening/diagnostic algorithm, serologic tests, transcription-mediated amplification.

INTRODUCTION

Viral infection, including hepatitis C virus (HCV), has been transmitted through cadaver organ donors [1,2]. Indeed the likelihood of HCV transmission is greater from an organ donor than from a blood donor, because the prevalence of HCV infection is 10-fold higher in the former group [3–6]. The Food and Drug Administration (FDA) recommends that all human tissue intended for transplantation be procured from donors who are negative to antibodies for HCV. Currently most organ procurement organizations (OPOs) use serologic tests, specifically enzyme immunoassays followed by confirmatory recombinant immunoblot assays, to screen potential donors for HCV infectivity [7]. Although these tests are generally performed in the critical time period between donor identification and transplantation, they cannot confirm active infection and are therefore limited in their ability to determine donor infectivity.

In addition, serologic tests have other limitations. There is an average window period of 68 days between the time of HCV infection and the development of antibodies during which the serologic tests will be nonreactive [8]. Individuals who clear virus may remain antibody reactive for decades [9] during which time their serology tests will be reactive, but their nucleic acid tests will be nonreactive. Conversely, individuals recently infected or those unable to mount an antibody response may have nonreactive serology but will most likely have reactive nucleic acid tests. Also, both enzyme immunoassays and confirmatory recombinant assays may provide indeterminate results, necessitating additional

Abbreviations: HCV, hepatitis C virus; FDA, Food and Drug Administration; OPOS, Organ procurement organization; TMA, transcription-mediated amplification.

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testing [10]. Finally, not all serologic tests have been approved for use in cadaver samples and the accuracy of these tests may be affected by microbial contaminants, haemolysis, and/or haemodilution secondary to premortem resuscitation attempts [3,11].

Until recently, transmission of HCV infection to transplant recipients was thought only to occur from seropositive donors [12]. However, the Centres for Disease Control and Prevention have documented HCV transmission from a seronegative donor whose premortem serum later showed detectable HCV RNA by nucleic acid testing [1] and a recent French study documented detectable HCV RNA among 0.2% of their HCV seronegative organ donors [13]. The prevalence of viraemia among seronegative cadaver donors in the U.S. has yet to be determined, and to date no test that might identify these individuals has been incorporated in the donor screening algorithm.

Currently, nucleic acid testing and core antigen testing are the only methodologies capable of confirming active HCV infection as the former directly detects the RNA or the virus [10] and the latter detects the antigen [14]. Minipool nucleic acid testing is now routine for blood transfusion services and has helped prevent the transmission of over 50 HCV infections annually in the US [15]. Although the need for this state-of-the-art testing in the transplant setting has been recognized [11], it has yet to be universally adopted for individual screening of potential cadaver organ donors because of the need to test samples on an individual basis and the limited time allowed between organ procurement and transplantation. The FDA recently proposed nucleic acid testing for living donors using FDA-licensed nucleic acid blood screening tests for HCV and HIV and may extend this recommendation to cadaver donors in the future [16]. In the past few years, the VERSANT® HCV Qualitative Test based on transcription-mediated amplification [HCV Qual transcription-mediated amplification (TMA)] (manufactured by Gen-Probe, San Diego, CA, USA and marketed by Bayer Healthcare LLC, Tarrytown, NY, USA) has been introduced to aid diagnosis of active HCV infection and monitoring of treatment and follow-up. This assay can be completed in just over 5 h, has a sensitivity of ≤9.6 IU/mL, and it is not affected by any of the inhibitors found in cadaver blood samples [17–20].

At the National Institute of Transplantation in Los Angeles, we screen sera from cadaver organ donors sent from several California OPOs. We are continually looking for ways to improve our ability to determine the potential infective status of donors prior to transplantation. In the past we have successfully used the HCV Qual TMA assay to detect viraemia in both our HCV seropositive and seronegative dialysis patients [21]. In this study, we assessed the performance and practicality of using the HCV Qual TMA assay to detect active HCV infection in cadaver organ donors. We determined the range of viral levels in samples from seropositive and seronegative organ donors with detectable HCV RNA and assessed the HCV genotype of the samples.

MATERIALS AND METHODS

Study design

Between November 2001 and February 2004, sera from 1445 potential organ donors were screened at the National Institute of Transplantation laboratories, Los Angeles, California. In almost all cases, specimens were obtained from heart-beating donors. All samples were tested for anti-HCV antibodies; 726 samples had sufficient volume for additional testing and were also tested for the presence of HCV RNA as described below. HCV genotype and quantitative HCV RNA testing was performed on samples with detectable HCV RNA when sufficient sample volume was available. Qualitative HCV RNA testing was performed in batch on a weekly basis. None of the assays used in this study have been approved for use on cadaver samples. Results from these tests were not intended for patient management. The study samples were not originally linked to the donors and the laboratory was blinded both to donor identity and recipient outcome. Results of nucleic acid testing were reported to the OPOs near study completion. Institutional Review Board approval was granted for additional antibody and HCV RNA testing of donor samples.

Anti-HCV antibody screening methods

Nine hundred and sixty-four unique specimens were tested with the Abbott HCV EIA-2.0 (EIA-2.0; Abbott Laboratories, Chicago, IL, USA) between November 2001 and October 2002, and 481 unique specimens were tested with the Ortho® HCV Version 3.0 ELISA (ELISA-3.0; Ortho-Clinical Diagnostics, Raritan, NJ, USA) between October 2002 and February 2004. In addition, 170 of the 964 specimens originally tested with the EIA-2.0 were subsequently retested with the ELISA 3.0. Results from 45 samples reactive by either assay and confirmed with the supplemental recombinant immunoblot assay, Chiron RIBA®HCV 3.0 SIA (RIBA-3.0; Chiron Corporation, Emeryville, CA, USA) were available.

Qualitative HCV RNA testing

All specimens with sufficient volume (63 seropositive specimens and 663 seronegative specimens) were tested for the presence of HCV RNA with the HCV Qual TMA assay, including 199 specimens that were tested by EIA-2.0 only, 357 specimens tested by ELISA-3.0 only, and 170 specimens tested by both EIA-2.0 and ELISA-3.0. We have no reason to believe that the 663 seronegative specimens with sufficient volume for testing represented significantly different donors than the 705 seronegative specimens with insufficient

volume. The assay, which is based on TMA, was performed according to the manufacturer's instructions. Data are reported both as calculated relative light units and as signal-to-cut-off ratios where a specimen is considered reactive, or having detectable HCV RNA, when the signal-to-cut-off ratio is >1. The assay has a 95% limit of detection of ≤9.6 IU/mL, and its design and performance have been described [17,22–24]. The HCV Qual TMA assay is not inhibited by heparin, a substance found in cadaveric samples which inhibits PCR reactions [19,25].

All samples that gave discordant results (i.e. seropositive/HCV RNA nonreactive, or seronegative/HCV RNA-reactive) were retested in duplicate with the HCV Qual TMA assay and, when sufficient volume was available, with ELISA-3.0.

Quantitative HCV RNA testing

Seventeen seropositive and six seronegative samples with sufficient volume also were assessed by the VERSANT®HCV 3.0 (bDNA) Assay (Bayer Healthcare LLC) according to manufacturer's instructions. This assay has a quantification range of 615–7,770,000 IU/mL and has been previously described [26].

Five of the six seronegative specimens with detectable HCV RNA by the HCV Qual TMA assay were assessed also by the HCV TMA Quantitative Assay, an analyte specific reagent assay that was validated at the Bayer Reference Testing Laboratory (BRTL, Berkeley, CA, USA) using samples from live donors. This assay has a quantification range of 9.3–3,571 IU/mL (BRTL, unpublished observations). This assay is distinct from the HCV Qual TMA assay which is approved for *in vitro* diagnostic use by clinical professionals.

HCV genotyping

Specimens from seropositive and seronegative donors with detectable HCV RNA and sufficient volume were genotyped using the VERSANT® HCV LiPA assay (Bayer Healthcare LLC) which is for research use only, following HCV RNA detection with the HCV Qual TMA assay as previously described [27].

RESULTS

Among the 1445 samples from potential organ donors screened at our centre by EIA-2.0 and/or ELISA-3.0, 77 (5.3%) were seropositive and 1368 (94.7%) were seronegative. The 5.3% prevalence of seropositivity observed among our donor population is consistent with the 5.1–5.7% prevalence reported for other cadaver donor populations [28–30].

Concordant results between EIA-2.0 and ELISA-3.0 were observed for all but one of the 170 samples that were tested by both assays (99.4% concordance) The single discordant sample tested seronegative by EIA-2.0 but seropositive by

ELISA-3.0, and was considered seropositive for the purpose of this study. This sample (#2900) also had undetectable HCV RNA by HCV Qual TMA. This pattern of discordant test results may indicate that the donor had an HCV infection in the distant past, which resulted in waning antibody and serum viral clearance [9].

A total of 726 samples had sufficient volume to test for the presence of HCV RNA. As shown in Table 1, HCV RNA was detected in samples from both seropositive and seronegative organ donors. Sixty-three seropositive donors had sufficient volume for additional testing and 52 of 63 (82.5%) had detectable HCV RNA. Results from RIBA-3.0 testing were obtained for 45 of these seropositive samples. As shown in Table 2, HCV RNA was detected in 37 samples that were confirmed seropositive by RIBA-3.0 as well as in two samples that gave indeterminate RIBA-3.0 results.

The HCV RNA was not detected among the vast majority of seronegative donors (99.1%), although six were found to be viraemic (0.9%, 95% CI 0.4–1.4%). Table 3 summarizes the complete test results for these six samples. Five samples that were seronegative by ELISA-3.0 had signal-to-cutoff ratios ranging from 0.01 to 0.11. The remaining sample (#3424) had an EIA-2.0 signal-to-cutoff ratio of 0.16. The presence of HCV RNA in all six samples was confirmed by repeat testing with HCV Qual TMA yielding signal-to-cutoff ratios of eight or above, which are considered strongly positive, although signal-to-cutoff ratios are not linearly correlated with the amount of virus present. Moreover, HCV genotypes were determined for five of these samples further verifying the presence of HCV RNA. The viral level in sample

Table 1 Correlation of hepatitis C virus (HCV) Qual transcription-mediated amplification and EIA-2.0/ELISA-3.0 results

	Seropositive by EIA-2.0 and/or ELISA-3.0 (n = 63)	Seronegative by EIA-2.0 and/or ELISA-3.0 (n = 663)
HCV RNA reactive	52 (82.5%)	6 (0.9%)
HCV RNA nonreactive	11 (17.5%)*	657 (99.1%)

*Includes one sample that was seropositive by ELISA-3.0 but seronegative by EIA-2.0.

Table 2 Correlation of hepatitis C virus (HCV) Qual transcription-mediated amplification and RIBA-3.0 results

	Seropositive by RIBA-3.0 (n = 41)	Indeterminate by RIBA-3.0 (n = 4)
HCV RNA reactive	37 (90%)	2 (50%)
HCV RNA nonreactive	4 (10%)	2 (50%)

Table 3 Characterization of seronegative specimens with detectable hepatitis C virus (HCV) RNA

| Specimen no. | EIA-2.0 (signal-to-cutoff ratio) | ELISA-3.0 (signal-to-cutoff ratio) | HCV Qual TMA§ | | HCV RNA TMA quantitative (IU/mL) | HCV 3.0 (bDNA) (IU/mL) | HCV genotype |
			Initial test (signal-to-cutoff ratio)	Repeat test (signal-to-cutoff ratio)			
# 3043	NT	0.11	4	22	9.4	<615	2b
# 3109	0.26	0.02	20	20	18.5	<615	1
# 3113	0.11	0.03	20	20	4120	11 961	1
# 3116	0.20	0.01	21	8	<9.3	<615	†
# 4119	NT	0.02	22	23	NT*	1 464 799	1
# 3424	0.16	NT	24	24	<9.3	<615	3a

NT, not tested.

*Specimen 4119 did not have sufficient volume for testing with the HCV RNA TMA Quantitative assay.

†no HCV genotype could be assigned to specimen 3116, which gave an indeterminate pattern upon repeat testing with the VERSANT® HCV LiPA assay.

§A signal-to-cutoff ratio of 3 or more is considered strongly reactive, although this ratio does not correlate linearly with the amount of virus in the sample.

#3116 may have been too low to obtain a genotype as it was below the detection limit of the HCV TMA Quantitative assay. This pattern of discordant results may indicate active HCV infection with a very low viral load, which could be observed either in an immunosilent carrier or in acute infection prior to the viral ramp-up phase that occurs before seroconversion [8, 15].

Sufficient volume was available to evaluate genotype in the six seronegative/HCV RNA reactive samples as well as in 51 of the seropositive samples in which HCV RNA was detected. As shown in Table 4, HCV genotype was successfully determined for 56 of the 57 samples tested. As expected for a North American patient population, the majority of the samples were genotype 1 and most of the remaining samples were genotypes 2 and 3. A single sample of genotype 4 and a single sample of mixed genotype were also observed.

A total of 23 samples with detectable HCV RNA by HCV Qual TMA had sufficient volume for quantitative HCV RNA testing. As shown in Fig. 1, the 17 seropositive samples showed HCV RNA concentrations ranging from 128,123 to >7,692,316 IU/mL, and the six seronegative samples showed HCV RNA concentrations ranging from <9.3 to 1,464,799 IU/mL. Overall, the seronegative samples had viral loads that were 3–4 $\log_{10}$ values lower than those of the seropositive samples.

DISCUSSION

As the Centres for Disease Control reported HCV transmission to multiple organ recipients from a seronegative donor who was subsequently shown to have detectable HCV RNA by a nucleic acid test, the current practice of screening organ

Table 4 Genotype distribution among seropositive and seronegative samples with detectable hepatitis C virus RNA

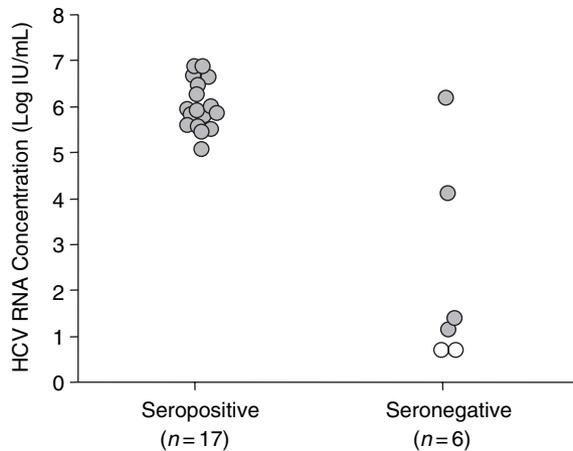| Genotype and subtype	No. seropositive samples	No. seronegative samples	Total no. (% of genotyped samples)
Genotype 1	41	3	44 (78.6%)
Subtype 1a	2	0	–
Subtype 1b	17	0	–
Subtype 1a/1b	1	0	–
Subtype not obtained	21	3	–
Genotype 2	4	1	5 (8.9%)
Subtype 2b	1	1	–
Subtype not obtained	3	0	–
Genotype 3a	4	1	5 (8.9%)
Genotype 4	1	0	1 (1.8%)
Mixed genotype 1a/1b/3a	1	0	1 (1.8%)
Genotype not determined	0	1	–

Fig. 1 Viral load distribution of specimens that had detectable hepatitis C virus (HCV) RNA by HCV Qual TMA. HCV RNA levels in all 17 seropositive specimens and two of the seronegative specimens were quantified with the VERSANT HCV 3.0 (bDNA) assay. HCV RNA levels in two of the remaining four seronegative specimens were quantified with the HCV RNA TMA Quantitative Assay. Filled circles indicate specimens with quantifiable HCV RNA; open circles indicate specimens that were below the quantification limit (9.3 IU/mL) of the HCV RNA TMA Quantitative Assay.

donors only with serologic tests has come under scrutiny [1]. That this current practice may be inadequate is supported by our findings of six of 663 (0.9%, 95% CI 0.4–2.2%) seronegative donors with detectable HCV RNA. Our findings agree with a recent French study using a similar nucleic acid test to document HCV RNA in five of 2119 (0.2%, 95% CI 0.09–0.4) seronegative donors [13]. The higher prevalence of viraemic seronegative donors in our population may in part be explained by the higher prevalence of HCV antibodies in both the general US and our cadaver donor populations compared with the comparable French populations (1.8 and 5.3% *vs* 1.1 and 4.4%, respectively) [13, 31]. Moreover, our study used the HCV Qual TMA assay with a limit of detection of ≤9.6 IU/mL, whereas the French study used the Procleix® HIV-1/HCV Assay manufactured by Gen-Probe and marketed by Chiron Blood Testing (Emeryville, CA, USA). The Procleix assay, which also is based on TMA, has a limit of detection of 50 IU/mL for HCV RNA and has been approved for use in both heart beating and nonheart beating donors [32]. Had we detected only samples with viral loads of 50 IU/mL or higher in our study, we theoretically would not have detected HCV RNA in four of the six seronegative/HCV RNA reactive donors and the prevalence of viraemia among our seronegative donors would have been 0.3%, almost identical to that found in France.

We believe the seronegative/HCV RNA-reactive donor samples from both the French study and our study to be true positives for a number of reasons. First the nucleic acid tests used in these studies, both of which are based on TMA and

manufactured by Gen-Probe, have been shown to be highly specific. The HCV Qual TMA assay used in our study has a reported specificity of 99.6% that was demonstrated by testing approximately 2500 seronegative samples [17]. The Procleix assay used in the French study has also been shown to be highly specific through testing of approximately 37 million units of blood from major laboratories in the US [15]. Even though up to 0.4% of specimens identified by the HCV Qual TMA as HCV RNA reactive could be false positives, we have additional reasons to believe that the seronegative viraemic specimens identified among our donor population likely represent true positives. Five of six seronegative viraemic specimens were tested with ELISA-3.0 and three of six had sufficient volume for testing by both EIA-2.0 and ELISA-3.0. We confirmed our nucleic acid test results in duplicate, using separate aliquots of all six of these discordant samples. Also, HCV genotypes were determined for all but one of the six seronegative/HCV RNA reactive samples, and the virus was quantified using branched DNA technology in two of these six samples and by a different TMA-based assay in the remaining four.

The commonly cited explanation for the finding of viraemia in seronegative specimens is that these specimens may have been obtained from an individual in the window period. However, the estimated prevalence of viraemia among our seronegative donors (0.9%, CI 0.4–2.2) and that of the French study (0.2%, CI 0.09–0.4) is difficult to explain using data based on volunteer blood donors estimated to be in the window period. One would have to assume that far more potential organ donors are in the window period than the reported *c.* 0.0005% of volunteer blood donors [5]. This may be in part true as, unlike blood donors, cadaver organ donors cannot be directly screened for life style factors and have higher prevalence than blood donors of risk factors for parenterally transmitted viruses, such as unsuspected intravenous drug use or sexual promiscuity [28]. In one study, intravenous drug users were found to have a long seronegative window period (range 13–94 months, mean 40.8 months) prior to seroconversion [33]. Following exposure, these infected individuals were found to have low, transiently detectable levels of HCV RNA which were thought to be insufficient for antibody production. However, aside from donors being in the window period, there could be other factors that may explain the lack of detectable antibody in the cadaver organ donor population.

There are other examples of seronegative, viraemic individuals in the literature. In a US study of community acquired hepatitis C, 10% of the identified adults lacked HCV antibodies but had evidence of HCV infection [34]. HCV infection acquired in infancy may persist for years without the development of antibodies [34]. Investigators also have documented a chronic healthy carrier HCV state in volunteer blood donors with no risk factors for hepatitis and no evidence of antibodies over several years [35,36]. Recently three of 67 (4%) of HCV seronegative/HCV RNA-reactive

blood donors followed from between 1.5 to over 3 years did not show ALT elevations nor did they seroconvert [15]. Any of the four seronegative donors in our study with low viral loads might represent such immunosilent infected donors; however, without serial samples from these donors it is impossible to ascertain whether they are silent carriers or are newly infected who have not yet reached the ramp-up phase normally observed in the window period. One donor had a very high viral load which is characteristic of the postramp-up phase of the window period. It is possible that some of these seronegative/HCV RNA-reactive samples could represent false negative antibody tests as none of the antibody tests used in this study have been approved for use in cadaver samples, which may be haemodiluted, haemolysed, or contain microbial contaminants [3,11,37].

Our study also estimated the prevalence of active infection among our seropositive organ donors in the US to be 82.5%, which is within the 57–96% range previously reported among cadaver organ donors [11] and commensurate with the reported *c.* 80% rate of chronic infection among the general infected population [38]. Among our seropositive cadaver organ donor population who had sufficient volume for additional testing, 17.5% were not viraemic. At present the safety of transplanting organs from seropositive/HCV RNA nonreactive donors has not been determined. One study demonstrated that both seropositive and seronegative recipients of seropositive organs had decreased survival. However, the study did not discriminate between viraemic and nonviraemic seropositive donors [39]. Although the likelihood of HCV transmission from an HCV RNA-nonreactive donor is low [40], HCV transmission has been reported from a donor probably in the very early stage of infection whose serum was HCV RNA-nonreactive by HCV Qual TMA, but who later seroconverted [41]. To date, there is no single test to determine if a patient has truly cleared virus or is harbouring an occult infection that might be transmitted.

Current practice does not include nucleic acid or core antigen testing to screen potential cadaver organ donors because of the expense, time and difficulty of testing a single sample within a few hours of receipt. Prior to the CDC report, it was believed that a negative result from an EIA-2.0 test was sufficient to exclude possible HCV infectivity from a potential donor. A previous, small study using a home made PCR of unknown sensitivity did not detect HCV RNA among EIA-2.0 seronegative cadaver donors [12]. In light of our findings and those of the French study, the use of nucleic acid testing to screen potential organ donors should be considered. Nucleic acid testing has been shown to reduce the window period to 7 days for HCV and should greatly reduce the probability of donor viraemia if used to screen individual donors [4]. In addition, the nucleic acid test used in this study can be performed in just over 5 h. If started simultaneously with an ELISA test, then information regarding both the anti-HCV antibody and active infective status of the donor could be delivered to the OPO within our current 8-h target. Core antigen testing for HCV also has been shown to shorten the window period and might appear an attractive alternative, as the core antigen EIA is somewhat easier to perform than nucleic acid testing [42,43]. However, the core antigen test has an estimated limit of detection of 27,000 IU/mL [14], which is considerably less sensitive than either the HCV Qual TMA or the Procleix assay. Moreover, the core antigen test missed 7.5% of specimens shown to be viraemic by HCV Qual TMA in a large collection of seropositive samples. [14].

In summary, we have demonstrated both the utility and the feasibility of using nucleic acid tests, specifically HCV Qual TMA, to confirm active infection in cadaver organ donors. Our findings of detectable virus in both the seropositive and seronegative donor populations suggest the time has arrived for nucleic acid testing of cadaver organ donors, and we are in the process of incorporating nucleic acid testing into our screening/diagnostic algorithm. It is expected that the automation of nucleic acid tests will improve in the coming years, making these tests more suited for the rapid turnaround requirements of the pretransplant setting. In the near future, organ donor screening should reach the same standard as blood donation screening.

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