Multicenter Beta Trial of the GeneXpert Enterovirus Assay[▽]

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The GeneXpert Dx system (Cepheid, Sunnyvale, CA) is a fully integrated and automated nucleic acid sample preparation, amplification, and real-time detection system. It consists of an instrument, a personal computer, and disposable fluidic cartridges. The analytical sensitivity and specificity of the GeneXpert enterovirus assay (GXEA) were determined with a panel of 63 different enterovirus serotypes and 24 other microorganisms, respectively. The potential for blood, hemoglobin, white blood cells, and excess protein to interfere with the assay was also assessed. The performance parameters of the GXEA were determined at three sites with 102 cerebrospinal fluid (CSF) samples obtained from patients with suspected meningitis. All samples were tested for enterovirus RNA with locally developed reverse transcription-PCR (RT-PCR) assays at the trial sites and with a seminested RT-PCR and an analyte-specific reagent (Cepheid) at a reference laboratory. The 5' nontranslated region was the target for all of the PCR assays except the seminested RT-PCR, which amplified a VP1 sequence. The VP1 amplicon was sequenced to identify the enterovirus types. Consensus reference laboratory RT-PCR results were used to classify cases of enteroviral meningitis. The GXEA detected all of the enterovirus serotypes and none of the other microorganisms tested except rhinovirus 16. The assay was unaffected by moderate amounts of blood or blood components. Thirty-six (35%) of the CSF samples tested had at least one positive PCR result. Eleven different enterovirus serotypes were identified in the positive samples. The GXEA had a sensitivity of 97.1% (95% confidence interval [CI], 84.7 to 99.9%) and a specificity of 100% (95% CI, 94.6 to 100%) for the diagnosis of enteroviral meningitis.

Enteroviruses are responsible for a wide variety of diseases in children and adults, including nonspecific febrile illnesses and meningitis (14). These infections are very common, with an estimated 5 to 10 million symptomatic infections occurring annually in the United States, primarily in infants and young children (15). Enteroviruses may cause up to 90% of aseptic meningitis cases for which an etiology is identified. Occurring mainly in the summer and fall, enteroviral meningitis leads to a large number of hospitalizations of both children and adults. Enteroviral meningitis may be difficult to differentiate from partially treated bacterial meningitis because the cerebrospinal fluid (CSF) pleocytosis may have an early predominance of neutrophils. Many patients with enteroviral meningitis are hospitalized and treated with parenteral antibiotics until the clinical picture improves and the bacterial cultures of blood and CSF are negative after 48 h of incubation.

CSF viral cultures lack sensitivity, rarely provide results in a clinically relevant time frame, and require high-level technical expertise to perform. Nucleic acid amplification tests (NATs) for enterovirus RNA in CSF have emerged as the new gold standard for diagnosis of enteroviral meningitis (10–13). These tests have better sensitivity than culture, the results can be available within hours of specimen collection, and the costs are similar to those for viral cultures (9).

Significant health care resources are utilized in diagnosing

and caring for infants and young children with enteroviral infections. Several investigators have demonstrated that early diagnoses provided by NATs can improve the management and decrease the costs of caring for children with enteroviral meningitis by decreasing length of hospital stay, hospital charges, and antibiotic use (2, 5, 7, 8). Despite the enhanced performance characteristics and potential for improving the paradigms for diagnosis and patient management, there are no FDA-approved tests for enterovirus RNA.

The GeneXpert Dx system performs hands-off sample processing and real-time, multiplex PCR for detection of DNA or RNA. In this platform, sample preparation, amplification, and real-time detection are all fully automated and completely integrated. The system consists of an instrument, a personal computer, and disposable fluidic cartridges that have been designed to complete sample preparation and reverse transcription-PCR (RT-PCR) of enteroviruses in about 2.5 h (Fig. 1). Each instrument contains four randomly accessible modules that are each capable of performing separate sample preparation and RT-PCR tests. Each module contains a syringe drive for dispensing fluids, an ultrasonic horn for lysing cells or spores if necessary, and an ICORE thermocycler for performing reverse transcription, real-time PCR, and amplicon detection.

The patented single-use cartridges contain the following: 11 chambers for holding sample, reagents, or other materials; a valve body composed of a plunger and syringe barrel; a rotary valve system for controlling the movement of fluids between chambers; a capture matrix for binding the RNA; a sample preparation control in the form of a dry bead; dry reagents for

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FIG. 1. The GeneXpert Dx diagnostic system (top) and Xpert enterovirus self-contained cartridge (bottom).

reverse transcription and real-time PCR; and an integrated PCR tube that can be automatically filled by the instrument. To eliminate test-to-test contamination, all fluids including amplicons are contained within the disposable cartridge. The instrument never comes into contact with any fluids within the cartridge. Up to four different targets or groups of targets can be simultaneously detected in each sample by employing multiplex PCR techniques and real-time fluorescence technologies such as TaqMan, molecular beacon, or scorpion probes.

In this study, the analytical sensitivity and specificity of the GeneXpert enterovirus assay (GXEA) were determined with a

panel of 63 different enterovirus serotypes and 24 other microorganisms, respectively. The potential for blood and blood components to interfere with the assay was investigated. The performance parameters of the GXEA were also determined at three sites with CSF samples that were obtained from patients with suspected meningitis. All CSF samples were also tested for enterovirus RNA with locally developed RT-PCR assays at the trial sites and with a seminested RT-PCR (RT-snPCR) method and an analyte-specific reagent (Cepheid) at a reference laboratory.

(The results of this study were presented in part at the 106th

General Meeting of the American Society for Microbiology, Orlando, FL, 21 to 25 May 2006 [3a, 13a].)

MATERIALS AND METHODS

Analytical sensitivity. Prototypes of 63 different enterovirus serotypes were obtained from the ATCC and CDC (Table 1). Virus stocks were 10-fold serially diluted in enterovirus-negative CSF, and the least amount of virus detected by the GXEA was determined. The results were recorded as the lowest detectable log₁₀ dilution and either the 50% tissue culture infectious dose (TCID₅₀) or the 50% lethal dose (LD₅₀)/ml and are the averages from three runs.

Analytical specificity. Nucleic acids were isolated from organisms known to cause meningitis-like symptoms and tested for cross-reactivity at approximately 10⁶ copies/test, using the same reagents employed in the GXEA and a Smart-Cycler (Cepheid). In addition, suspensions of intact organisms were made in enterovirus-negative CSF and tested with the GXEA cartridge. The panel included Epstein-Barr virus, herpes simplex virus type 1, herpes simplex virus type 2, human herpesvirus 6, human herpesvirus 7, varicella-zoster virus, adenovirus type 2, measles virus, mumps virus, parainfluenza virus type 1, parainfluenza virus type 2, parainfluenza virus type 3, influenza A virus, influenza B virus, *Streptococcus agalactiae*, *Haemophilus influenzae* type b, *H. influenzae* non-type b, *Escherichia coli*, *Neisseria meningitidis*, *Citrobacter freundii*, and *Citrobacter koseri*. Rhinoviruses 7 and 16 were also tested because of the known nucleic acid sequence homology of the 5' nontranslated regions (NTRs) of rhinoviruses and enteroviruses.

Interfering substances. Samples positive for coxsackievirus A9 at the limit of detection were spiked with increasing concentrations of substances commonly found in CSF specimens. These substances included whole blood (5,000 to 2.5 × 10⁶ red blood cells/mm³), hemoglobin (0 to 3.6 g/dl), white blood cells (0 to 7,140 cells/mm³), and protein (bovine serum albumin and gamma globulin) (0 to 1,071 mg/dl).

GXEA. Binding, wash, elution, and lysis buffers were added to the appropriate ports on the microfluidic cartridge. After addition of the buffers, 140 µl of CSF was added to sample chamber. The cartridge was then loaded into a GeneXpert Dx module to complete sample preparation, target amplification, and product detection. The total analysis time was approximately 2.5 h. The assay targets the 5' NTR of the enterovirus genome (nucleotides 452 to 596), and the primer and probe sequences are shown in Table 2. Any samples with values exceeding the preset fluorescence threshold were considered positive, and the cycle threshold (C_T) was recorded.

The assay includes a sample preparation control/internal control to verify adequate processing of the target virus and to monitor for the presence of assay inhibitors. It consists of an armored RNA (Ambion, Austin, TX) containing a proprietary sequence. The amount is strictly controlled and optimized so that it does not affect target sensitivity but is responsive to the presence of inhibitors. The assay also includes a probe check control step to verify reagent rehydration, probe integrity, and reaction tube filling in the cartridge. Prior to thermal cycling, fluorescence readings are obtained from the TaqMan probe at multiple temperatures, and the readings are compared to the default setting established by the manufacturer. Positive and negative external controls provided by the manufacturer were run weekly to validate the performance of the system.

Locally developed RT-PCR assays. Locally developed RT-PCR assays for enterovirus RNA were run in parallel with GXEA at the three beta trial sites. These locally developed assays were the standards of care at the trial sites. Sites A and B used the QIAGEN QIAmp viral RNA minikit (QIAGEN, Valencia, CA), and site C used MagNA Pure LC (Roche Applied Science, Indianapolis, IN) for nucleic acid extraction. The target for all of the locally developed assays was the 5' NTR of the enterovirus genome. An end point RT-PCR (Light Diagnostics Pan-Enterovirus OligoDetect assay; Chemicon, Temecula, CA) was used at site A, and real-time RT-PCR was used at sites B and C. The primer and probe sequences used in the assays performed at sites B and C are shown in Table 2. The sequences targeted in the Chemicon assay are proprietary. The thermal cyclers employed were a GeneAmp 9600 (Applied Biosystems, Foster City, CA) at site A, a SmartCycler (Cepheid) at site B, and ABI 7000 and 7500 instruments (Applied Biosystems, Foster City, CA) at site C.

Reference laboratory RT-PCR assays. Frozen aliquots of CSF samples were tested at the CDC using two different NATs, an enterovirus primer and probe set analyte-specific reagent (ASR) (Cepheid), and an RT-snpPCR. RNA was extracted for both with QIAGEN QIAmp viral RNA minikit.

The ASR consists of two types of lyophilized beads, the enterovirus bead and the sample preparation control bead. The enterovirus beads contain primers, a 6-carboxyfluorescein-labeled probe, and an Alexa 532-labeled probe for detection of the 5' NTR. The primer and probe sequences used in the ASR are shown in

TABLE 1. Limits of detection for GXEA, expressed as lowest dilution and lowest nominal concentration detected for each member of the analytical sensitivity panel

Species	Serotype	Lowest detectable log ₁₀ dilution	Concn (TCID ₅₀ or LD ₅₀ /ml ^a)
A	Coxsackievirus A2	-8	NA ^b
	Coxsackievirus A3	-4	5.01
	Coxsackievirus A4	-6	NA
	Coxsackievirus A5	-7	12.59
	Coxsackievirus A6	-7	12.59
	Coxsackievirus A7	-8	3.33*
	Coxsackievirus A8	-8	NA
	Coxsackievirus A10	-7	2.81
	Coxsackievirus A12	-5	19.95
	Coxsackievirus A14	-6	0.1
	Coxsackievirus A16	-6	0.02
	Enterovirus 71	-6	0.16
B	Coxsackievirus A9	-8	20
	Coxsackievirus B1	-8	4
	Coxsackievirus B2	-9	0.02
	Coxsackievirus B3	-9	0.03
	Coxsackievirus B4	-8	0.4
	Coxsackievirus B5	-8	0.04
	Coxsackievirus B6	-8	0.01
	Echovirus 1	-8	0.1
	Echovirus 2	-8	0.03
	Echovirus 3	-7	200
	Echovirus 4	-8	0.00032
	Echovirus 5	-8	0.032
	Echovirus 6	-6	200
	Echovirus 7	-8	2
	Echovirus 9	-8	2
	Echovirus 11	-7	40
	Echovirus 12	-7	1.58
	Echovirus 13	-7	0.01
	Echovirus 14	-7	0.0005
	Echovirus 15	-7	0.0032
	Echovirus 16	-7	0.0005
	Echovirus 17	-7	0.05
	Echovirus 18	-8	0.0002
	Echovirus 19	-6	2.51
	Echovirus 20	-7	0.032
	Echovirus 21	-6	1
	Echovirus 24	-7	0.02
	Echovirus 25	-6	0.05
	Echovirus 26	-6	0.032
	Echovirus 27	-7	0.00032
	Echovirus 29	-6	5.01
	Echovirus 30	-7	0.005
	Echovirus 31	-7	0.0032
	Echovirus 32	-5	0.1
	Echovirus 33	-7	0.05
	Enterovirus 69	-8	0.0002
C	Coxsackievirus A13	-6	13.3*
	Coxsackievirus A11	-6	0.105*
	Coxsackievirus A15	-7	0.0032
	Coxsackievirus A17	-6	1.58
	Coxsackievirus A18	-7	0.02
	Coxsackievirus A19	-8	0.03*
	Coxsackievirus A20	-7	0.0019
	Coxsackievirus A21	-7	0.03
	Coxsackievirus A22	-7	0.02
	Coxsackievirus A24	-7	0.1
D	Enterovirus 68	-4	200
	Enterovirus 70	-7	2
Poliovirus	Poliovirus 1	-8	2
	Poliovirus 2	-8	0.4
	Poliovirus 3	-8	20

^a *, LD₅₀/ml.

^b NA, not available.

Table 2. In addition, each enterovirus bead contains primers and a Texas Red-labeled probe for a separate sample-processing control sequence. The sample-processing control bead contains armored sample preparation control RNA, RNase inhibitor, and poly(A) RNA. RT-PCR was performed with the SuperScript One-Step kit (Invitrogen, Carlsbad, CA) on a SmartCycler (Cepheid) real-time PCR instrument according to the manufacturers' instructions. If the 5'

TABLE 2. Nucleic acid sequences and locations of the primers and probes used in the enterovirus 5' NTR RT-PCR assays

Assay	Primer or probe	Nucleotide sequence (5' to 3')	Position ^a
Site B	Forward primer	GGC CCC TGA ATG CGG CTA AT	449–468
	Reverse primer	ATT GTC ACC ATA AGC AGC CA	577–596
	Probe	AAA CAC GGA CAC CCA AAG TAG TCG G	537–561
Site C	Forward primer	GCC CCT GAA TGC GGC TAA	450–467
	Reverse primer	ATT GTC ACC ATA AGC AGC CA	577–596
	Probe	AAA CAC GGA CAC CCA AAG TAG TCG	538–561
ASR	Forward primer	CCC CTG AAT GCG GCT AAT C	451–469
	Reverse primer	AAG GAA ACA CGG ACA CCC AA	546–565
	Probe A	TCC GCC ACA GAC TTG CGC ATT ACG A	511–535
	Probe B	TCC GCT GCA GAG TTG CCC GTT ACG A	511–535
GXEA	Forward primer	CCC TGA ATG CGG CTA ATC C	452–470
	Reverse primer	ATT GTC ACC ATA AGC AGC CA	577–596
	Probe	AAA CAC GGA CAC CCA AAG TAG TCG G	537–561

^a Poliovirus genome numbering (GenBank accession no. NC_002058).

NTR sequence is present in a sample, it will be detected by either the 6-carboxyfluorescein probe or the Alexa 532 probe but not by both. The sample-processing control sequence will be detected by the Texas Red probe in all adequately processed samples.

Enterovirus virion protein 1 (VP1) sequences were amplified using an RT-snPCR as previously described (4). The 350- to 400-bp PCR amplicons were separated and visualized on agarose gels containing ethidium bromide and then were purified from the gel for sequencing. The amplicons were sequenced with the BigDye Terminator v1.1 Ready Reaction cycle sequencing kit on an ABI Prism 3100 automated sequencer (Applied Biosystems, Foster City, CA). Amplicon sequences were compared with the VP1 sequences of enterovirus reference strains by script-driven sequential pairwise comparison using the program Gap (Wisconsin Sequence Analysis Package, version 10.2; Accelrys, Inc., San Diego, CA), as described previously (6).

Beta trial protocol. CSF samples of ≥ 500 μ l that were submitted to trial sites for enterovirus RT-PCR were included in the study. The samples were collected from patients at the trial sites in Atlanta, GA; Dallas, TX; and Denver, CO during May, June, and July 2005. Two separate 150- μ l aliquots of study samples were made and frozen at -20°C for subsequent testing. Each sample was tested at the trial site with its own locally developed RT-PCR and with the GXEA. The third aliquot was sent to the reference laboratory for testing with the SmartCycler ASR and RT-snPCR for enterovirus RNA. The GXEA, SmartCycler ASR, and RT-snPCR were performed within the same freeze-thaw cycle of the sample. Consensus reference laboratory results were used to classify cases of enteroviral meningitis. The reference laboratory tests were used to classify cases based on the requirements for superior analytical sensitivity (ASR) and amplification of an alternative target site (VP1). No other patient information or results of any other laboratory tests were recorded. The trial was conducted with the approval or exemption of the local Institutional Review Boards at the trial sites and reference laboratory.

RESULTS

The GXEA detected enterovirus nucleic acid from all of the 63 enterovirus serotypes tested, with limits of detection ranging from 0.0002 to 200 TCID₅₀/ml or LD₅₀/ml (Table 1). When coxsackievirus A9 was used as a template, the GXEA detected an 8-log dynamic range of concentrations, with a limit of detection of 20 TCID₅₀/ml (data not shown). No cross-reactivity was found between the enterovirus primers and probes used in the GXEA and nucleic acid isolated from other microorganisms known to cause meningitis-like symptoms. However, positive results were obtained with rhinovirus 16. This cross-reaction resulted from homology of 5' NTR sequences of rhinovirus 16 and enteroviruses in the regions where the primers and probe bind. The GXEA tolerated up to 125,000 cells/

mm³ of whole blood, 7,140 cells/mm³ of white blood cells, 3.6g/dl of hemoglobin, and 1,071 mg/dl of excess protein before the inhibition of the internal control was observed.

Three tests for enterovirus RNA were performed on 102 CSF samples. The results of the locally developed RT-PCRs and GXEAs performed at the three trial sites are shown in Table 3. The total numbers of positive samples detected by the locally developed RT-PCRs and GXEA were 31 and 34, respectively. Complete concordance between the locally developed RT-PCRs and the GXEA was observed at sites A and C. The GXEA detected three additional positive samples at site B (GXEA C_T s, 30.3, 35.3, and 40.7). The three samples were retested at site B with the GXEA, and all were confirmed as positive. The GXEA sample preparation control reaction failed for one sample (0.98%). It was repeated, and fluorescent signals were obtained for both the enterovirus target (C_T , 34.3) and the internal control (C_T , 32.3). The overall agreement between the results of the locally developed RT-PCRs and the GXEA was 97.1%.

The numbers of positive samples detected at the reference laboratory by the RT-snPCR and ASR were 34 and 36, respectively. All of the ASR-positive samples were detected with the Alexa 532 probe. The C_T s for the two samples positive only with the ASR were 38.2 and 37.8. Both samples were tested again with both tests, and the initial results were confirmed. For two samples for which the reference laboratory tests dis-

TABLE 3. Results of enterovirus RNA tests performed at the beta trial sites

Trial site	No. of CSF samples tested	No. of positive enterovirus RNA tests	
		Locally developed RT-PCRs	GeneXpert
A	13	1	1
B	54	19	22
C	35	11	11 ^a
Total	102	31	34

^a Includes one sample with that was initially negative for enterovirus RNA but for which the test was repeated because of a sample preparation control failure.

TABLE 4. GeneXpert enterovirus assay agreement with reference laboratory tests for 102 CSF samples

Reference laboratory results	No. of samples		Total
	With GeneXpert result:		
	Positive	Negative	
SmartCyler ASR positive, RT-snPCR positive	33	1	34
SmartCyler ASR positive, RT-snPCR negative	1	1	2
SmartCyler ASR negative, RT-snPCR negative	0	66	66
Total	34	68	102

agreed, one was positive (C_T , 40.7) and one was negative with the GXEA. Sequence analysis of the VP1 product of the RT-snPCR was able to identify the enteroviruses present in 34 of the positive samples. The enterovirus type assignments were based on matches of >74% to reference sequences as follows: 14 for echovirus 30; 9 for echovirus 18; 2 for echovirus 6; 2 for enterovirus 71; and 1 each for echovirus 3, echovirus 5, echovirus 9, echovirus 11, echovirus 13, coxsackievirus B4, and coxsackievirus B5.

The agreement between the results of reference laboratory tests and the GXEA are shown in Table 4. The GXEA results were 99% concordant with the consensus reference test results. A single sample was positive with both the ASR and RT-snPCR and negative with the GXEA. Two of the three samples detected only by the GXEA at site B were positive with both of the reference tests, and the remaining sample was positive with only the ASR.

The performance characteristics of the GXEA for the diagnosis of enteroviral meningitis were determined by comparison with consensus reference laboratory test results. The two samples with discordant reference laboratory test results were considered indeterminate. The two indeterminate cases could not be resolved since no patient information was collected as part of this study, and these cases were excluded from the calculations. The GX test had a sensitivity of 97.1% (95% confidence interval, 84.7 to 99.9%), a specificity of 100% (95% confidence interval, 94.6 to 100%), a positive predictive value of 100%, and a negative predictive value of 98.5% for the diagnosis of enteroviral meningitis.

DISCUSSION

The GeneXpert Dx system is an important new development in the field of molecular diagnostics. It automates all of the steps of a NAT in a disposable, microfluidic cartridge. It is a moderately complex test, but is simple enough to be performed reliably by individuals without a background in nucleic acid diagnostics. Sample preparation reagents and sample are all that are required to be added to the cartridge by the user. It has independently controlled and operated analysis modules that facilitate testing of individual samples in a random-access mode. The test incorporates an internal control that ensures that the entire test system is functioning properly, and a probe check control step is performed before PCR to verify reagent

rehydration, probe integrity, and PCR tube filling in the cartridge. The system is uniquely suited for clinical applications of molecular diagnostics when on-demand testing and rapid-result capability are needed. The diagnosis of enteroviral meningitis is one such application.

The primers and probes used in the GXEA detected all of the serotypes of enterovirus tested, with an analytical sensitivity of as low as 0.0002 TCID₅₀/ml for some enterovirus serotypes. Among the 64 recognized enterovirus serotypes, only coxsackievirus A1 was not tested. The wide range of sensitivities observed with the different enteroviruses is likely due to the imprecision associated with biological methods of virus quantification rather than serotype bias of the GXEA. Enterovirus RNA reference standards are not currently available.

Nonspecific amplification of nucleic acid preparations from other organisms associated with central nervous system infections was not observed. The cross-reaction with rhinovirus 16 was not unexpected given the homology between some rhinoviruses and enteroviruses in the 5' NTR region. This homology and cross-reactions with rhinoviruses in RT-PCR assays that target similar regions in the 5' NTR have been noted by others (3, 4). Potential cross-reactions with rhinovirus are not a significant concern when testing CSF samples, because rhinoviruses do not cause infections of the central nervous system. This would, however, have a significant impact on testing of nasal or oropharyngeal samples.

Moderate levels of whole blood, white blood cells, hemoglobin, or excess protein did not adversely affect the GXEA. In addition, the internal positive control failed for only one CSF sample during the beta trial. On retesting, both the enterovirus target and the internal positive control were detected. Taken together, the data demonstrate the robust nature of the GXEA.

The GXEA was performed on CSF samples from 102 patients with suspected aseptic meningitis at three trial sites and the results compared to the results of three different RT-PCR assays for enterovirus RNA performed on the same samples. Consensus reference laboratory RT-PCR results were used to classify the patients. The GXEA results were highly concordant with the reference laboratory test results, with a sensitivity of 97.1% and a specificity of 100% for diagnosis of enteroviral meningitis. Although the beta trial was conducted over a single enterovirus season, 11 distinct enterovirus types were present in the samples, confirming the broad reactivity of the GXEA.

The major limitations of this beta trial include the relatively small number of positive samples and lack of clinical information on the patients, including other CSF parameters and clinical diagnoses. Viral culture was not included as a comparator because of its poor sensitivity and sample volume constraints. A larger full-scale clinical trial of the GXEA that addressed these limitations has recently been completed and the data submitted to the U.S. FDA for evaluation.

Although NATs for enterovirus RNA are used widely for the diagnosis of enteroviral meningitis, there are currently no FDA-cleared tests. In practice, a wide variety of laboratory-developed methods and commercial kits assays are in use. In a recent College of American Pathologists (CAP) proficiency testing survey (2006 ID-03), 63% of the 83 participant laboratories used laboratory-developed methods. This lack of standardization leads to concerns about the interlaboratory repro-

ducibility of test results. In a proficiency testing study of nucleic acid amplification methods for detection of enterovirus that involved 59 European laboratories, 34% were found to perform inadequately (16). In contrast, only 4.2% of laboratories participating in the 2006 ID-03 CAP enterovirus challenge gave an incorrect response. The European proficiency testing program was more rigorous than the CAP program and may better demonstrate the magnitude of the problem. The GXEA detected three (9%) more positive cases than the locally developed RT-PCRs used for diagnostic purposes at the trial sites. An easy-to-use, commercially available assay for enterovirus RNA with well-defined performance characteristics should contribute to the optimization and standardization of molecular diagnostics for enteroviral meningitis.

False-positive reactions due to target and amplicon cross-contamination remain a significant concern for NATs. Real-time amplification and detection methods reduce these concerns because the reaction vessel is not opened after addition of the sample. Since the GX cartridge is completely self-contained and performs the all of the PCR steps, including sample preparation, concerns about false-positive results due to cross-contamination are essentially eliminated. No false-positive GXEA tests were encountered in this trial.

The on-board positive control in the GXEA ensures that the sample is adequately prepared, critical reagents are functioning properly, and the sample is free of interfering substances. External positive and negative controls were run weekly during this trial at the three sites, with no failures. However, the optimal frequency for testing external controls with test systems such as the GXEA has not been established and is not adequately addressed in the Center for Medicare and Medicaid Services final rule for laboratory requirements (1). The strategy for the use of external controls will have a large impact on the cost of performing the GXEA with individual samples or in small batches.

The anticipated costs of for the GXEA cartridge and four-module analyzer are \$US 65 and \$US 68,000, respectively. The price for the disposables will be higher than that of other competing nucleic acid amplification technologies, but the labor component of the GXEA will be much less because it is completely automated. The cost of the analyzer is similar to that of other real-time PCR instruments, with the added advantage that no additional instrumentation is required to automate nucleic acid extraction.

The GXEA could shift the paradigm for the diagnosis and management of enteroviral meningitis. NATs for enterovirus can significantly reduce health care costs through earlier discharge, less antibiotic use, and fewer orders for ancillary diagnostic tests if the results are available within 24 h of admission (2, 5, 7, 8). The NATs in use currently are labor-intensive, are technically complex, and require 4 to 8 h to complete the analysis. Consequently, the tests are usually batched, and many laboratories struggle to provide these important results within a 24-h window.

The complete automation and rapid-result capability of the GXEA make it uniquely suited for on-demand testing. It is

simple enough to be performed as a stat test in clinical laboratory sections that are typically staffed around the clock and by individuals with no training in molecular biology or virology. The results of rapid, on-demand testing for enteroviruses could affect the decision to admit patients with suspected meningitis to the hospital. Those patients with positive tests could be managed more conservatively than those with negative tests, without the need for hospitalization. Since enteroviral meningitis is a leading cause for hospitalization of children in the summer months, this strategy could save significant health care resources.

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