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Comparison of the NucliSens Basic Kit (Nucleic Acid Sequence-Based Amplification) and the Argene Biosoft Enterovirus Consensus Reverse Transcription-PCR Assays for Rapid Detection of Enterovirus RNA in Clinical Specimens

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Samples were tested for enterovirus by nucleic acid sequence-based amplification (NASBA) (NucliSens Basic kit; BioMerieux), reverse transcription-PCR (RT-PCR) (Enterovirus Consensus RT-PCR kit; Argene Biosoft), and virus isolation. Eighty-two samples were tested, and 44 were positive, 34 by both NASBA and RT-PCR and 5 each by NASBA or RT-PCR only. Two nasopharyngeal samples positive only by RT-PCR were determined to be rhinovirus. Of 42 enterovirus-positive samples, NASBA detected 39 (92.9%) and RT-PCR detected 37 (88.1%). The NucliSens Basic kit and the Argene Biosoft RT-PCR had comparable sensitivities for detection of enterovirus RNA, and both molecular methods were more sensitive than culture, which detected only 60.5% of positive samples. NASBA could be completed in 6.5 h versus 9 h for the Argene Biosoft RT-PCR kit.

Enterovirus (EV) infections are extremely common, with an estimated 10 to 30 million infections occurring annually in the United States alone (15). While most infections either are asymptomatic or result in minor illnesses, aseptic meningitis and neonatal sepsis syndrome bring many patients to the hospital (16–18).

For the past 50 years, the mainstay of EV diagnosis has been virus isolation in cell culture (5). The optimal cell culture systems for the isolation of more than 60 recognized EV serotypes differ. To increase recovery, ideally five different cell systems should be employed (2, 5, 7). Use of E-mix cells has been reported to reduce the number of tubes inoculated and to increase recovery (1). However, the turnaround time for cell culture is usually 2 to 7 days for positive results and 10 to 14 days for negative reports. Lastly, many coxsackie group A serotypes require suckling mouse inoculation (5).

Nucleic acid amplification techniques can detect most serotypes, including those that grow poorly or not at all in cell culture, can provide results within 24 h, and, consequently, can significantly alter patient management (13, 14, 20). Molecular methods also require a smaller volume of cerebrospinal fluid (CSF) than comprehensive culture, an important advantage. To inoculate five cell culture systems, 1 ml of CSF is needed, but this amount is often not available. In contrast, only 0.2 ml is needed for nucleic acid extraction. In addition, other pathogens that cause viral meningitis, such as herpes simplex virus (HSV) type 2, can also be detected by using the same nucleic acid extract.

In the 1990s, several studies were published validating the AMPLICOR reverse transcription-PCR (RT-PCR) enterovirus kit from Roche (12, 15, 19, 22). Subsequently, however, the AMPLICOR kit was withdrawn from the market. More recently, studies using real-time PCR for EV diagnosis have been reported (10, 21). However, many smaller laboratories may not have access to expensive real-time PCR equipment and may lack the expertise needed to develop molecular techniques in-house. Instead, they rely on commercial kits to bring molecular technology to the patients they serve.

We and others have recently reported on nucleic acid sequence-based amplification (NASBA) using the NucliSens Basic kit for the diagnosis of EVs (3, 9; F. Zhang, C. C. Ginocchio, A. Malhotra, and C. Chakrabarti, presented at the 18th Annual Clinical Virology Symposium, 2002). Since most laboratories have the experience and equipment for RT-PCR, rather than NASBA, we have compared NASBA to the Enterovirus Consensus RT-PCR kit from Argene Biosoft for EV detection in clinical specimens. Some of the NASBA and culture data from 2001, but none of the RT-PCR data, have been reported previously (9).

MATERIALS AND METHODS

Samples and patients. Eighty-two samples submitted to the Clinical Virology Laboratory at Yale New Haven Hospital for EV diagnosis from July to December 2001 and July to November 2002 were tested by both EV NASBA and RT-PCR. For 76 of these samples, a sufficient amount remained for inoculation into cell culture. Culture and NASBA were performed within 3 days of sample receipt. Remaining RNA extracts were saved at −70°C for as long as 3 months and batched for RT-PCR testing. Samples for RT-PCR testing were selected to represent a range of NASBA-positive, NASBA-negative, and invalid results and also included a number of NASBA-negative CSF samples with elevated levels of nucleated cells.

The 82 samples from 76 patients consisted of 58 CSF samples, 13 nasopharyngeal (NP) or throat swabs, 9 rectal or stool specimens, one mouth swab, and one serum sample. Swabs were submitted in viral transport medium (M4; MicroTest, Inc., Lilburn, Ga.). Spinal fluids and stools were submitted in sterile containers. Patients ranged in age from 6 days to 93 years, and 28% of patients were over the age of 18 years.

Sample processing. CSF samples and NP and rectal swabs in viral transport medium were vortexed. For stool samples, a 10% suspension (wt/vol) in phosphate-buffered saline with antibiotics was vortexed and centrifuged at 2,000 × *g*

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TABLE 1. NASBA and RT-PCR results according to sample type

Sample type	No. tested	No. of samples with the following result:								Total positive
		NASBA positive, RT-PCR positive	NASBA positive, RT-PCR negative	NASBA negative, RT-PCR positive	NASBA positive, RT-PCR invalid	NASBA invalid, RT-PCR positive	NASBA invalid, RT-PCR negative	NASBA negative, RT-PCR invalid	NASBA negative, RT-PCR negative	
CSF	58	26	1	1	2	0	0	2	26	30
NP/throat	13	3	0	3[a]	0	0	4	0	3	6[a]
Rectal/stool	9	5	2	0	0	1	0	0	1	8
Other	2	0	0	0	0	0	1	0	1	0
Total	82	34	3	4[a]	2	1	5	2	31	44[a]

[a] Two RT-PCR-positive NP samples were subsequently determined to be rhinovirus and not EV.

for 15 min, and the clarified supernatant was used for testing. Aliquots of samples were then added to NucliSens lysis buffer for RNA extraction. If molecular testing was delayed, specimens in lysis buffer were frozen at −70°C. RNA was isolated with the reagents provided in the Nuclisens kit.

Virus isolation. From July through October, one tube each of five cell systems—primary rhesus monkey kidney (RhMK), human embryonic lung fibroblast (MRC-5), human epidermoid tumor (A549), rhabdomyosarcoma (RD), and Buffalo green monkey kidney (BGMK) cells (Viromed Laboratories, Minneapolis, Minn.; Diagnostic Hybrids, Athens, Ohio)—was inoculated with 0.1 to 0.2 ml of sample when sample volume permitted. From November to December, RD and BGMK cells were not available. Culture tubes were incubated at 35°C in a roller drum for 14 days. Cultures showing cytopathic effect were passaged to fresh culture tubes and were identified by immunofluorescence using EV monoclonal antibody pools (Chemicon International, Temecula, Calif.).

NASBA procedure. The NucliSens Basic kit (bioMerieux, Durham, N.C.) was utilized for RNA detection, and the EV NASBA protocol available on the NucliSens website was followed. The details have been published previously (9). Briefly, 5 μl of the extracted nucleic acid was taken for amplification. An EV-specific internal control (IC) was coamplified with the test sample, using the same primers, and amplification products were detected by hybridization using two different probes, one specific for the wild-type (WT) EV product and the other specific for the IC product, both containing a generic ruthenium-labeled electrochemiluminescent (ECL) detection probe. Results were obtained by analysis of the ECL hybridization products using the NASBA QR system and the NucliSens Basic kit software program. Specimens with WT signals of ≥650 ECL units were considered positive for EV RNA, regardless of the IC signal. Specimens with WT signals of <450 ECL units and IC signals of ≥50,000 ECL units were considered negative for EV RNA and not inhibitory. Specimens with WT signals of <450 ECL units but IC signals of <50,000 ECL units were considered inhibitory to amplification. These results were deemed "invalid." WT signals of 450 to 649 ECL units would be considered indeterminate and retested (9).

EV RT-PCR. EV RT-PCR was performed by using the Enterovirus Consensus kit (Argene Biosoft, Varilhes, France), according to the manufacturer's instructions, except that the RNA was extracted by using NucliSens reagents as described above, instead of Qiagen reagents. NucliSens and Qiagen extraction methods have been determined to be equivalent for recovery of RNA (11).

(i) RNA amplification. RNA was amplified using a two-step RT-PCR method. For reverse transcription, samples were denatured for 10 min at 60°C and returned to 37°C, after which reverse transcriptase was added. After incubation for 45 min, the reaction was stopped by heating at 90°C for 5 min. For PCR, the primers were directed to the 5′ noncoding region of the EV genome and generated a 425-bp amplicon. PCR was carried out in a PE9600 thermal cycler by using HotStartTaq and the following parameters: cycle 1, 94°C for 15 min; cycles 2 to 6, 94°C for 15 s, 52°C for 1 min, and 72°C for 50 s; cycles 7 to 41, 94°C for 15 s, 54°C for 30 s, and 74°C for 30 s.

(ii) Inhibition controls. After RNA extraction and reverse transcription, patient samples were amplified in duplicate tubes. To one of the tubes, a positive-control plasmid containing a Coxsackie B4 virus sequence targeted by the EV primers, but containing a different internal sequence, was added to detect inhibition of amplification.

(iii) Amplicon detection. Amplicons were detected by a microplate hybridization assay. Denatured amplicons were mixed with a coating solution and then pipetted into the wells of a microtiter plate and incubated for 1 h at 37°C. Then the wells were emptied, and a biotinylated probe specific for the EV group was added. In addition, a probe specific for the inhibition control was assayed in parallel with the EV probe. A conjugate containing streptavidin peroxidase was then reacted with the captured probes. Color development was accomplished by exposing the conjugate to a 3,3′,5,5′-tetramethylbenzidine (TMB) substrate. Optical densities (OD) of all wells were read at 450 nm with a microplate reader.

(iv) Interpretation. A cutoff OD was calculated based on the mean of the negative detection control OD. Specimens with an OD higher than 1.1 × cutoff were determined to be positive for EV RNA. Specimens with an OD lower than 0.9 × cutoff were considered negative for EV RNA provided that the inhibition control well had an OD of >0.800. Specimens with an OD lower than 0.9 × cutoff, but with an inhibition well OD of <0.800, were considered to be inhibitory, and the results were considered invalid. Specimens with an OD between 0.9 × cutoff and 1.1 × cutoff were considered indeterminate.

Parechovirus RT-PCR. One EV-like virus isolate from an NP sample that was negative both by NASBA and by RT-PCR was tested by parechovirus RT-PCR at the Centers for Disease Control and Prevention (CDC).

Resolution of discrepancies and definition of true positives. Samples from which EV was isolated and confirmed by subculture and immunofluorescence were considered true positives. Samples positive by both NASBA and RT-PCR, but either negative or not tested by viral culture, were also considered true positives. A sample positive by either molecular test, but for which culture was either negative or not done, was considered a true positive if one of the following conditions was met: (i) EV was detected by culture and/or both molecular assays in another sample from the patient; (ii) in adults, the clinical course and CSF profile were typical for aseptic meningitis, Gram staining and bacterial cultures were negative, and HSV PCR was negative; or (iii) in young infants, fever and lethargy or irritability were present, no other pathogens were detected by viral or bacterial cultures or by rapid respiratory virus direct immunofluorescence (DFA) testing, and the clinical course was most compatible with viral infection.

RESULTS

Comparison of EV NASBA and RT-PCR results. Of the 82 samples tested by both methods, 44 were positive: 34 by both molecular tests and 5 each by NASBA or RT-PCR only (Table 1). Thus, 10 of 44 positive samples were positive by only one of the two molecular tests. Ten samples were invalid due to inhibitors: six (four throat swabs, one mouth swab, and one stool sample) were invalid by NASBA, and four CSF samples were invalid by RT-PCR. No samples were invalid by both tests. Three invalid samples ultimately tested EV positive, one by RT-PCR and culture, two by NASBA (see Table 2).

Results of virus isolation. Seventy-six of 82 samples, including 40 of the 44 that were positive by one or both molecular tests, were inoculated into cell culture. Of these, 23 were positive for EVs. Two RT-PCR-positive samples were positive for rhinovirus, not EV. One EV-like isolate was recovered from an NP sample negative by both NASBA and RT-PCR. This isolate was subsequently identified as parechovirus by RT-PCR at the CDC. Thus, culture detected only 23 of 38 EV-positive samples, resulting in a culture sensitivity of 60.5%. Of the 15

TABLE 2. Characteristics of samples with discrepant molecular test results

Patient	Age	Sample	Result[a] by:			No. of nucleated cells/μl of CSF[d]	Clinical findings	Final diagnosis[f]
			EV-NASBA (ECL units)[b]	EV RT-PCR (index)[c]	Culture			
1	2 mo	Stool	Invalid	Pos (7.22)	EV	615	Aseptic meningitis[e]	True EV positive
2	7 wk	Rectal	Pos (6,747)	Neg (0.64)	EV	ND	Fever, irritability	True EV positive
3	16 yr	Stool	Pos (1,072)	Neg (0.70)	EV	ND	Fever, headache, vomiting	True EV positive
4	6 mo	NP	Neg (47)	Pos (8.79)	Rhinovirus	ND	Premature infant with respiratory distress	False EV positive
5	52 yr	NP	Neg (1)	Pos (10.2)	Rhinovirus	ND	Common cold	False EV positive
6	17 days	CSF	Pos (4,437)	Invalid	Negative	1,100	Fever, lethargy, irritability	True EV positive
7	2 mo	CSF	Pos (12,221)	Invalid	Negative	1,650	Fever, irritability, vomiting	True EV positive
8	9 yr	CSF	Neg (1)	Pos (2.6)	Negative	500	Aseptic meningitis[e]	True EV positive
9	5 wk	NP	Neg (84)	Pos (7.1)	Negative	7	Fever, lethargy, apnea, prematurity	True EV positive
10	10 wk	CSF	Pos (6,428)	Neg (0.57)	Negative	0	Fever, irritability, mottled skin, sepsis	True EV positive

[a] Pos, positive; Neg, negative; invalid, amplification of IC did not reach minimum value.
[b] Positive values by EV NASBA, ≥650 ECL units.
[c] Positive scores on EV RT-PCR index, ≥1.10.
[d] Normal range, <6 nucleated cells/μl of CSF. ND, not done.
[e] Positive by throat and stool EV culture.
[f] For definition of true EV positives, see Materials and Methods.

EV positives missed by culture, 14 were CSF samples and one was an NP swab. Only 2 of the 15 samples were inoculated into five cell systems; three were inoculated into only two cell systems, and five were inoculated into only one. Hence, culture was generally suboptimal for EV false-negative samples.

Resolution of discrepancies between NASBA and RT-PCR. The characteristics of the 10 samples positive by one molecular test, and negative or invalid by the other, are shown in Table 2. Three samples (patients 1 to 3) were confirmed as true positives by isolation of EV. Rhinoviruses were isolated from two RT-PCR-positive, NASBA-negative NP samples (patients 4 and 5). For these two rhinoviruses, the EV RT-PCR OD were fairly high, at 8.79 and 10.2 times the cutoff. Patients 6 to 8 had CSF profiles and clinical features suggestive of aseptic meningitis, and patient 8 had EV recovered from both throat and stool samples. Patients 9 (RT-PCR positive) and 10 (NASBA positive) were more problematic. They had unremarkable CSF profiles, but had summer febrile illnesses and clinical syndromes associated with EV infection in young infants (lethargy and apnea; irritability and "sepsis"), and no other pathogens identified by routine bacterial cultures, viral cultures, or rapid respiratory virus DFA tests (17, 20). Furthermore, lack of CSF pleocytosis is common in young infants with documented EV infections (20). Both cases were accepted as true positives. Thus, 8 of 10 samples were considered true EV positives. Of note, while RT-PCR OD for most negative samples were 0.2, the RT-PCR false negatives had OD of 0.57 to 0.70, indicating some reactivity, though below the indeterminate range. Likewise, one of the samples with a false-negative NASBA result had a signal of 84 ECL units (case 9), where most NASBA negatives had signals of 1 ECL unit. Thus, the true EV positives among the discrepant samples either were invalid by one test (one by NASBA and two by RT-PCR) or were low-level positives.

After resolution of discrepancies, 42 samples were determined to be true EV positives. NASBA detected 39 (92.9%), and RT-PCR detected 37 (88.1%) (Table 3). The difference between NASBA and RT-PCR in detecting EV-positive samples was not significant ($P = 0.7126$ by Fisher's exact test). However, virus isolation was significantly less sensitive than NASBA ($P < 0.001$) and RT-PCR ($P < 0.01$) by Fisher's exact test. With no false positives, both NASBA and culture had specificities of 100%. Of 40 true EV negatives, RT-PCR found 36 negative, 2 positive, and 2 invalid. Due to the erroneous identification of the two rhinovirus-positive samples as EVs, RT-PCR had a specificity of only 94.7%.

Validity and ease of interpretation of NASBA results. As previously reported (9), a positive EV result by NASBA was indicated by a WT signal of ≥650 ECL units. For a valid negative result, an IC signal of ≥50,000 ECL units was required. For all 39 NASBA-positive samples, the WT signal was >1,000 ECL units; for 37 of these samples (94.8%), the WT signal was >2,000 ECL units. The range of positive values was 1,072 to 1,358,702 ECL units, with a median of 58,804 ECL units.

Of 34 samples positive by both molecular tests, 30 (88.2%) had ECL values of >10,000. For five samples positive only by NASBA and not by RT-PCR, ECL values were 1,072 to 12,221 (median, 6,428 ECL units). Of 40 true negatives, 5 were invalid by NASBA, 26 had an ECL value of 1, and 9 had values of 11 to 105 ECL units. The two NASBA false-negative samples had values of 1 and 84.

TABLE 3. Number of true EV positives detected by each method

Method	Total no. tested	No. of true EV positives tested by the indicated method[a]	No. (%) of EV detected by the indicated method[b]	Specificity (%)
NASBA	82	42	39 (92.9)	100
RT-PCR	82	42	37 (88.1)	94.7
Culture	76	38	23 (60.5)	100

[a] RT-PCR gave positive results for two NP samples subsequently shown by culture to contain rhinovirus. Culture detected a parechovirus-positive sample that was EV negative by both NASBA and RT-PCR.
[b] The difference in sensitivity between NASBA and RT-PCR was not significant ($P = 0.7126$ by Fisher's exact test). Culture was significantly less sensitive than NASBA ($P < 0.001$) and RT-PCR ($P < 0.01$) by Fisher's exact test.

Validity and ease of interpretation of RT-PCR results. The 34 samples positive by both RT-PCR and NASBA had OD of 2.1 times the cutoff to >12 times the cutoff, with a median OD of >12. Twenty-one of 34 (61.7%) had OD of >10, and only 2 of 34 samples positive by both tests had values of <5 times the cutoff. The two samples positive only by RT-PCR had indices of 7.1 and 2.6. Of 38 true negatives, excluding the two rhinovirus false positives, 2 were invalid by RT-PCR, 35 had indices of <0.3, and only 1 had an index of 0.54. In contrast, three RT-PCR false-negative samples had indices of 0.57 to 0.70.

DISCUSSION

In this study of two commercial kits, the EV NASBA application of the NucliSens Basic kit from bioMerieux and the Enterovirus Consensus RT-PCR kit from Argene Biosoft had comparable sensitivities for detection of EV RNA (92.9 and 88.1%, respectively), and both were significantly more sensitive than culture, which detected only 60.5% of EV positives. The sensitivity of the Argene RT-PCR was identical to that recently reported by Buck et al. (1). False-negative cultures were a particular problem with CSF samples, since insufficient sample volumes did not allow inoculation of the spectrum of cell types needed to recover multiple EV serotypes. Since the samples most commonly submitted to the laboratory for EV diagnosis are CSF samples from young children, and often are of low volume, molecular methods have a critical advantage.

The RT-PCR was performed retrospectively, using RNA extracts stored a few days to 3 months at −70°C. Loss of EV RNA during storage was a concern. In several discrepant cases, where RT-PCR was negative but the original NASBA had been positive, the NASBA was repeated to be sure that the RNA had not degraded, and the samples still tested positive by NASBA. In addition, two samples that were NASBA negative upon initial testing were RT-PCR positive after 3 months of storage. On one of these (sample 8 in Table 2), NASBA was repeated, and the sample was found to be a low positive upon repeat testing; however, the original negative NASBA result was used in the data analysis. Samples were selected to represent a range of NASBA-positive, -negative, and -invalid results but also to include several NASBA-negative CSF samples with elevated levels of nucleated cells, a NASBA-invalid stool from which EV was isolated, and two rhinovirus-positive NP swabs. Thus, sample selection may have increased the discrepancies between the two tests, yet they remained comparable in sensitivity.

As expected, neither molecular method detected a parechovirus-positive sample (6). EV RT-PCR, but not NASBA, gave high positive index values for two rhinovirus-positive NP samples. Amplification of rhinoviruses by EV primers is a recognized problem, since both EVs and rhinoviruses are members of the picornavirus family and share conserved sequences in the 5′ nontranslated regions of their genomes (8). In many reports, either a differential hybridization or restriction enzyme digestion step is needed to distinguish EV from rhinovirus amplicons (8). These two rhinovirus-positive NP samples were included to test the specificity of both assays, and they lowered the specificity of the Argene RT-PCR more than is likely warranted. If samples tested for EV are largely limited to CSF, stool, and serum samples, the ability to detect rhinovirus will not pose a problem, since rhinovirus is found only in respiratory samples. However, EVs have been reported as a cause of pneumonia in bone marrow transplant patients. Thus, testing of respiratory samples may be indicated (4). One could also argue that since the only therapy available on compassionate plea for EV or rhinovirus is pleconaril, the distinction between the two groups may not be clinically relevant (18).

The EV NASBA procedure could be completed in 6.5 h, whereas 9 h was required to complete the EV RT-PCR. The shorter time to results with NASBA was an advantage if samples received in the morning were to be completed before the end of the day shift.

Separation of positive from negative results with both kits was clear-cut. However, three RT-PCR false-negative samples showed reactivities higher than those of the vast majority of true-negative samples. More work is needed to determine whether lowering the indeterminate range would enhance sensitivity, without reducing specificity.

Invalid results were a concern and were slightly more frequent with NASBA than with RT-PCR. Surprisingly, of the 10 invalid samples, none were invalid by both methods, despite the fact that the same RNA extract was used for both tests. The ICs provided by the kits were critical in recognizing amplification failure, thus allowing for reextraction and/or reamplification. Three samples with invalid results ultimately tested EV positive. Inexperience with the extraction method, as well as with the kit protocols, likely contributed, since invalid results were much more frequent in 2001 than in 2002. Laboratories will benefit from the use of ICs to detect amplification failure, especially when they are initially obtaining experience with the methods and when new employees are trained.

The RT-PCR protocol specified addition of the IC after extraction and the reverse transcription step, but prior to PCR. Amplification of the RT-PCR IC was performed separately in a duplicate tube. In contrast, the IC in the EV NASBA kit was added to the sample prior to extraction and was coamplified with the patient's sample in a single tube. Thus, the NASBA IC was a better monitor of the procedure from extraction through amplification.

An advantage for the smaller hospital laboratory is that the NASBA format is more suitable for testing small runs than the RT-PCR procedure. However, personnel must be trained in NASBA methodology, and additional bench space to accommodate NASBA equipment must be available. Making up NASBA primers and probes was inconvenient and required some practice. A "starter pack" of NASBA primers and probes was provided when we experienced difficulty but was not routinely provided to customers. Technical service for NASBA is provided via the Nuclisens website and was slower than direct telephone communication. NASBA technical experts were located at Boxtel, The Netherlands, and the difference in time zones added to the delay in communication. Lastly, technical service for EV NASBA was also limited by the fact that it was one of many applications of the Nuclisens Basic kit and not a complete kit.

Fewer technical problems occurred with RT-PCR, likely due to our familiarity with the methodology. Since more laboratories routinely perform PCR and already have the expertise and the equipment, implementing an additional PCR assay should require less effort. However, the Enterovirus Consensus RT-

PCR protocol was unnecessarily complicated. Kit directions were very convoluted and difficult to understand. The hybridization procedure involved laborious pipetting, making large runs especially tedious. Testing all samples in duplicate in order to perform an IC increased labor, reagent costs, and the complexity of the test. To improve the Enterovirus Consensus RT-PCR kit, we would suggest the following: simplify the written instructions, combine the two-step reverse transcription and PCR into a single step, streamline the hybridization pipetting, provide an IC that can be coamplified with the patient sample in a single tube, and add the IC to the sample prior to extraction in order to better monitor the entire process.

The cost per test depends on the number of patient samples per run, the negotiated price, and whether equipment rental is included in the reagent costs. For our laboratory, NASBA reagent costs were 50% higher than Argene RT-PCR reagent costs, at least in part because the former included equipment rental. However, the Argene RT-PCR procedure entailed 50% greater labor costs. Thus, the total costs for reagents and labor in our laboratory, including controls, were equivalent for the two kits at about $118 per patient sample for a run size of three patient samples. Even at this small run size, use of these kits was appreciably less costly than sending the samples to a reference laboratory ($150 to $200). Furthermore, to have a clinical impact, it is most useful to perform EV molecular tests daily in-house, since patients are usually discharged after 2 days (13, 20).

Many recent reports focus on real-time RT-PCR assays, which have the great advantage of faster results (10, 21). Real-time assays are not inherently more sensitive than conventional RT-PCR, however, and require time and expertise to set up and optimize. Many of the smaller hospital laboratories may have neither the technical resources for molecular test development nor the funds to purchase a real-time instrument. Thus, the availability of the two commercial kits compared in this report may fill an important niche. Of note, however, both these kits do require in-house validation (e.g., against culture), since they are not FDA approved.

In summary, both the EV NASBA and the EV RT-PCR kits were significantly more sensitive and more rapid than culture methods, especially for CSF samples. EV NASBA had a shorter assay time, but RT-PCR employed more widely used technology and equipment. Importantly, both kits provided ICs to monitor amplification efficiency. Clinical laboratories without the time or expertise to develop in-house molecular methods can now choose between two kits and two methods that can provide rapid and sensitive molecular diagnosis of EV infections.

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