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Evaluation of a Commercial DNA Enzyme Immunoassay for Detection of Enterovirus Reverse Transcription-PCR Products Amplified from Cerebrospinal Fluid Specimens

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We evaluated the DiaSorin DNA enzyme immunoassay (DEIA) kit for detection of enteroviral reverse transcription-PCR (RT-PCR) products amplified from cerebrospinal fluid. By use of an optical density of 0.05 as the absorbance cutoff, 35% of 198 specimens were PCR positive, whereas 16% were culture positive. DEIA was rapid and sensitive and can help implement enterovirus RT-PCR in clinical laboratories.

Human enteroviruses are believed to be responsible for as many as 80% of cases of viral meningitis (7). Although enterovirus infections themselves are generally benign, clinical features of enteroviral meningitis can overlap those of bacterial infections and herpes simplex virus infection, resulting in prolonged hospital stays and presumptive treatment until a diagnosis is established.

Reverse transcription-PCR (RT-PCR) is a rapid and sensitive alternative to cell culture for evaluating cerebrospinal fluid (CSF) samples (5, 6, 8, 10). Hybridization-based detection of PCR products has generally been required in order to achieve the sensitivity required for optimal clinical utility (3, 7). This study evaluates the use of a DNA enzyme immunoassay (DEIA) produced by DiaSorin (Stillwater, Minn.) for enterovirus RT-PCR product detection. In addition to its being hybridization-based, favorable attributes of this assay include the lack of a requirement for radioactivity and the potential for universal application to any PCR assay, based on the fact that the capture probe can be changed while all other components remain the same.

To detect amplified products, the DEIA utilizes a biotinylated capture probe bound to the bottoms of the wells of a 96-well plate. Denatured PCR products are added, and hybridization to the capture probe is detected by a monoclonal antibody that binds specifically to double-stranded DNA. Addition of an enzyme-conjugated antibody and a substrate-chromogen causes color development, which is read by spectrophotometry. Enterovirus detection takes approximately 8 h, including 1.5 h for DNA extraction, 3 h for RT-PCR, and 3 h for the DEIA.

During May through September, 1998, CSF specimens submitted for viral culture to the virology laboratory at St. Louis Children's Hospital were also tested for the presence of enteroviral RNA using RT-PCR. If the volume of CSF submitted was less than 1.4 ml, the specimen was brought to that volume with Eagle's minimal essential medium (BioWhittaker, Walkersville, Md.) prior to any studies. RNA was extracted from 140 μl of CSF using the QIAamp Viral RNA Mini Kit (Qiagen, Chatsworth, Calif.) according to kit directions, and RT-PCR was performed in a two-step process using primers described by Rotbart (7), Promega avian myeloblastosis virus reverse transcriptase, and *Taq* polymerase (Promega Inc., Madison, Wisc.). The procedure comprised a 1-h incubation at 42°C, a 5-min incubation at 95°C, cooling to 4°C, and a PCR consisting of 35 cycles of 94°C for 1 min, 50°C for 1 min, and 72°C for 1 min. After PCR amplification, DEIA was performed according to the manufacturer's directions. Briefly, PCR products were denatured by incubation at 94°C for 15 min and were added, together with hybridization buffer, to the well of a detection plate that had been previously coated with the specific biotinylated enterovirus capture probe (5′ biotin-AAAAGGAAAC ACGGACACCCAAAGTAGTCGGTTCC-3′). Hybridization was carried out for 1 h at 50°C, after which the wells were washed with an automatic plate washer. A series of three 30-min room temperature incubations and washes was then performed in the following order: anti-double-stranded DNA antibody, enzyme conjugate, and chromogen-substrate. Following the addition of a blocking reagent, the absorbance was read in a spectrophotometer at 450 and 630 nm. The run was considered valid when the ratio of the DEIA kit positive control to the mean of the DEIA negative controls was greater than or equal to 10. For each batch of specimens processed, a negative control consisting of sterile, RNase-free water and a positive control consisting of a 10^{-5} dilution of ECHO 11 stock were processed along with the patient specimens. In addition, at least one negative control was processed and run for every fourth specimen. Viral CSF culture consisted of one culture tube each of MRC-5 fibroblasts, human neonatal kidney cells, and primary African green monkey kidney cells.

A total of 198 CSF samples were available for culture and PCR analysis. The concordance of culture and PCR results is shown in Table 1. Viral culture was positive in 32 (16%) of the 198 specimens analyzed. The cutoff for defining a positive DEIA, as suggested by the manufacturer, is 0.15 above the mean absorbance of the negative controls. Using this cutoff, enterovirus RNA was detected in 59 (30%) of the samples, yielding a sensitivity and specificity of RT-PCR relative to culture of 91 and 82%, respectively. We also evaluated a cutoff of 0.05, which was in excess of 6 standard deviations above the mean of the negative controls of all runs (data not shown). Using this cutoff, 70 (35%) of the CSF specimens were positive for enteroviral RNA, yielding a sensitivity and specificity of 100 and 77%, respectively.

The detection of enterovirus RNA in samples with negative culture results is similar to the findings of other studies com-

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TABLE 1. RT-PCR versus cell culture for detection of enterovirus in CSF

RT-PCR result	No. of specimens with the indicated result(s)[a]			Sensitivity (%)	Specificity (%)
	Culture positive	Culture negative	Total		
Absorbance cutoff, 0.15					
Positive	29	30	59	91	82
Negative	3	136[b]	139		
Absorbance cutoff, 0.05					
Positive	32	38	70	100	77
Negative	0	128[b]	128		

[a] Based on 198 patients whose CSF was tested by culture and RT-PCR.
[b] Nine patients were diagnosed on the basis of clinical criteria (neonatal hemochromatosis, IVIG-induced aseptic meningitis, lymphoproliferative disease) or were given laboratory-confirmed diagnoses (herpes simplex virus meningitis, varicella encephalopathy, listeriosis, *Escherichia coli* urosepsis, toxic encephalopathy secondary to *Salmonella* infection, meningococcal meningitis).

paring RT-PCR to culture, and is very likely a reflection of the poor sensitivity of enterovirus culture (1–5, 8, 9). Because few patients had enteroviral cultures performed on specimens from other body sites, such as stools and nasopharyngeal secretions, it was not possible to use results of cultures from these sites to resolve discrepancies between CSF culture and RT-PCR. Therefore, we compared the presence of CSF pleocytosis in subjects with enterovirus RNA and negative CSF cultures with that in patients with enterovirus RNA and positive CSF cultures. In this analysis, 89% of patients with negative cultures had CSF pleocytosis, compared to 96% of patients with positive cultures ($P = 0.3$ by Fisher's exact test). These two groups did not differ with respect to DEIA absorbance, patient age, extent of CSF pleocytosis, or protein or glucose levels (data not included). These observations are consistent with, but do not prove, the concept that the negative cultures for patients with enteroviral RNA represent false-negative cultures. Negative controls were consistently negative, further lessening the concern that discrepant results occurred because of contamination or nonspecific amplification.

We also investigated whether the presence of CSF pleocytosis could be used as a "screen" for specimens likely to contain detectable enterovirus RNA. As shown in Table 2, which compares the presence of CSF pleocytosis with the results of RT-PCR, we found that the absence of pleocytosis has a high negative predictive value (98%) for individuals 2 years old or older but not for those younger than 2 (77%) ($P < 0.01$). The implication is that laboratories could use the absence of pleocytosis to discourage RT-PCR testing of older children and adults but should not use this criterion for children below the age of 2 years. The presence in both age categories of a substantial number of patients with CSF pleocytosis but negative RT-PCR results is also noteworthy. This could indicate suboptimal sensitivity of the RT-PCR assay or the presence of clinical entities other than enteroviral meningitis as the explanation for the pleocytosis. RT-PCR may be of value in focusing attention on this group of patients and directing a search for other possible etiologic explanations for CSF pleocytosis, including other viral infections, infections caused by nonviral agents, and pleocytosis related to noninfectious conditions.

TABLE 2. Effect of patient age on the relationship between the presence of CSF pleocytosis and the detection of enterovirus RNA in CSF by RT-PCR

Age (yrs)	Pleocytosis	No. (%) with the following RT-PCR result[a]:		Total no.
		Positive	Negative	
<2	Present	13 (28)	11 (24)	24
	Absent	5 (11)	17 (37)	22
≥2	Present	51 (41)	17 (14)	68
	Absent	1 (2)	54 (44)	55

[a] Based on 169 patients whose CSF was tested by culture and RT-PCR and whose ages and CSF cell count results were available. The data are based on an absorbance cutoff (optical density) of 0.05. For patients below the age of 2 years, the sensitivity, specificity, and positive and negative predictive values were 72, 61, 54, and 77%, respectively. For patients 2 years old and older, the corresponding parameters were 98, 76, 75, and 98%, respectively.

We conclude that DEIA is a sensitive, specific, and practical means by which laboratories performing enteroviral RT-PCR can achieve hybridization-based detection of reaction products. Laboratories may choose to discourage enteroviral RT-PCR testing for older children and adults without CSF pleocytosis. The group of patients with CSF pleocytosis and negative RT-PCR results represents an intriguing diagnostic challenge.

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