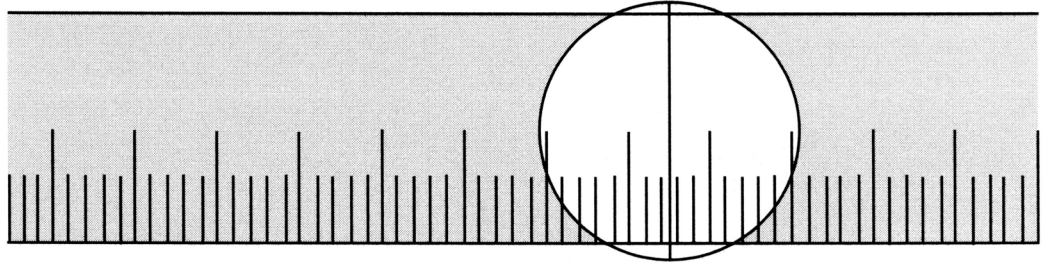


LAB NEWS



From the Department of Laboratory Medicine - Yale-New Haven Hospital Medical Center

Clinical Virology Laboratory Newsletter

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Rapid Enterovirus RNA Detection by Real-time TaqMan RT-PCR

Enteroviruses are a diverse group of small, non-enveloped RNA viruses that are transmitted largely by the fecal-oral route and replicate in high titer in the GI tract. Enteroviruses rarely cause significant GI disease. Rather, they are carried by the blood to target organs where further replication and pathology can occur. Enterovirus infections occur worldwide and are most prevalent during warm weather. Thus in temperate zones, infections are most prevalent in summer and early fall. Several serotypes circulate concurrently, but predominant types will vary from year to year.

Clinical Manifestations: Enteroviruses are associated with a multitude of clinical manifestations. While most infections are subclinical, serious illness and death can occur. Enterovirus (EV) meningitis is common in the U.S., and usually has a benign outcome. Nevertheless, it leads to a large number of hospitalizations of both children and adults, mainly because it is difficult to distinguish clinically from bacterial meningitis. The ability to rapidly differentiate EV meningitis from bacterial illness can reduce hospitalization time, antimicrobial usage and diagnostic tests (1). In addition to meningitis, other neurologic manifestations include encephalitis, poliomyelitis, Guillain-Barre, transverse myelitis, cerebellar ataxia, peripheral neuritis and Reye's syndrome. Neonates and young infants can present with a "sepsis" picture during the viremic phase of enterovirus infection. Febrile illness, rash, and myopericarditis also occur.

Laboratory diagnosis: Enterovirus isolation in cell culture requires 2-7 days, and some coxsackie group A serotypes require suckling mouse inoculation. Nucleic acid amplification techniques can provide results within 24 hours, can detect serotypes that grow poorly in cell culture, and may significantly alter the medical care offered to patients (1). Since 2001, the Virology Laboratory at YNH has been performing NASBA, a highly sensitive RNA amplification method, to detect enterovirus RNA (2,3).

Real-time TaqMan RT-PCR: In 2005, we have changed to real-time TaqMan PCR (4), a method currently used for other viruses in our laboratory. Sensitivity varies somewhat for different serotypes, but is similar to NASBA and generally in the range of < 1 to 10 TCID₅₀. See reverse for principle.

Pitfalls: Parechoviruses cause similar illnesses, but are not detected by enterovirus molecular tests (2). Improper handling of CSF, or CSF collected late in illness, can yield false-negative results.

Samples: **1) Neurologic disease:** 0.5-1.0 mL CSF. **2) Neonatal sepsis:** CSF, urine, and serum. **3) Other:** Throat swab and stool. Note: Enteroviruses can be shed in high titers in stool for prolonged periods. While testing stool can be very helpful, a positive result in stool alone may not correlate with current disease.

Time to result: Generally within one working day, excluding weekends and holidays (when staffing is limited and molecular tests are not performed).

CAUTION-Dual Infections: Clinicians must also be aware that dual infections with enteroviruses and bacteria can rarely occur. If clinical features are not compatible with a benign viral meningitis, the finding of an enterovirus in the CSF should not lead to discontinuing antibiotics.

Clinical Virology Laboratory Enterovirus NASBA Results from 2004

Month	Jan	Feb	March	April	May	June	July	Aug	Sept	Oct	Nov	Dec	Total
No. Pos.	0	0	0	0	1	3	6	29*	7	8	4	1	59
No. done	2	6	5	7	16	27	37	65	50	48	34	16	313

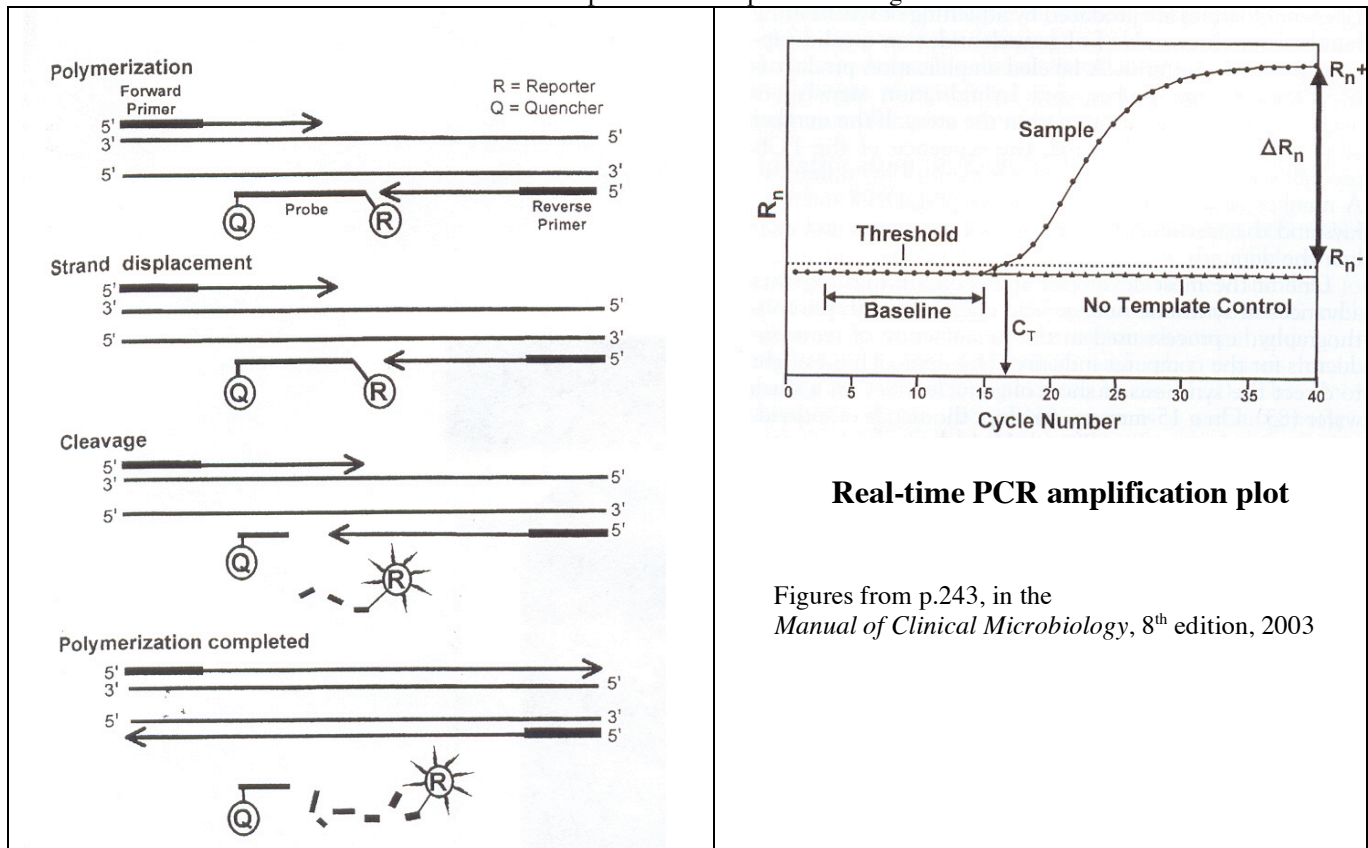
*Includes samples from an EV outbreak in a church youth group returning from Mexico.

Patient Ages: Of 59 EV positive patients, 23 (39%) were less than one year old, 29 (49%) were 1-18 years old, and 7 (12%) were 19 or older (i.e. 19, 22, 23, 27, 45, 50 and 63 years of age).

Spinal fluid profiles: Despite meningitis, CSF protein levels were often in the normal range, especially in older children (68%) and adults (57%). Normal leukocyte counts however, occurred only in infants less than 3 months old (5).

Principle of Real-time PCR using TaqMan chemistries

1. Specific probe hybridizes to target DNA in the sample (see left panel below).
2. When in close proximity on the intact probe, the Quencher dye prevents detection of the fluorescent Reporter dye
3. When specific primers anneal and extend, the hybridization probe is degraded by the exonuclease activity of *Taq* DNA polymerase and the Reporter dye is released and separated from the Quencher dye
4. After “N” cycles of amplification, sufficient Reporter molecules have been released to cross the threshold that separates positive from negative results (see right panel below).
5. The Cycle number at which the sample becomes positive is called the “cycle threshold” (C_T). The lower the cycle number, the higher the amount of target DNA in the patient’s sample.
6. Thus, amplification is detected “in real-time”, and separate hybridization and detection steps are not needed.
7. Real-time amplification provides a shorter time to result, ability to quantify target DNA, and a reduction in cross-contamination since reaction tubes are not opened once amplification begins.



Figures from p.243, in the
Manual of Clinical Microbiology, 8th edition, 2003

References:

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2. Landry ML, Garner R, Ferguson D. Rapid enterovirus RNA detection in clinical specimens using nucleic acid sequence based amplification (NASBA). *J Clin Microbiol* 41:346-350, 2003.
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