

FLETCHER ALLEN HEALTH CARE

PATHOLOGY & LABORATORY MEDICINE

TEST UPDATE: VIRAL DETECTION

ASSAY INFORMATION:

Viral culture at Fletcher Allen Health Care has been performed by traditional cell culture techniques: inoculation of monolayers in tubes, examination for cytopathic effect, and identification of the isolate with fluorescein-tagged monoclonal antibodies. The one exception was cytomegalovirus, for which we additionally performed a spin-amplification technique.

Our laboratory is moving at a steady pace towards viral diagnosis by molecular methods, which provide more sensitive and more timely answers¹. Now the spectrum of viruses that are not covered by molecular techniques and that can be isolated in cell culture is greatly reduced. We are therefore moving to spin-amplification for all viral culture. **This change means that we MUST know which virus(es) you are considering in order to perform testing**

The test will be performed in the Clinical Microbiology Laboratory once daily.

CLINICAL APPLICATION:

Viral diagnostic techniques are essential for management of select patients with infectious diseases, particularly those who are immunosuppressed. The following table lists the most common agents and the appropriate technique:

Agent	Technique	CPT
Influenza viruses	PCR	87798 x2
Respiratory syncytial virus (RSV)	PCR	87798
Parainfluenza viruses **	Culture, hemadsorption	87253 (possibly x2), 87140 (possibly x3)
Adenovirus	Shell-vial	87254 (possibly x2)
Human metapneumovirus	PCR	87798
Herpes simplex virus	PCR	87529 x2
Varicella-zoster virus	Shell-vial; Direct immunofluorescence	87254 (possibly x2)
Enterovirus (CSF)	PCR	87498
Cytomegalovirus	Shell-vial	87254

** PCR will replace culture once testing receives FDA approval. CPT 87798 x3.

Please note that in several important clinical situations PCR will detect the great preponderance of etiologic agents:

1. Acute respiratory infection: Influenza A, Influenza B, RSV
2. Genital: Herpes simplex virus
3. CSF: Enterovirus, Herpes simplex virus

It should not be necessary to order viral culture except in special circumstances, such as immunosuppressive disease or chemotherapy.

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METHOD:

The spin-amplification (shell vial) technique was first developed for the isolation of *Chlamydia trachomatis*. Smith and colleagues later applied the technique to the isolation of cytomegalovirus (CMV) and demonstrated a high sensitivity compared to culture, particularly in urine specimens. CMV is a notoriously slow growing virus, requiring 10-21 days in traditional cell culture for detection, compared to 48 hours in shell vials. Subsequently, other microbiologists have applied the technique to a variety of other viruses. A further refinement came with the development of mixtures of cell that would support the growth of specific viruses or groups of viruses.

In the shell vial technique a monolayer of cells is grown on a small, round glass coverslip, which is then placed in the bottom of a flat-bottomed tube. The specimen is centrifuged onto the monolayer, increasing the contact between virus and cell. Subsequently, the vial is incubated at 37°C for a period of time appropriate for the virus of interest. The coverslip is then retrieved and stained with a fluorescein-conjugated monoclonal antibody to the virus of interest. The sensitivity of the spin-amplification is similar to conventional culture and results are obtained more quickly (although not as rapidly as with molecular amplification techniques). **Because the cell monolayer and the monoclonal antibody are specific for the virus of interest, it is absolutely essential to know which virus(es) are suspected.**

ORDERING INFORMATION:

Test Name:	Viral Detection
Test Code:	VIRD
Sample Requirements:	Collect sample and place in VCT broth or in a sterile container, 0.5 mL fluid, 0.5 gram tissue or a swab refrigerated.
Sample Note:	Testing cannot be performed until suspected virus(es) are indicated, please note on laboratory requisition.
Expected Value:	No virus recovered
Days Performed:	Daily
Analytical Time:	Report available within 2-7 days, depending on virus.
Patient Price:	Call Laboratory Customer Service for pricing information (847-5121 or 1-800-991-2799).
Effective Date:	October 19, 2009

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