



Porter Hospital Laboratory

115 Porter Drive
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TEST ANNOUNCEMENTS

Attached you will find several updates and new test announcements received from Fletcher Allen Health Care you need to be aware of as the specimen requirements and methodology may have changed.

- Viral Detection
- Syphilis Serology
- Vitamin D, 25-OH (Total)
- Lyme Antibody (*Borrelia burgdorferi*)

Also the following tests have had specimen requirement changes:

| Test | Specimen Required | Transport Temperature |
|----------------------------------|--|-----------------------|
| Amitriptyline & Nortriptyline, S | Collect blood 12 hours after last dose in red top . Spin and send 3 mL of serum | Refrigerate |
| Desipramine, S | Collect blood 12 hours after last dose in red top. Spin and send 1 mL of serum | Refrigerate |
| Imipramine & Desipramine, S | Collect blood 12 hours after last dose in red top . Spin and send 1 mL of serum | |
| Insulin Antibodies, S | 0.5 to 1.0 mL Serum | Refrigerate |
| Iodine, S | Draw blood in a plain, royal blue-top Monoject trace element blood collection tube. Spin down and send 1 mL of serum | Refrigerate |
| Nortriptyline, S | Collect blood 12 hours after last dose in red top . Spin and send 1 mL of serum | Refrigerate |
| Phenytoin Total and Free, S | 2 ml serum from red top | Refrigerate |
| Primidone & Phenobarbital, S | 1 ml serum from red top | Refrigerate |

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.

FAHC laboratory is moving at a steady pace towards viral diagnosis by molecular methods, which provide more sensitive and more timely answers. 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 FAHC 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 | Units | Patient Price/Unit |
|-----------------------------------|--|--------------|--------------|---------------------------|
| Influenza viruses | PCR | 87798 | 2 | \$176.79 |
| Respiratory syncytial virus (RSV) | PCR | 87798 | 1 | \$176.79 |
| Parainfluenza viruses ** | Hemadsorption Fluorescent AB | 87253 | Possibly x2 | \$103.57 |
| | | 87140 | Possibly x3 | \$57.93 |
| Adenovirus | Shell-vial | 87254 | Possibly x2 | \$95.00 |
| Human metapneumovirus | PCR | 87798 | 1 | \$250.00 |
| Herpes simplex virus | PCR | 87529 | 2 | \$170.60 |
| Varicella-zoster virus | Shell-vial; Direct immunofluorescence | 87254 | Possibly x2 | \$125.00 |
| Enterovirus (CSF) | PCR | 87498 | 1 | \$210.00 |
| Cytomegalovirus | Shell-vial | 87254 | 1 | \$95.00 |

** 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.

Test Update: Viral Detection (cont.)

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:

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|----------------------|--|
| 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. |
| Price: | See table for pricing and CPT codes. |

Test Update: Syphilis Serology

Assay Information:

Effective November 3, 2009, the Chemistry Laboratory will begin performing a *Treponema* assay as initial screening for syphilis. The assay will be performed on the DiaSorin Liaison, which utilizes a sandwich chemiluminescence immunoassay for the qualitative determination of total antibodies directed against *Treponema pallidum* in serum. If you have any questions regarding this change in testing, please contact Dr. Greg Sharp in the laboratory (847-5115) or by email (greg.sharp@vtmednet.org).

Method:

The DiaSorin Liaison *Treponema* assay is a one-step sandwich chemiluminescence immunoassay. *Treponema pallidum* antibodies present in the patient sample bind to magnetic particles coated with recombinant *Treponema pallidum* antigens as well as the isoluminol-antigen conjugate (recombinant *Treponema pallidum* antigen conjugated to isoluminol). A wash cycle removes unbound material. Starter reagents are added to induce a flash chemiluminescent reaction. The light signal, and hence the isoluminolantigen conjugate, is measured by a photomultiplier as relative light units and is indicative of *Treponema pallidum* total antibody concentration.

Clinical Application:

Syphilis is a disease, generally sexually transmitted, caused by an infection with the spirochete *Treponema pallidum*. The natural course of syphilis is divided into three phases. After an incubation period of about three weeks, a skin lesion (chancre) appears and is often associated with regional lymphadenopathy (primary phase). The disease progresses into the secondary phase, which is disseminated and accompanied by general mucocutaneous lesions and lymphadenopathy. If syphilis is allowed to progress into the late phases of the disease, a subclinical infection (latent syphilis) can only be detected by serological tests.

Reversal of the traditional syphilis screening sequence from initial testing with a nontreponemal test such as RPR, followed by a treponemal assay when positive, to initial testing with a treponemal assay, followed by a RPR when positive, was suggested by the CDC a few years ago.

Treponemal assays detect antibodies specific to *Treponema pallidum*. A reactive treponemal result indicates that treponemal infection has occurred at some point in the past but can not differentiate between treated and untreated infections. Treponemal assays can produce reactive results for life even after treatment for syphilis. Nontreponemal assays such as the RPR detect antibodies to cardiolipin and are not specific for treponemal infection. Nontreponemal assays are more likely than treponemal assays to produce nonreactive results after treatment. When results are reactive by both the treponemal and RPR tests, persons should be considered to have untreated syphilis unless it is ruled out by treatment history. When results are reactive by the treponemal assay but nonreactive by RPR, samples will be sent to the State Laboratory for a FTA (fluorescent treponemal antibody) assay.

Test Update: Syphilis Serology (cont.)

Assay Limitations:

- This assay screens for the presence of *Treponema pallidum* total antibodies. It detects both recent and past infections, but it can't differentiate between antibody classes.
- Detection of *Treponema pallidum* total antibodies may indicate recent, past or successfully treated syphilis. Therefore, this assay can not discriminate between active and treated disease.
- The Liaison Treponema assay may produce reactive results than are nonreactive by RPR because it detects *Treponema pallidum* antibodies that persist for life. RPR tests usually produce negative results in the absence of recent infection because they detect heterophilic antibodies that are present in only the early phase of infection.
- Grossly hemolyzed, icteric or lipemic samples are not acceptable for analysis.

Test Name: Syphilis Serology

Test Code: SYPH

Sample Requirements: Collect 2.5 mL of blood in either a SST or Red Top Tube.
Submit 0.8 mL of serum refrigerated.
The minimum volume is 0.5 mL.

Test Note: Samples that are reactive by the Treponema assay will reflex an RPR. If the RPR is negative, the sample will be sent to the State Laboratory for a FTA assay. You have the option to decline reflex testing if you believe it is not medically necessary.

Days performed: Monday-Friday

Analytical Time: Same day

Expected Value: Non reactive

Patient Price: \$33.73

CPT Code: 86592

Effective Date: November 3, 2009

Test Update: Vitamin D, 25-OH (Total)

Assay Information:

As of November 3, 2009, the Chemistry Laboratory will begin performing a 25-OH Vitamin D (Total) assay. The assay will be performed on the DiaSorin Liaison, which utilizes a direct competitive chemiluminescence immunoassay for the quantitative determination of total 25-OH Vitamin D. If you have any questions regarding this testing, please contact Dr. Greg Sharp in the laboratory (847-5115) or by email (greg.sharp@vtmednet.org).

Method:

The DiaSorin Liaison 25-OH Vitamin D assay is a direct competitive chemiluminescence immunoassay. The 25-OH Vitamin D in the patient serum competes with 25-OH Vitamin D conjugated to an isoluminol derivative for binding sites on the anti-25-OH Vitamin D coated magnetic particles. Starter reagents are added to initiate a flash chemiluminescent reaction. The observed light signal is measured by a photomultiplier as relative light units and is inversely proportional to the patient's 25-OH Vitamin D concentration.

Clinical Application:

Vitamin D plays an important role in bone and mineral metabolism and has recently been recognized as a prohormone that has multiple roles in maintaining good health. Vitamin D3 (cholecalciferol) and Vitamin D2 (ergocalciferol) are the most abundant forms of Vitamin D in the body. Vitamin D3 is synthesized in the skin from 7-dehydrocholesterol in response to sunlight. Dietary sources of Vitamin D3 are oily fish such as salmon and mackerel. Dietary sources of Vitamin D2 are from some vegetables, yeast and fungi. Vitamin D is converted to 25-OH vitamin D in the liver. Measuring 25 OH Vitamin D concentrations in the serum gives the best indication of Vitamin D status.

Assay Limitations:

- The antibody utilized in this assay will demonstrate cross-reactivity to many dihydroxylated metabolites of Vitamin D. These compounds are naturally present in picomolar concentrations in humans.
- Grossly hemolyzed, icteric or lipemic samples are not acceptable for analysis.
- The effect of heterophilic antibodies has not been evaluated.

References:

Liaison 25 OH Vitamin D Total Assay product insert, DiaSorin Inc., January, 2009.

Test Update: Vitamin D, 25-OH (cont.)

Test Name: Vitamin D, 25-OH (Total)
Test Code: VITD
Sample Requirements: Collect 2.5 mL of blood in either a SST or Red Top Tube.
Submit 1.0 mL of serum refrigerated.
The minimum volume is 0.5 mL.
Days performed: Monday – Friday
Analytical Time: Same day
Expected Value: <10 ng/ml Deficiency
10-30 ng/ml Insufficiency
30-100 ng/ml Sufficiency
>100 ng/ml Toxicity
Patient Price: \$120.00 **CPT Code:** 82306

Test Update: Lyme Antibody (Borrelia burgdorferi)

Assay Information:

As of November 3, 2009, the Chemistry Laboratory will begin using a new method for the Lyme Antibody assay. The new method is an indirect chemiluminescence immunoassay performed on the DiaSorin Liaison. This replaces the current method, which is an Immunitics C6 *Borrelia burgdorferi* ELISA assay. If you have any questions concerning this change, please contact Dr. Greg Sharp in the laboratory (847-5115) or by email (greg.sharp@vtmednet.org).

Method:

The DiaSorin Liaison Lyme Antibody (*Borrelia burgdorferi*) assay is an indirect chemiluminescence immunoassay. In the first step of the reaction, anti-*Borrelia burgdorferi* antibodies present in the patient serum bind to magnetic particles coated with VIsE antigen. During the second step of the reaction, the conjugate (mouse monoclonal antibodies to human IgG and human IgM conjugated to isoluminol) reacts with patient anti-*Borrelia burgdorferi* IgG and IgM antibodies that have bound to the VIsE antigen coated magnetic particles. Starter reagents are then added to induce a chemiluminescent reaction. The light signal, and hence the amount of isoluminol antibody conjugate, is measured by a photomultiplier as relative light units and is indicative of the concentration of *Borrelia burgdorferi* antibodies present in the patient serum.

Clinical Application:

Lyme disease is caused by the tickborne spirochete *Borrelia burgdorferi*. Lyme borreliosis is a multisystemic disorder that can affect several organs, such as large joints, cardiovascular system, skin and nervous system. Despite the fact that the *Borrelia* spirochetes elicit a strong immune response, the bacteria survive and persist in the circulation of infected patients. Lyme borreliosis generally progresses through several different stages, from early to late infection.

The DiaSorin Liaison assay features a solid phase coated with VIsE (variable major protein-like sequence, expressed) antigen from two different strains of the *Borrelia burgdorferi* sensu lato complex (*Borrelia burgdorferi* and *Borrelia garinii*). VIsE is an outer surface lipoprotein that is believed to play a major role in the immune response to Lyme disease. Lyme disease patients consistently produce a strong antibody response against VIsE, in all stages of the disease, including the early stages.

Assay Limitations:

- Grossly hemolyzed, icteric or lipemic samples are not acceptable for testing.
- Some patient samples may be reactive with the DiaSorin Liaison *Borrelia burgdorferi* assay but not reactive with the Western Blot test due to the use of different antigens in the Western Blot.
- Testing should be performed on patients with clinical symptoms of Lyme disease or when exposure is suspected.
- Potential assay interference due to circulating antibodies against Human Ehrlichiosis (HGE) and Tick Borne Relapsing Fever (TBRF) has been observed. Interpret results from these patients with caution.

Test Update: Lyme Antibody (cont.)

Assay Limitations (cont.):

- Samples from individuals vaccinated with a licensed OspA vaccine (LYMERix) have been assayed using the Liaison *Borrelia burgdorferi* assay and were found to be negative. The assay performance has not been determined on samples from recipients of other Lyme vaccines.
- Potential assay interference due to HAMA (human anti-mouse antibodies) exists.

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| Test Name: | Lyme Antibody |
| Test Code: | LYMAB |
| Sample Requirements: | Collect 2.5 mL of blood in either a SST or Red Top Tube. Submit 0.8 mL of serum refrigerated. The minimum volume is 0.5 mL. |
| Test Note: | Samples with results of Positive or Equivocal will reflex Lyme Disease Antibody Western Blot analysis (CPT 86617 x 2) to be sent to Mayo Medical Laboratories. You have the option to decline reflex testing if you believe it is not medically necessary. |
| Days performed: | Monday-Friday |
| Analytical Time: | Same day |
| Expected Value: | Negative |
| Patient Price: | \$74.98 |
| CPT Code: | 86618 |