



JULY/AUG 2008

PATHOLOGY & LABORATORY MEDICINE NEWSLETTER

NEWS YOU CAN USE

New Tube Needed for HIV-1 Viral Load. Our new assay for HIV-1 viral load uses a platform which automates both extraction of the RNA as well as quantitation. The dynamic range extends from 50 to 10 million copies/ml so no dilution is required for samples with high viral load. However, the new assay requires a dedicated 6 ml of EDTA plasma. We no longer accept yellow-top (ACD) or blue-top (Citrate) tubes for this test.

ABO/Rh Initiative Launched. In order to reduce the risk of a mismatched transfusion due to errors in patient identification or specimen labeling, a new procedure is in place. All requests for type-and-screen or type-and-crossmatch will be reviewed. If there is no previous ABO/Rh result in our laboratory information system, the Transfusion Service will notify the ordering unit and ask them to send a second specimen. This confirmatory test (which requires a special requisition and special orange-coded collection tube) is a reflex test that does not need a physician order and for which there is no additional charge.

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OLIGOCLONAL BANDS Markers of Neuroimmunological Disorders

Jim Faix MD - Medical Director of Clinical Chemistry & Immunology

Laboratory testing of cerebrospinal fluid (CSF), usually obtained by lumbar puncture, provides important information to help diagnose a number of neurological disorders. At LPCH and Stanford Hospital, the CSF is divided into four tubes as it is removed from the patient. The first is considered "dirty" and is used for routine Chemistry tests such as total protein and glucose. The second is considered "clean" and is sent to the Microbiology section for Gram stain and routine culture. The third is considered free of any blood associated with the procedure and is used for cell count and differential in the Hematology section. The fourth may be reserved for additional studies (such as Virology or Molecular testing or Cytopathology).

Other Chemistry tests sometimes ordered include tumor markers and, when multiple sclerosis is suspected, an examination for "oligoclonal banding". We have always performed this procedure in-house, but quantitative measurement of CSF IgG and albumin (and calculation of the CSF IgG index) was sent to an outside reference laboratory. We recently brought this additional testing in-house and have combined the orders into one: CSF Protein & Immunofixation Electrophoresis (CPIE).

ABNORMAL QUANTITY OF CSF IMMUNOGLOBULIN

CSF is produced by the choroid plexus primarily by filtration of blood, but there is also some active transport (especially of glucose). Relatively little protein normally gets through this barrier, however. Newborns have higher protein levels because it takes some time for structural changes in the arachnoid villi to allow increased CSF drainage (reflected in our different reference ranges for infants). Elevated CSF total protein is the most common abnormality and it may be associated with a number of problems including infection, tumor, inflammation and obstruction of CSF flow.

Because it is associated with increased production of antibody within the central nervous system, multiple sclerosis should be suspected when the elevated CSF total protein includes a significant amount of IgG. However, simply measuring the CSF IgG concentration is not sufficient. Any significant disturbance of the blood-CSF barrier will result in increased IgG levels. Consequently, it is necessary to show that the CSF IgG is elevated beyond what one would expect if there were no intrathecal synthesis.

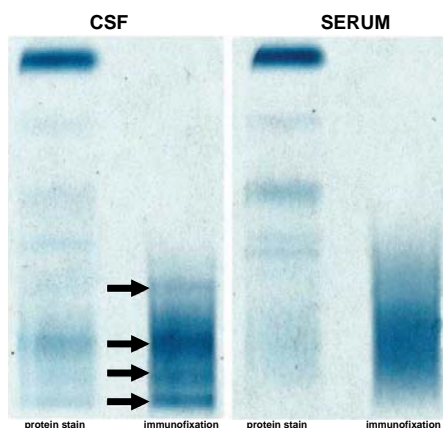


Fig. 1: CSF Oligoclonal banding

Paired CSF and serum specimens from a patient with multiple sclerosis. Several abnormal bands are present in the gamma region of the CSF protein electrophoresis, highlighted by the immunofixation procedure. No bands are seen in the patient's serum.

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NEWS YOU CAN USE (CONT.)

Rapid Testing On Vacation.

With “flu” season over, we no longer offer rapid testing for either RSV or influenza A and B antigens. Due to the low prevalence of these viruses during the summer, the predictive value of these tests is markedly reduced. We still offer year-round direct fluorescent antibody exam (DFA), culture and gene chip analysis for a variety of respiratory viruses. Rapid testing for RSV and influenza will resume in October.

Tuberculosis Testing Now Done in Primary Tube.

Our alternative to the traditional skin test for exposure to *Mycobacterium tuberculosis* requires *in vitro* stimulation of the person’s lymphocytes with specific mycobacterial antigen, followed by measurement of the cytokine interferon-gamma by immunoassay (ELISA). This test (QuantiFERON®) previously required culture in a microtiter plate, followed by transfer of culture fluid to a separate tube for ELISA. Now, both culture and assay for interferon-gamma can all be performed in the same primary tube. Only laboratory personnel may draw this test, using the new specialized blood collection tubes, and pediatric patients will need pre-approval from Pediatric Infectious Disease.

LABletter

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The most common way to show this is the **CSF IgG/albumin index**. The ratio of IgG to albumin in the CSF is compared with the same ratio in serum. If this index is elevated (>0.8), one should suspect that intrathecal antibody production is underway. Another approach to this determination is the so-called “CNS IgG synthesis rate” in mg/day. Most reports consider the two approaches to be equivalent, with a sensitivity for multiple sclerosis of approximately 75%.

ABNORMAL QUALITY OF CSF IMMUNOGLOBULIN

It is well known that the abnormal immune response in multiple sclerosis also results in a qualitative abnormality in the CSF immunoglobulin. Unlike conventional immune responses in which a relatively large number of B cell clones are stimulated to produce antibody, the immune response in multiple sclerosis is relatively restricted. Instead of the diffuse pattern characteristic of a polyclonal gammopathy, one sees discrete bands when the CSF is examined by electrophoresis. This pattern has been referred to as **oligoclonal banding**. It is not specific for multiple sclerosis but may be seen in a number of neuroimmunological disorders. In fact, it was first described in subacute sclerosing panencephalitis, an abnormal response to early infection with the measles virus. The oligoclonal bands in this disorder have been shown to be anti-measles antibody; the oligoclonal bands in multiple sclerosis may be antibodies against a similar inciting (but, as yet, unknown) virus.

Although electrophoresis using isoelectric focusing (IEF) with subsequent immunoblotting has been recommended as the “gold standard” for detecting the presence of oligoclonal bands

in the CSF, we continue to use high-resolution agarose gel electrophoresis combined with immunofixation. We will likely switch to IEF in the near future, using the same instrument we currently use for serum and urine protein electrophoresis and using an adaptation of our current immunofixation procedure. In the meantime, we will use the CSF IgG/albumin index as a guide to help us determine if the oligoclonal banding procedure needs to be repeated using more concentrated CSF.

	Reference range:
CSF IgG concentration	<6mg/dl
CSF IgG/albumin ratio	<0.3
CSF IgG/albumin index: $\frac{\text{CSF IgG/albumin}}{\text{serum IgG/albumin}}$	<0.8
CNS IgG synthesis rate:	<3 mg/d
$\left[\left(\frac{\text{IgG}_{\text{CSF}} - \frac{\text{IgG}_{\text{Serum}}}{369}}{\text{Alb}_{\text{CSF}} - \frac{\text{Alb}_{\text{Serum}}}{230}} - \frac{\text{IgG}_{\text{Serum}}}{\text{Alb}_{\text{Serum}}} \right) (0.43) \right] \times 5$	

Fig. 2: CSF IgG parameters

We are using the CSF IgG/Albumin index to help determine whether elevated CSF IgG is due to true intrathecal synthesis or breakdown in the blood-CSF barrier.

CPiE: CSF PROTEIN & IMMUNOFIXATION ELECTROPHORESIS

The new order code CPiE includes both the CSF IgG/albumin index and the analysis for oligoclonal banding. We are using only one charge code for all of this testing, which will include an interpretative comment. There are five classic patterns described for the appearance of the CSF electrophoresis when viewed with its paired serum specimen. If oligoclonal banding (or a single band) is present, the report will describe these findings.

CSF Protein and Immunofixation Electrophoresis

Order Code: CPiE

Synonyms:	CSF oligoclonal banding; CSF IgG index or synthesis rate
Specimen Type:	Cerebrospinal fluid and Serum/Plasma
Container Type:	Sterile CSF tube for CSF; SST Gold-top, Plain Red-top or Mint-top for Serum or Plasma
Required Volume:	1.0 ml for both CSF and Serum/Plasma
Minimum Volume (Pediatric):	0.5 ml for both CSF and Serum/Plasma
Methodology:	Protein electrophoresis, immunofixation and nephelometry
Components:	CSF protein electrophoresis, CSF immunofixation, CSF and Serum/Plasma IgG and Albumin and calculation of CSF IgG/Albumin Index
Standard Run Times:	Daily (Monday-Saturday)
Turnaround Time:	12 hours
Special Handling:	None
CPT Codes:	83916
Causes For Rejection:	None