

## **CSF ANALYSIS IN METABOLIC DISORDERS:** **INDICATIONS FOR LUMBAR PUNCTURE**

Many of the clinical features seen in inherited neurotransmitter disorders are non-specific, although for some specific disorders there are a characteristic set of clinical manifestations.

Investigation of some neuro-metabolic disorders requires biochemical analysis of CSF for routine biochemistry, amino acids, neurotransmitters and pteridines. Such disorders fall into the following groups:

**Lactic acidoses:** CSF lactate measurement. A normal CSF lactate argues strongly against, but does not exclude, a mitochondrial encephalopathy.

**Glucose transporter:** (GLUT-1 defect) Paired CSF and plasma glucose analyses. CSF/plasma glucose ratio < 0.4 is suggestive.

**Non-ketotic hyperglycinaemia, and serine deficiency disorders:** Paired CSF and plasma amino acid analysis.

**GABA metabolism disorders, dopamine, serotonin and catecholamine disorders and pteridine disorders:** All require CSF neurotransmitter and pteridine analyses.

### **PRINCIPAL INDICATIONS FOR LUMBAR PUNCTURE TO DIAGNOSE GENETIC METABOLIC ENCEPHALOPATHY**

CSF neurotransmitter and pteridine studies should be performed in the following clinical settings (particularly in patients with a combination of these clinical features):

Neonatal and infantile seizures of unknown cause

Clinical features suggestive of dopamine and serotonin deficiency (hypersalivation, swallowing difficulties, abnormalities of temperature regulation, pinpoint pupils, oculogyric crises, hypokinesia, distal chorea, truncal hypotonia, peripheral hypertonia, reduced spontaneous movements [incl. mask-like face], growth retardation)

Dystonia (with or without diurnal variation, with or without a response to L-dopa)

Other movement disorders (incl. chorea, tremor, myoclonic jerks, ballistic movements)

Hyperekplexia

“Primary” postural hypotension

Also in carefully selected cases with:

cerebral palsy, especially with an athetoid component; microcephaly or macrocephaly combined with other features; psychomotor retardation; difficult to control seizures; raised urinary vanillyl-lactic acid.

## **CLINICAL AND BIOCHEMICAL FEATURES**

**Lactic acidoses:** Widely variable clinical features. Elevated CSF lactate in disorders with cerebral expression.

**Glucose transporter defect:** Hard-to-control seizures, developmental delay, low CSF glucose and lactate, with CSF: plasma glucose ratio < 0.40

**Non-ketotic hyperglycinaemia:** Neonatal onset: hypotonia, seizures, apnoeic attacks, lethargy, hiccoughs. Late-onset: nonspecific neurological symptoms. Burst-suppression EEG. Elevated CSF glycine, with CSF: plasma ratio > 0.05 (lower in late-onset).

**Serine deficiency disorders:** Microcephaly, psychomotor retardation, and intractable seizures. Low CSF serine, and usually glycine.

**Folinic acid responsive seizures:** Very early seizures. Rapid response to folinic acid. Unknown compound in CSF

**Disorders of GABA metabolism:** (GABA quantitation not available at our laboratory)

**Glutamic acid decarboxylase:** Some B6-responsive seizures may be due to defective Glutamic acid Decarboxylase. CSF GABA is low while CSF glutamine may be elevated in some.

**GABA transaminase deficiency:** Only three cases reported: Feeding problems, axial hypotonia, seizures, hyperreflexia, severe psychomotor retardation accelerated growth. CSF GABA,  $\beta$ -alanine, homocarnosine increased. Same profile seen with Vigabatrin therapy.

**Succinic semialdehyde dehydrogenase deficiency:** Variable psychomotor retardation, delayed speech, hypotonia, ataxia, seizures, aggression, oculomotor apraxia, nystagmus, choreoathetosis. Gamma-hydroxybutyrate elevated in urine, plasma and CSF

**Hyperekplexia:** Stiffness on handling, excessive startle response. Disorder in glycine receptor,  $\alpha$ -1 subunit. Response to clonazepam.

### **Inborn errors of monoamines:**

#### **1. Affecting dopamine metabolism**

**Tyrosine hydroxylase deficiency** 1<sup>st</sup> year presentation: extrapyramidal signs: dystonia (lower limbs), tremor, or hypokinetic-rigid Parkinsonian syndrome. Some have diurnal variation. Low CSF HVA and MHPG; normal 5-HIAA.

#### **2. Affecting dopamine and serotonin metabolism**

**Aromatic l-amino acid decarboxylase deficiency:** Extrapyramidal movement disorder, axial hypotonia, abnormalities of eye movement, including oculogyric crises. Autonomic features, increased startle, sleep disturbance, etc Low HVA, MHPG, and 5-HIAA in CSF. High levels of L-dopa, 5-HTP, 3-O-methyl-L-dopa, vanillylactic acid in CSF. Elevated vanillylactic acid in urine. Pteridines normal.

### Other disorders

Many other disorders have been described in one or two families, or are putative defects, yet to be described. These include:

**Monoamine oxidase deficiency:** Depression, ADHD, aggression

**Catechol-O-methyltransferase deficiency**

**Dopamine  $\beta$ -hydroxylase deficiency:** orthostatic hypotension in adolescents and adults

**Tryptophan hydroxylase deficiency**

**Dopamine transporter defect**

**Serotonin transporter deficiency**

Blood, plasma and urine as well as CSF may be required for investigation of these disorders.  
For more details contact:

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## **CEREBROSPINAL FLUID NEUROCHEMISTRY**

This protocol allows cerebrospinal fluid (CSF) to be collected for a variety of tests in addition to neurotransmitter analyses minimises the volume of fluid required and is thus suitable for both children and adults. Where there is diurnal variation, CSF should be collected when symptoms are the most severe.

- Avoid therapy with drugs affecting neurotransmitter turnover for ten days prior to collection.
- CSF for neurotransmitter analysis must be collected first.
- Follow all details for collection, storage, preservation and transport to reduce variations resulting from the lumbar-ventricular gradient, to minimise breakdown of neurotransmitters and their metabolites and to maintain consistency with our reference intervals.
- The volumes of CSF collected should not be varied according to the age of the patient. Age-related differences are accounted for by the use of age-specific reference intervals.

Gather all your requirements before attempting the collection, and identify volumes required for tests, where to take samples and how they should be handled. Five micro-tubes are provided by the laboratory. Other tubes as required. "Wet" ice (a paper cup containing ice blocks from the ward refrigerator is suitable). Any samples contaminated by blood must be rapidly centrifuged at 5 degrees Celsius, and the supernatant transferred to a clean labelled tube before freezing.

Samples for neurotransmitters should be frozen within 5 minutes of collection. Additional CSF samples may be collected in the standard tubes for a variety of other analyses and stored at room temperature or as required by the relevant laboratory. Generally the minimum volume required for neurotransmitters and other tests will be 4.0 – 5.0 ml CSF, although additional amounts may be required for a variety of PCR tests and for TB screening.

## **CEREBROSPINAL FLUID NEUROCHEMISTRY** **COLLECTION PROTOCOL**

Collect the following set of micro-tubes first.

Collect onto "wet" ice and send within 5 minutes to the Biochemistry Laboratory. If the sample is contaminated with blood, the sample must be immediately centrifuged in the Biochemistry Laboratory, and the supernatant transferred to a fresh tube.

Micro-tube 1	0.5 mL CSF for Lactate, Glucose and Pyruvate <sup>1</sup>
Micro-tube 2	0.5 mL CSF for Amino Acids <sup>2</sup>
Micro-tube 3	0.5 mL CSF for HVA, 5-HIAA and other metabolites <sup>3</sup>
Micro-tube 4	0.5 mL CSF for Pteridines <sup>4</sup>
Micro-tube 5	0.5 mL CSF for MHPG, 3-MDOPA and storage for future studies
Micro-tube 6	0.5 mL CSF for Immunology (Collected for CHW patients only)

Ideally CSF should be collected at a time when symptoms are most severe, especially for DRD with diurnal variation. Collect all tubes to ensure consistency in sampling volume. For example if lactate, pyruvate, amino acids are not required, it is still essential to collect 0.5ml into microtubes 1 and 2 and store or redirect for other analyses. A paired blood sample for glucose, lactate, pyruvate and amino acids should be collected onto ice and sent immediately to the Biochemistry Laboratory.

Collect the remainder in standard tubes.

Collect at room temperature and send immediately to the Microbiology Laboratory.

Tube 1	1.0 mL CSF for Bacteriology, Glucose & Protein <sup>5</sup>
Tube 2	1.0 mL CSF for Virology or Cytospin
Tube 3	Further CSF as required for DNA studies or TB screening <sup>6</sup>

### **Notes:**

1. Micro-tube 1 is most likely to be blood-contaminated. The laboratory can centrifuge this sample, but this must take place immediately, with the clear CSF supernatant being transferred to a fresh tube.
2. Sample will be passed on to the NSW Biochemical Genetics Service for amino acid analysis. A paired blood sample is also required for glycine analysis.
3. This sample must be free of preservative to facilitate metabolite analyses
4. This tube contains a special preservative consisting of diethylenetriaminepentaacetic acid and dithioerythritol to minimise breakdown of tetra-hydrobiopterin.
5. Sample will be processed rapidly by Bacteriology and supernatant passed on to Biochemistry for analysis of glucose and protein.
6. Virology Department at The Children's Hospital at Westmead requires 10mL of CSF for TB screening.

## **CEREBROSPINAL FLUID NEUROCHEMISTRY - MEDICATION EFFECTS**

A list of all drugs being taken by the patient should be provided to the laboratory including any medication/anaesthesia for performing a lumbar puncture. The presence of certain drugs may be important in assessing CSF abnormalities, or it may be necessary to modify analysis conditions to avoid interference from various drugs and their metabolites.

The following drugs should be avoided for ten days prior to CSF collection.

### Metabolic Intermediates

(Produce high levels of CSF amines and metabolites)

L-DOPA	3,4-DIHYDROXYPHENYLALANINE, MALDOPAR, SINEMET
5-HTP	5-HYDROXYTRYPTOPHAN

### Mono-amine Oxidase Inhibitors

(Prevent formation of metabolites)

Selegiline	ELDEPRYL, SELGENE
Moclobemide	ARIMA, AURORIX
Tranylcypromine	PARNATE
Phenelzine	NARDIL

### Catechol-O-Methyltransferase Inhibitors

(Prevent formation of metabolites)

Entacapone	COMTAN
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### Re-uptake inhibitors

(May raise or lower amines and metabolites)

Fluoxetine	ZACTIN, AUSCAP, EROCAP, FLUOHEXAL, LOVAN, PROZAC, ZACTIN
Citalopram	CIPRAMIL
Fluvoxamine	FAVERIN, LUVOX
Paroxetine	AROPAX
Sertraline	ZOLOFT
Venlafaxine	EFEXOR

For further information contact Dr Sushil Bandodkar. Ph: 61-2- 9845-3289 Fax: 61-2-98453332

## **Information for Referring Laboratories.**

### **Storage & packaging:**

CSF specimens should be stored below -20deg. C. prior to packaging and transport. Samples should be transported with sufficient dry ice to ensure samples remain frozen for the whole time the samples will be in transit. Dry ice must not be placed inside a sealed container and packaging must comply with IATA standards: International Air Transport Association, Dangerous Goods Regulations 1999, Section 3.6.2.4, p 80-81.

### **Request Form:**

A request form must accompany each set of patient samples, setting out details of the patient, doctor, referring institution, billing information, clinical notes, details of the patient's medication, clinical details, brain imaging, and preliminary CSF chemistry.

### **Transport:**

Address samples to: Dr Sushil Bandodkar, Clinical Biochemistry, The Children's Hospital at Westmead, Hawkesbury Road, Westmead, NSW. Australia. In practice it is best to send specimens from interstate or overseas early in the week so they will arrive between 9:00 am and 2:00 pm on Monday - Thursday. On several occasions, samples arriving in Sydney on a Friday afternoon have not been delivered until the following Monday morning, by which time the specimens had completely thawed.

### **Prepared by:**

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### **References:**

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Wevers R.A., de Rijk-van Andel J.F., et al. A review of biochemical and molecular genetic aspects of tyrosine hydroxylase deficiency including a novel mutation (291delC). . *J Inher Metab Dis.* 1999; 22; 364 – 373.

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Hyland K., Arnold L.A. Value of lumbar puncture in the diagnosis of genetic metabolic encephalopathies. *J Child Neurol* 1999; 14 (suppl. 1); S9 – S15.

Earl J. Neurotransmitter diseases. *Clin Biochem Rev* 2000; 21; 3 – 13.

## Request Form

MRN: \_\_\_\_\_ Date of Birth: \_\_/\_\_/\_\_ Female  Male   
Name: (FAMILY) \_\_\_\_\_ (GIVEN) \_\_\_\_\_  
Address: \_\_\_\_\_

### **Billing Status:**

- Hospital Patient in a recognised hospital  Private Patient in a recognised hospital  
 Private Patient in an approved day hospital facility  Patient from outside Australia  
 Compensable - Transcover Pre July 1989  Compensable - Other  
 Compensable - Motor Accident Authority, from July '89

**CSF Collection:** Date: \_\_/\_\_/\_\_ Time: \_\_:\_\_

- Lumbar  Ventricular  Other (specify) \_\_\_\_\_

Requesting Institution: \_\_\_\_\_ E-mail: \_\_\_\_\_

Institution Address: \_\_\_\_\_

Requesting Doctor: \_\_\_\_\_ (Signed) \_\_\_\_\_ Phone \_\_\_\_\_

AMO: \_\_\_\_\_ Provider No. \_\_\_\_\_

### **Tests Requested:**

- HVA, 5-HIAA, Pterins  Amino Acids  L-DOPA  5-HTP  Other \_\_\_\_\_

**Indications/ Clinical Notes:** \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

**Medication:** Anticonvulsants/Anaesthetic agents/other medication \_\_\_\_\_  
\_\_\_\_\_

L-DOPA therapy:  None  Ceased for \_\_\_ days prior to collection.

### **MRI:** Normal

- Brain size/shape abnormalities: \_\_\_\_\_  
 Ventricle/ fluid abnormalities: \_\_\_\_\_  
 Demyelinating disease: \_\_\_\_\_  
 Leukodystrophy: \_\_\_\_\_  
 Neurodegenerative condition: \_\_\_\_\_  
 Brainstem changes: \_\_\_\_\_  
 Other conditions: \_\_\_\_\_

### **Laboratory Studies already performed:**

CSF Appearance:  Bloodstained  Clear  Turbid  Coloured \_\_\_\_\_

CSF Cell Counts: RBC \_\_\_\_\_ Polymorphs \_\_\_\_\_ Mononuclear \_\_\_\_\_

CSF Glucose: \_\_\_\_\_ Protein: \_\_\_\_\_ Lactate: \_\_\_\_\_ Pyruvate: \_\_\_\_\_

CSF Phenylalanine: \_\_\_\_\_ Tyrosine: \_\_\_\_\_ Tryptophan: \_\_\_\_\_

Phenylalanine Load Test Result:  Normal.  Abnormal  Not Performed