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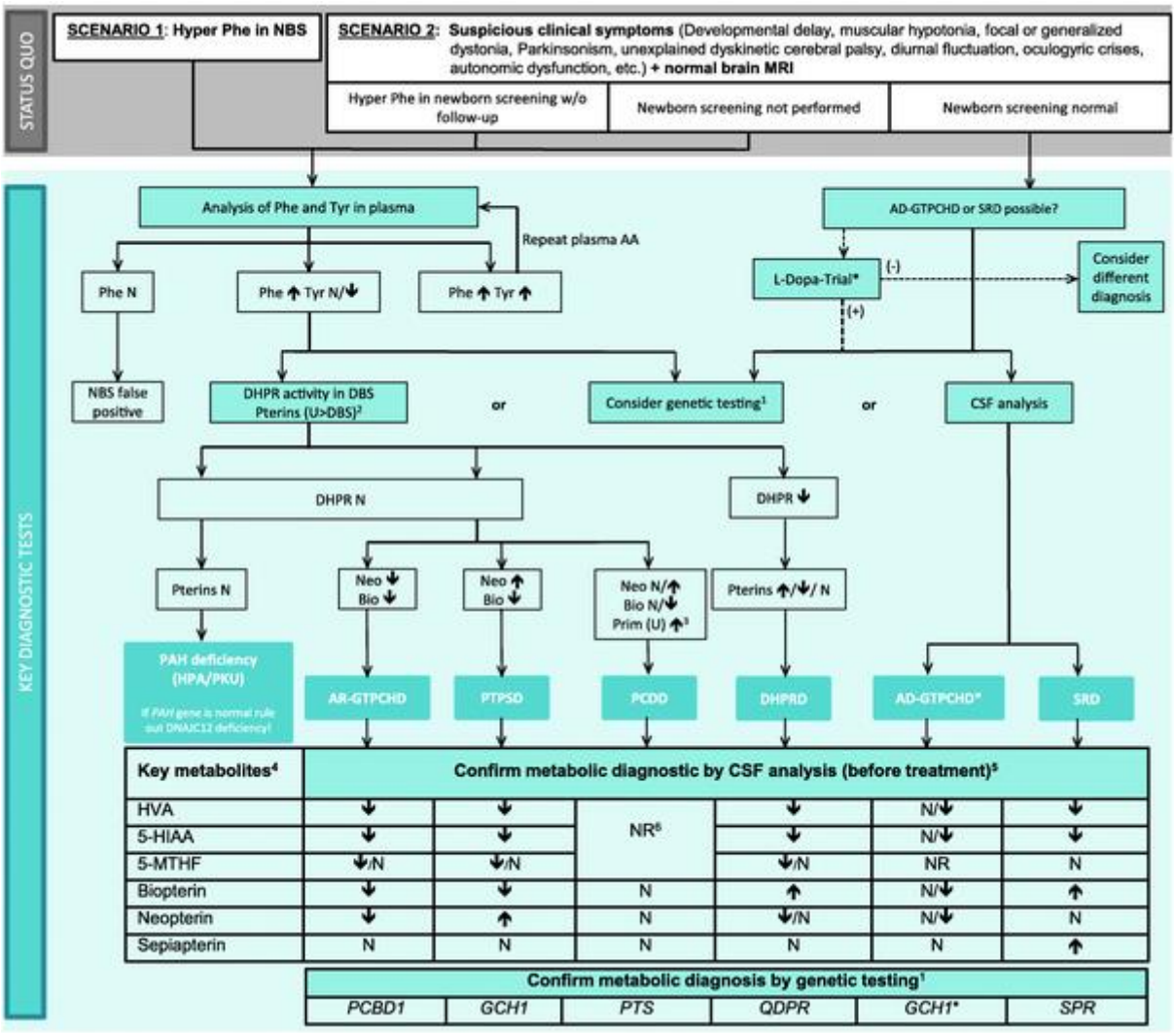
Urinary Pterins, Biogenic Amine Metabolites and BH4 Loading Test Information

Useful For

Differential diagnosis of tetrahydrobiopterin related pterin disorder with or without hyper phenylalanine. See the diagnostic flowchart shown below:

Diagnostic flowchart for differential diagnosis of BH_4Ds with and without HPA.



Ref: Consensus guideline for the diagnosis and treatment of BH4 deficiencies. Orphanet J Rare Dis. 2020; 15: 126

Test Code(s)

UPKUR: Urine Biogenic amine metabolite and Pterins panel (HVA, 5HIAA, VMA, NEO, BIO, PRI, SEP, and BIO ratio)

UCRR: Urine Creatinine

Test frequency

Batched and run at least twice a month
Urgent requests must be formally notified via email or phone call by the referrers.
Urgent UPKUR requests are normally reported within two weeks. Completion of a report may be delayed in instances when further investigation is required or information is sought from the referring clinician/metabolic centre prior to report finalisation. Specialised assays such as these require time and consideration when reporting to provide the clinician with the information that is personalised to each individual patient.

Specimen collection and handling

CHW In-Patients:

Random urines (10-20 mL) are collected using 50 mL yellow top urine jars or 11 mL urine tubes. If a newborn urine collector is used to collect urine, the urine once collected in the urine bag must be transferred into a 50 mL yellow top urine jar without delay whilst ensuring protection from light.
No preservatives are required for this collection.

Date of collection, time of collection, if applicable “Pre BH4 load” or “Post BH4 load” must be noted on the sample jar and the accompanying request form. Properly labelled urine containers are then wrapped in foil to protect from light.

Samples are transported in ample amount of ice and hand delivered to Pathology Specimen Reception. Samples must arrive cold.

Referred samples from other Laboratories/Hospitals/Metabolic Clinics:

Random urines (10-20 mL) are collected using 50 mL yellow top urine jars or 11 mL urine tube. If a newborn urine collector is used to collect urine, the urine once collected in the urine bag must be transferred into a 50 mL yellow top urine jar without delay whilst ensuring protection from light.

Date of collection, time of collection, and if applicable “Pre BH4 load” or “Post BH4 load” must be noted on the sample jar and the accompanying request form. Please fill in all details requested on the form – doing so will expedite reporting and a differential diagnosis.

Labelled urine containers are then wrapped in foil to protect from light, stored on ice and transported to the local Pathology Specimen Reception- Sendaway Department/Collection Centre. Samples must frozen immediately once receipted in the Laboratory.

Sendaway department of the referring laboratory/hospital/clinic must organise transport of samples to CHW Neurochemistry preferably Monday to Thursday.

Sample Transport

Ensure the Urine Pterin-Biogenic Amine Request Form is filled in.
Transport the frozen sample covered in foil to:

Department of Clinical Biochemistry -Neurochemistry Laboratory
Pathology Level 2
Corner Hawkesbury Road and Hainsworth Street
Westmead NSW 2145, Sydney, AUSTRALIA

Sample Reception During normal working hours

Once these urine samples are received in the CHW Pathology Specimen Reception area, they must be quickly inspected (must be correctly labelled, frozen and covered in foil), logged in and promptly transferred to the Neurochemistry -40 degrees Ultrafreezer Room 52PA017. These samples are stored at -40 degrees until assayed.

Documents received with the patient samples are inspected and scanned in and Sample acknowledgement forms faxed back to the referral laboratory. Samples which are not compliant with our collection, storage and transportation protocol are handled appropriately and the referral laboratory is contacted for remedial action.

Sample Reception During Out-of-Hours including weekends and public holidays

Urine samples are receipted by Biochemistry/Out of hours staff, stored overnight in the -40 degrees underbench freezer in the Corelab. These samples are logged in on the following working weekday and transferred to the Neurochemistry -40 degrees freezer in Room 52PA017. Documents received with the patient samples are scanned in and any sample acknowledgement forms faxed back to the referral laboratory.

Once received in Neurochemistry Laboratory, all new samples are logged into storage tracking in Pathnet under Storage Tracking/CHW Neurochemistry -40C/ Shelf/Received Neurolab. Logging of samples in neurochemistry is conducted when a scientist is allotted on the bench. Any missing samples or any sample integrity issues are quickly escalated to the clinical scientist.

Urine samples once analysed are stored in the -70 Ultrafreezer for long term storage.

Analytical Methodology

Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) for Pterins, Biogenic Amine Metabolites and Creatinine.

Ordering Guidance

Random urine collections are preferred. No preservatives such as HCl are required for this collection.

Unlabelled samples, unprotected samples from light or heat and contaminated samples will be rejected or will lead to significant delay in reporting and diagnosis.

It is possible for this laboratory to detect if urine samples were not protected from light and not stored frozen through the measurement of total Biopterin in untreated (unoxidized) samples. Presence of higher levels of total Biopterin in untreated samples may indicate poor sample integrity. Only small amounts of Biopterin is usually present in urine samples, the majority being the reduced forms BH4 and BH2.

Request form must be filled in completely and must accompany the samples for analysis. This will assist speedy turn-around-times and hopefully quicker differential diagnosis.

Urine samples contaminated with blood or faecal matter are not accepted for testing. Recollect clean sample.

Patient Preparation:

Patients with infection or other inflammatory disease may have raised neopterin levels consequent to their inflammatory condition giving a low Biopterin Ratio. In this case, a urine sample should be recollected for repeat analysis after the patient has recovered from their infection.

Patients undergoing BH4 Loading tests – Check the information on BH4 Loading Test given below

Specimen Stability Information

Frozen Samples (-40 degrees) protected from light are stable for at least 1 month

Report TAT

14 to 21 days once sample is receipted

Billing

Patients meeting the criteria for Medicare will continue to be bulk billed for eligible items only and referring institutions will be expected to pay for the non-MBS components. For patients not eligible for Medicare, the full cost will be payable by the referring institution. Urinary Pterins are not covered by the Medicare schedule. Random, Pre-BH4 and Post-BH4 samples are individual episodes and will be billed separately.
Write to sushil.bandodkar@health.nsw.gov.au for a copy of the current price list.

BH4 Loading Test Procedure

Newborn screening detects babies with elevated phenylalanine levels in the first few days of life. The most common reason for this is due to a deficiency in phenylalanine hydroxylase (PAH), an enzyme that breaks down phenylalanine to tyrosine. Tetrahydrobiopterin (BH4) is a co-factor in this chemical reaction within the body.
Elevated phenylalanine levels, with corresponding normal to low levels of tyrosine, are also an indicator of pterin defects in the body. This could be due to several different enzyme deficiencies within the BH4 recycling metabolic pathway.

Determination of Tetrahydrobiopterin (BH_4)-responsiveness in patients with Hyperphenylalanine (HPA) can be done by oral BH_4 loading test. The loading test is used to discriminate between patients with elevated phenylalanine (Phe) levels due to PAH deficiency and patients with elevated Phe levels due to BH_4 deficiencies.

A significant decrease in Phe concentration within the first 8–12 h is observed following BH_4 load in AR-GTPCHD, PTPSD and PCDD. In contrast, patients with DHPRD show a less prominent Phe reduction during the same time period.

The procedure usually consists of baseline assessment of Phenylalanine concentration in blood at times − 24 h, − 12 h, and 0 h (=basal measurement). This is followed by the oral administration of 20 mg/kg BW of Sapropterin dihydrochloride once daily, taken with a regular meal on two consecutive days. Phe concentration in DBS should be tested every 8 h for 72 h after exposure. Please refer to “Consensus guideline for the diagnosis and treatment of tetrahydrobiopterin (BH4) deficiencies” https://doi.org/10.1186/s13023-020-01379-8

Preparation for BH4 Load Procedure at CHW

Arrange bed for day stay admission if <2hrs away from the hospital or overnight admission if >2hrs away as soon as practical (ideally before baby is 2 weeks of age).
A medical record number will likely need to be created
eRFA completed
Parents notified of place, date and time of admission
Powerchart orders
2x urine samples for random urine pteridine and creatinine

Drug order for Sapropterin 20mg/kg once only via NG tube (contact pharmacy to ensure they have supply in stock)
Have all equipment on hand.

Special Considerations
Urine does not need to be sterile but should be a clean specimen.
Urine needs to be protected from light and heat as pteridine deteriorates quickly.
Check urine bag / nappy frequently.
Use foil to wrap urine container as soon as possible after collection.
Sapropterin is given via NG tube to ensure complete dose is given. Inadequate dosing affects interpretation of results.
Final dried bloodspot sample collected just before baby is due to feed.
Prescribed amino acid formula to commence only AFTER this sample is collected.
If final sample is collected at home, educate the family to allow the sample to dry then post to the laboratory via express post.

Equipment Required
Dried bloodspot cards
Lancets
Alcohol swabs
Sterile cotton wool or gauze swabs
Adhesive plasters (‘Band-Aids’)
Paediatric urine bag or collection device
Foil strips (pteridine deteriorates quickly with heat and light)
Nasogastric tube (FG6 for neonates)
Hyperfix
Universal indicator paper

BH4 Load Procedure
Complete information on dried bloodspot card and label with “Pre BH4 load”
Collect bloodspot sample and leave in safe place to dry
Place neonatal urine bag in-situ for baseline urine collection.
Immediately after urine is collected, label it “Pre-BH4”, note the date & time of collection on the label, wrap in foil and take to Pathology for freezing.
Insert NG tube and test location as per protocol.
ONLY AFTER BASELINE URINE IS COLLECTED, give correct dose of Sapropterin via NG tube.
1 hour post Sapropterin, take second dried bloodspot sample. Label with ‘1hr post BH4’. Alternate sites.
3-4 hours post Sapropterin, place neonatal urine bag in-situ for urine collection (and treat as previously). Label with “Post BH4”, note the date and time of collection on the label, wrap it in foil and take to Pathology for freezing.
4 hours post Sapropterin, take third dried bloodspot sample. Label with ‘4hrs post BH4’
8-10 hours post Sapropterin, take fourth dried bloodspot sample, pre-feed. Label with ‘8hrs post BH4’.
22-25 hours post Sapropterin, take fifth dried bloodspot sample, pre-feed. Label with ‘24hrs post BH4’.

Collection details

Urine Pterin Collection Details	**Random**	**Pre-BH4 Load**	**Post BH4-Load**	
			4Hr	**8Hr**
Labelled and covered in foil	Yes/No	Yes/No	Yes/No	Yes/No
Urine appearance				
Date of collection				
Collection time				
Referral Lab ID				

Clinical Symptoms

(please note that detailed clinical and drug therapy information is required for interpretation of test results; attach a separate page if required)

	Tick √		Tick √		Tick √
Autonomic dysfunction		**Hypokinesia**		**Parkinsonism**	
Cerebral palsy, unexplained dyskinetic		**Intellectual disability**		**Psychomotor delay**	
Developmental delay		**Microcephaly**		**Ptosis of eyelid**	
Diurnal fluctuation of symptoms		**Movement disorders including chorea, tremor, myoclonic jerks, ballistic movements**		**Rigidity**	
Dystonia, focal or generalised				**Seizure (Myoclonic)**	
Feeding difficulties				**Swallowing difficulties**	
Hypertonia, extremities				**Normal Brain MRI**	
Hypotonia, muscular axial		**Oculogyric crises**		**Neonatal Hyperphenylalaninaemia**	

Current Medication: **(please detail current treatment)**

☐ L-DOPA ☐ Sapropterin ☐ None ☐ Others

Required Laboratory Results

	Pre-BH4 Load	4 Hr Post BH4-Load	8 Hr Post BH4-Load
DBS Phenylalalanine			
DBS Tyrosine			
DHPR enzyme activity			

First line diagnostic test results if available

CSF Neurotransmitter (Biogenic Amines an Pterins) Studies
Genetic testing Results