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**Porphyria Testing Demystified**

***What is porphyria?***

Porphyrias are caused by various mutations in the enzymes that make up the heme synthetic pathway. Symptoms are exacerbated by certain drugs, particularly CYP450 inducers, stress and surgery, with sometimes fatal outcomes. Increased demand for the heme products leads to the accumulation of heme intermediate metabolites, including porphyrin precursors, porphobilinogen (PBG) and aminolevulinic acid (ALA), and/or porphyrinogens, depending on where the mutation occurs. The mechanism of the metabolite toxicity is not fully understood, but the literature most often suggests that PBG and ALA are neurotoxic, causing acute symptoms, (most commonly acute abdominal pain). In addition, it's thought that porphyrinogens, following oxidation to porphyrins, produce free radicals when exposed to UV light, resulting in the skin damage seen in the cutaneous porphyrias.

***Diagnosing porphyrias***

Although the porphyrias are rare, it is important to quickly and effectively exclude or diagnose the porphyrias when there is unexplained abdominal pain or cutaneous manifestation, and more common causes have been excluded. Diagnosis of porphyria can be made by combining clinical information with laboratory testing for the accumulation of heme intermediate metabolites in urine, stool and/or blood. The diagnostic strategy should initially determine whether the patient is presenting with acute abdomen or cutaneous symptoms, or both.

If acute porphyria is suspected, testing for urine PBG (commonly offered as the combined PBG/ALA test) is the recommended first line laboratory test. An increase in PBG (often close to 10 fold) is diagnostic of acute porphyria. Treatment can be started as soon as PBG elevation is detected, and further testing to determine the type of acute porphyria does not need to be rapid. However, it is best if all samples are collected prior to starting treatment, during an acute attack, before the metabolite levels normalize. Acute intermittent porphyria (AIP) is the most common acute porphyria. Variegate porphyria (VP) and hereditary coproporphyria (HCP) can present with cutaneous and/or acute symptoms. To differentiate between AIP, VP and HCP, urine PBG and both urine and fecal porphyrins need to be evaluated. Alternatively, plasma fluorescence emission spectroscopy can differentiate between these forms, however this test is not readily available.

LAB CONNECTIONS

***Your feedback, suggestions and new ideas are welcomed. Submit to the Editorial Office:***

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PBG deaminase is not the first-line test for acute porphyria, as it is diagnostic of AIP only, and levels can overlap between healthy individuals and AIP patients (due to partial enzyme deficiencies). In spite of its low performance, PBG deaminase is still recommended to aid in diagnosis of AIP between attacks (if urine PBG is inconclusive), or in family members of AIP patients. Testing other enzyme activities is rarely performed, due to limited availability and poor analytical performance.  $\delta$ -aminolevulinic acid dehydratase deficiency (ADP) is an extremely rare cause of isolated ALA elevation, therefore, any isolated ALA increase should be further investigated. Lead poisoning is a common cause of ALA elevation, and tyrosinemia type I, which can be excluded by organic acid testing, is a rare disorder with increased ALA.

Cutaneous porphyrias, porphyria cutanea tarda (PCT), hepatoerythropoietic porphyria (HEP) and congenital erythropoietic porphyria (CEP) can be differentiated by the presence of different porphyrin metabolites (fractions) on urine and fecal porphyrin screens followed by fractionation and quantitation. As mentioned above, VP and HCP can present with cutaneous and/or acute symptoms, therefore, they also require PBG testing. If erythropoietic protoporphyria (EPP) is suspected, total erythrocyte protoporphyrin (or free erythrocyte protoporphyrin, FEP) is the recommended diagnostic test.

It is controversial whether a random or 24h urine sample is better for urine PBG/ALA and urine porphyrin testing. The 24h urine sample is generally considered more sensitive, however it may delay diagnosis, and be compromised by inadequate urine collection. In addition, 24h urine specimens are associated with more false positive results. PBG/ALA and urine porphyrins are increased several fold in acute porphyrias, thus analytical sensitivity is usually not an issue during acute attacks. Currently the HRLMP recommends 24h urine sample as the preferred sample, and a random urine sample as acceptable in acute situations. Random urine porphyrin quantitation may be used to monitor improvement during treatment of acute porphyrias. Genetic analysis to identify the causative mutation is considered the gold standard, however rarely necessary for treatment, and not indicative of disease severity. It may be considered for confirmation, and in family members of a patient in whom a mutation has been identified.

### ***Simplifying the porphyria testing at HRLMP***

Porphyria testing can be a complex process, as multiple intermediates must be measured to accurately assess the array of possible mutations. This is further confused by the fact that porphyrins become less water soluble as they are processed through the heme synthetic pathway, resulting in different metabolites detected in different specimen types. This complexity leads to inappropriate test ordering and delays in testing. Thus, we are proposing an "all in one" porphyria testing option (**Figure 1**). The suggested Porphyria Panel is an order set that would require the collection of urine and fecal samples (+/- a blood sample if EPP is suspected), at the time of order. Ordering the tests as a panel is not expected to adversely affect current turnaround times (7-12 days). We anticipate the Porphyria Panel will streamline the process of porphyria testing, ensuring that all the necessary samples are collected during, or shortly after, the attack, when levels are likely to be the highest. There will be less opportunity for inappropriate ordering, which will result in more rapid diagnosis, and contribute to improved patient care and cost savings.

#### **We welcome any comments, questions or concerns.**

Kristin Hauff and Kika Veljkovic, Clinical Biochemistry Fellows

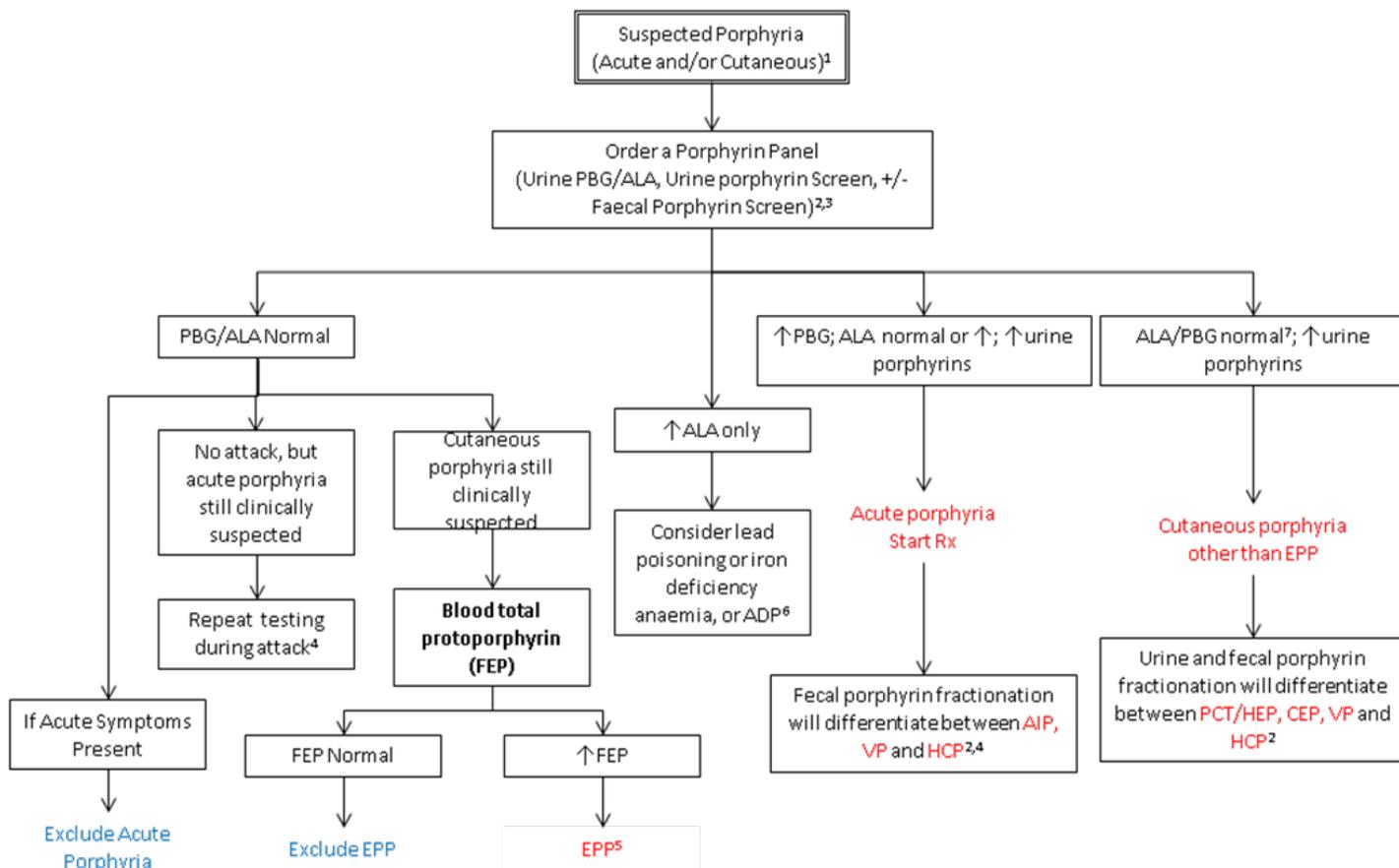
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Figure 1: The proposed HRLMP testing algorithm: Porphyrin Panel



1. Consider liver disease, alcohol and heavy metal poisoning, that may result in non-specific coproporphyrinuria; consider GI bleeding, and dietary heme that may result in non-specific proto excretion in feces.
2. Random or 24h urine samples are acceptable; it is best if both urine and fecal samples are collected, prior to the start of Rx; if fecal sample is unavailable, urine sample can be helpful, but cannot differentiate VP from HCP; plasma fluorescence emission spectroscopy can differentiate VP from HCP in this case.
3. Quantitation/fractionation is automatically performed for all positive screens.
4. Additional/alternative blood tests available upon consultation with biochemist: enzyme activity test (PBG deaminase is available in Canada) - used for confirmation, diagnosis between attacks or diagnosis in family members, however, there is an overlap in levels in porphyria patients and healthy individuals.
5. Consider lead poisoning or iron deficiency anemia in differential diagnosis, as they also result in increased total protoporphyrin.
6. ADP is an extremely rare form which results in increased ALA and normal PBG – if suspected, refer to genetic counselling services.
7. PBG can sometimes be increased in VP and HCP presenting with cutaneous symptoms.

## What else is in cocaine?

### Levamisole

- De-worming agent added to animal feed
- Added to cocaine in South America due to its similar appearance to cocaine and its co-stimulatory effect with cocaine
- Found in the majority of the cocaine in Canada
- May cause neutropenia or vasculitis in some people (seems to be based on HLA type)

**We have therefore modified our gas chromatography mass spectrometry method for confirmatory testing of cocaine metabolites in urine so that we can now detect and report the presence of levamisole.**

## Education News

Dr. Murray Potter has agreed to step into the role of DEC for the Department of Pathology and Molecular Medicine at McMaster.

We have 2 excellent Guest Speakers coming to our **Annual Resident Research Day** **May 24<sup>th</sup> 2012, MDCL 3020**

The topics reflect changing trends in the future of Pathology and will definitely be very exciting for pathologists, clinicians and radiologists.

### “Molecular pathology in a changing world of healthcare: SMART precision diagnostics”

**Dr. Jennifer L. Hunt, M.D. M.Ed**  
Chair of Pathology and Laboratory Medicine  
Aubrey L Hough, Jr. MD  
Endowed Professor of Pathology  
University of Arkansas for Medical Sciences,  
Little Rock, Arkansas

### “Endoscopic Microscopy: Bridging the Radiology-Pathology Divide”

**Dr. Guillermo Tearney M.D., Ph.D.**  
Professor of Pathology at Harvard Medical School,  
Affiliated Faculty member of the Harvard-MIT Division of Health Sciences and Technology (HST), Associate Director of the Wellman Center for Photomedicine, Massachusetts General Hospital

## Resident Training Programs

CaRMS results are in and the **Laboratory Medicine Resident Training Programs** are pleased to welcome the following residents for July 2012:

### Anatomical Pathology:

Paul Borowy-Borowski  
Jegan Jegathesan  
Jay Maxwell

### General Pathology:

Kak Ipshita

### Medical Biochemistry:

Janet Simons

### Medical Microbiology:

Diana Whellams

For information and the latest news on our residency training programs follow the link: <http://fhs.mcmaster.ca/pathres/news/index.html>

Information on the postdoctoral fellowship:  
<http://fhs.mcmaster.ca/pathology/education/postdoctoralfellowshiptraining.html>

## Laboratory Updates

The HRLMP is preparing for our second **OLA peer assessment** which is scheduled to start Monday April 23, 2012.

A new educational tool has been developed to aide nurses in determining **which tube to choose** when collecting blood:

<http://www.ltiq.hrlmp.ca/Which%20Tube%20to%20Choose.pdf>

## News from Chemistry

**Dr. Guillame Pare** has been recruited as a full time Medical Biochemist effective April 1, 2012. Dr. Pare is an outstanding clinician and researcher, and is a welcome addition to the HRLMP.

There will be a number of changes in chemistry as resulting from the move of the specialized chemistry laboratories and the core laboratory project. **Dr. Stephen Hill** will relocate to MUMC as a clinical biochemist with a paediatric focus. **Dr. Joe Macri** will supervise the new core-hub and will work with **Dr. Menaka Pai** for the quality mission of the core laboratories. **Dr. Johannes Zeidler** will lead TDM and toxicology and **Dr. Andrew Don-**

**Wauchope** will relocate to the JHCC. **Dr. Cynthia Balion** will relocate to SJHH as a POCT consultant.



#### Retirement party for Dr. Vijay Grey

After 36 years of dedicated service, **John Lafferty** has decided to retire, effective April 20. We wish him all the best in his retirement.

#### News from Pathology

**Dr. Hamidreze** will join the AP team at MUMC starting July 16, 2012. He completed his residency in Ottawa and is currently enrolled in a Surgical Pathology fellowship at the Mayo clinic. His fields of interest are Breast and Gynecological Pathology.

**Dr. Amal Abdel-Mesih** will begin working at St. Joseph's Healthcare on July 2. She is currently completing a fellowship in Head and Neck Pathology and has already begun research collaborations with our head and neck group.

#### News from Microbiology

Virology has repatriated HTLV serologic testing for the donor screening program. This will reduce turn-around time.

Congratulations to all of the microbiology technologists who staffed the HRLMP station at the MUMC annual review in March. The station was very well received again this year.

Hats off to all of the staff in Microbiology who took on a significant work load increase with no warning when Grand River Hospital was forced to shut down their microbiology facilities unexpectedly. All staff pitched in and gave their best effort.

#### News from Special Hematology

In Special Coagulation, we have said good-bye to **Betty Albay**, who retired March 31, 2012 after 35 years as an MLT. We welcome **Jennifer McNab** to our department.

In Molecular Hematology, we also said good-bye to **Judy Ireland** March 31, 2012, as she too has retired after 38 years as a technologist.

In Malignant Hematology, we welcome **David Kimmel** as our Technical Specialist in this area.

We wish Betty and Judy health and happiness and sincerely thank them for all their work and dedication over the many years that they've worked in the laboratory. We wish David and Jennifer all the best as they transition to their new positions within the HRLMP.



**Clinical Chemistry and Immunology consolidation** has been successfully completed after three years of dedicated work. The final result is the integration of testing from the General, MUMC and St. Joseph's campuses. This consolidation has been part of the HRLMP strategic plans since its inception in 1999.

This achievement is the result of a tremendous effort between the Clinical Chemistry technical staff, professional staff and administration. A dedicated consolidation team lead by our manager and MLT staff from each site was pivotal to the project success. The consolidation team was there from the beginning and actively involved in developing the consolidation project charter. They kept the project moving forward and provided a great conduit between the planning team and the technical staff.

From the early days of facility planning, the staff were participants in creating their new laboratory. Draft drawings moved from site to site to receive input and generate discussion. Working teams involved all staff and were created to plan for

operational changes and to develop and review work process. Teams addressed processes like turn around time planning, sample storage, specimen reception changes, purchasing and inventory, scheduling, RIA and training. Many of the teams are completing work as we continue to grow together as a consolidated team. **Through this collaborative work we will create the world class Clinical Chemistry and Immunology environment we have planned for.**

## Celebrating Research Success



**Dr. M. J. McQueen**

Since the 1970's, M. McQueen has been involved in research related to cholinesterase, markers of ischemic myocardial injury, lipids and lipoproteins.

In the early to mid-1980's, more effective pharmacological treatment of hyperlipidemia became available, presenting significant opportunities for academic laboratory involvement in clinical studies and clinical trials in the prevention and management of cardiovascular disease. This required increased capacity and the ability to meet good laboratory and clinical practice regulations, and so began what was later named the Clinical Research and Clinical Trials Laboratory (CRCTL).

The arrival in Hamilton of Dr. Salim Yusuf in the early 1990's and his HOPE study on the evaluation of the cardioprotective effects of the ACE-inhibitor Ramipril and Vitamin E in 9,300 patients in 19 countries, presented the research laboratory with an enlarged vision for clinical research. Collaboration increased as the Population Health Research Institute (PHRI) become an internationally renowned research centre under the leadership of Dr. Yusuf.

The CRCTL and Biobank occupy approximately 9000 sq. ft., with 18 staff who are totally supported by research funds. It is involved in many large, international, clinical, epidemiological and genetic studies relating to cardiovascular and renal disease, diabetes, hypertension and stroke. Samples and supplies are shipped to and from more than 70 countries. The biobank currently holds more than 2.5 million samples, stored in nitrogen vapour at -160 to -190°C with a

capacity of greater than 5 million samples.

During the past 15 years M. McQueen has been a co-investigator or principal investigator in 45 peer-reviewed grants for studies such as HOPE, DREAM, ON-TARGET, TRANSCEND, INTERHEART, INTERSTROKE. Three current research investigations include: investigation of societal and environmental determinants of lipids as risk factors for cardiovascular disease in 17 countries worldwide; integration of lipoprotein (a) concentration with genetic determinants and isoform size in assessment of risk of coronary heart disease; the role of genetics in understanding the development of chronic kidney disease in people with diabetes. Another very exciting project recently funded involves investigating more than 250 biomarkers from a single sample and their role in risk stratification in people with dysglycemia.

Selected Publications from approximately 200.

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