NeoGen Labs

NBS News 03 2009

This is a special issue of the newsletter to address questions from physicians asking us why we report the way we do. This is very important and we have a special 2 page NBS News to cover this topic in detail with examples.

Many physicians are familiar with laboratory reports providing analyte values with normal ranges specified. Because of the large number of analytes detected by Tandem Mass Spectrometry (MS/MS) and the sometimes complex interrelationships between them, conventional reporting of values and a reference range do not give a clear indication of a problem.

Specific values, reference ranges and cut offs are always available to the physician, if requested, but our reports do not carry them unless an abnormality is detected (in which case detailed values and our analysis are clearly presented)

Please take a few minutes to read through the reasoning we have presented.

Current best practice in MS/MS Analysis

Importance of pattern recognition and metabolite ratios in the interpretation of MS/MS results

Metabolic disorders are caused by block in a biochemical pathway, causing the accumulation of disease specific amino acids or acylcarnitines. With traditional newborn screening methods, samples are flagged when the quantity of a measured metabolite is above a certain value (cut off). With MS/MS, over 60 markers are detected at the same time. The increased number of metabolites monitored will result in increased number of results "flagged" as out of range, for which even though the pattern is not consistent with a metabolic disorder, another screen might be required. Premature infants and infants receiving intravenous hyperalimentation will have a higher rate of out-of-range results, due to the immaturity of their liver and other organs and/or to the components of intravenous fluids received.

To eliminate the frequent "flagging" of analytes requiring repeat specimens and ambiguous reporting, the latest interpretation of MS/MS results (based on the screening over 3.5 million samples) is based heavily on **pattern recognition**, while the measurement of the concentration of the different metabolites supports the interpretation (Chace, D.H. and Kalas, T.A., 2005, Clin. Biochem 38(4):296-309). In addition, the ability to detect multiple metabolites allows the use of **ratios of metabolites** to define whether an elevated value is due to a metabolic derangement or to the clinical and nutritional status of the newborn. These advances in interpretation methodologies have reduced the false positive rate to less than 0.3%.

The importance of pattern recognition in the interpretation of MS/MS results is illustrated in the following example:

Carnitine functions as a shuttle to transport long chain fatty acids inside mitochondria where they undergo beta-oxidation. In this process, the fatty acid is shortened, producing acetyl-CoA. In addition to this role, carnitine conjugates with organic acids to facilitate their removal. When one step in the metabolism of fatty acids or amino acids/organic acids is impaired, there is an increase in the corresponding acylcarnitine.

In MCAD deficiency, a defect in the metabolism of medium chain (6 to 10 carbon atoms) fatty acids, there is an increase in C8 (octanoyl carnitine), C6 (hexanoyl carnitine) and C10 (decanoyl carnitine) concentrations. However, the concentration of C8 (the marker commonly used for MCAD deficiency in newborn screening programs) is not always higher than the established cut off. The excessive formation of acylcarnitines in fatty acid oxidation disorders leads to depletion of carnitine resulting in lower concentration of all acylcarnitines and a possible decline of C8 levels below defined cut offs. However, the ratio of metabolites (C8/C16 or C8/C10) would indicate the presence of the disorder.

Interpretation of acylcarnitine profiles, therefore cannot be based only on cut-off values, because acylcarnitines might not be sufficiently higher than the cut-off value.

Furthermore, the same metabolite elevated in MCAD Deficiency, C8, is elevated in other diseases (Multiple Acyl-CoA Dehydrogenase Deficiency, MADD) or result from drug therapy (valproic acid) or dietary supplements (MCT oils used in special care formulas for premature infants).

In the same way that our complete amino acid profile aids in the detection of **true PKU**, the full acylcarnitine profile aids in the detection of disorders of fatty acid oxidation and organic acidemias.

Ratios of key metabolites, as well as absolute values of them are used to ascertain true positive results.

Within Normal Limits (WNL) - What does it mean?

Background: MS/MS application to newborn screening was first offered in 1994 in the US by **NeoGen Screening**. This company is now known as **PerkinElmer Genetics**. The company has screened over 3 million babies of all ethnicities. By analyzing millions of normal samples and tens of thousands of disease profiles, selective cut offs have been adopted for over 80 metabolically important analytes and ratios. The false positive rate is < 0.3%. The false negative rate is 0.0%, i.e., the technology, software and interpretation guidelines will never miss a case of

a listed metabolic disorder, if present* (See disclaimer).

Here is an example of how the technology works to screen for one of the 45 metabolic disorders, Maple Syrup Urine Disease (MSUD).

A. Re-Analysis Criteria

(Laboratory Criteria - When do we repeat a screen?)

- Leucine > 300 µM or
- Leu > 275 and Val > 275 or
- Leu > 250 and Leu/Ala > 1.5
- Leu> 225, Leu/Ala > 1.5, Leu/Phe > 5 or
- Leu > 200, Val > 275, Leu/Ala>1.5, Leu/Phe > 5

If any one of the above criteria is met, we process another dried blood spot and perform a repeat screen.

B. Notification criteria for MSUD screening

(What do we tell the Doctor/Hospital/Parent?)

Mean results

- Leu> 300
- Leu> 275 and Val> 275
- Leu> 250 and Leu/Ala>
- Leu> 225, Leu/Ala> 1.5, Leu/Phe> 5
- Leu> 200, Val> 275, Leu/Ala> 1.5, Leu/Phe> 5

Report Content: Send repeat sample

Mean results

- Leu> 400
- Leu> 350 and Val> 300
- Leu> 350 and Leu/Ala> 1.5
- Leu> 325, Leu/Ala>1.5, leu/Phe> 6
- Leu> 300, Val> 250, Leu/Ala>1.5, Leu/phe> 5

Report Content: Send repeat sample immediately

Mean results

- Leu> 500
- Leu> 425 and Val> 300
- Leu> 425 and Leu/Ala> 1.5
- Leu> 425, Leu/Ala> 1.5, Leu/Phe> 6
- Leu> 400, Val> 275, Leu/Ala> 1.5, Leu/Phe> 5

Report Content: Presumptive Positive

Note: Values have been changed to protect proprietary data

The proprietary list of **i)** primary and secondary analytes, **ii)** ratios and **iii)** cut off values have been determined by analysis of several million samples and has proven clinical utility across all ethnicities. As in the example above, every single one of the 80 analytes and ratios measured by MS/MS is evaluated by proprietary software algorithms to determine if the analyte concentration is 'normal' or 'abnormal'.

As is now obvious, it is impractical to give the above mentioned detailed results and multiple ranges for 80+ analytes and ratios. Also, specialized MS/MS training is required to interpret the results of a newborn screen.

Thus, a 'Within Normal Limits' result for the Acylcarnitine or Amino Acid Panel of metabolic disorders is a simple, direct way to signify that every analyte and/or ratio associated with the list of treatable metabolic disorders, has been evaluated in a thorough and rigorous fashion by best-in-the-world-class technology and by a trained expert in MS/MS interpretation, and found to be normal.

I strongly encourage a Doctor with doubts or questions to call Dr. Cariappa (+91 99006 55112) to discuss some or all aspects of the report. He will be happy to share specific data, cut offs and normal ranges if there is a suspicion of a certain metabolic disorder or class of disorders.

***Disclaimer:**

There are limitations to screening. It is a diagnostic tool used by physicians to assist them in diagnosis of the metabolic disorders. These disorders will be detected in the vast majority of affected individuals. Under no circumstance, however, can it be guaranteed that the screening process will detect the existence or non-existence of each of the potentially detectable disorders identified, due to factors such as genetic variability, age of patient at the time of specimen collection, quality of specimen, health status of the patient and other variables which are outside the parameters of the screening process

February 2009 Statistics

- 3 Cases of SCAD
- 1 Case of MADD/VLCADD
- 1 Case of IVA/MBCDD
- 1 Case of MMA/PA
- 2 Cases of HCY/Liver Disease

IMPORTANT: Administrative Notes

Many of you send screening samples to us for analysis with payment. Please ensure that the cheque or DD is made out to, **NeoGen Labs Private Limited** payable at Bangalore.

Screening Panels

- **First Step** (Over 50 IEMs for Rs. 3975)
- **First Step MS/MS** (45 IEMs, includes Fatty Acid Oxidation Disorders, Amino Acid Disorders, and Organic Acid Disorder panels for Rs. 3250)
- **First Step Bio** (5 IEMs which include CH, CAH, G6PD, GALT and Cystic Fibrosis for Rs.1500).

As always, we look for your feedback to improve this newsletter. If you are **unhappy for any reason** with our services (reporting, accuracy or any other subject), I would like you to call me so that I can rectify the situation immediately.

Thomas Mookken
T: +91 99006 55115 E: mookken@neogenlabs.com
www.neogenlabs.com