Newborn Screening by Tandem Mass Spectrometry (MS/MS)

Presymptomatic diagnosis by newborn screening for certain diseases has been shown to significantly decrease the morbidity and mortality. Although the number of diseases screened varies from state to state, phenylketonuria (PKU) and hypothyroidism are screened in all 50 states. The other commonly screened diseases include galactosemia, congenital adrenal hyperplasia (CAH) and hemoglobinopathies. The less commonly screened diseases are maple syrup urine disease (MSUD), homocystinuria, biotinidase deficiency, cystic fibrosis, and tyrosinemia. Missouri and Kansas perform newborn screening for phenylketonuria, hypothyroidism, galactosemia, and hemoglobinopathies. Missouri recently added screening for congenital adrenal hyperplasia.

In recent years, a few states have begun to expand newborn screening using tandem mass spectrometry (MS/MS). This powerful technique can screen for more than 20 disorders from a drop of blood on filter paper. Tandem mass spectrometry separates, identifies, and quantifies molecules based on their charge-to-mass ratio with high sensitivity and specificity in 2-3 minutes.

This is a screening assay only. The positives identified by MS/MS must be confirmed by more definitive methods, such as plasma amino acids and urine organic acids (both tests available at CMH), and generally require consultation with a specialist. The table shows the disorders detectable by MS/MS. Although the incidence of an individual disorder in this expanded screen is low, the combined incidence of these diseases is approximately 1:4000, which is equivalent to the incidence of hypothyroidism, currently a part of standard newborn screening in all states. Most MS/MS identifiable disorders are treatable and presymptomatic diagnosis and treatment result in improved prognosis in affected patients.

This is a new assay and many issues remain to be addressed. The equipment is expensive and expertise is limited, and the data on sensitivity and specificity is lacking. False positives exceed true positives. Many diseases identified by MS/MS do not respond consistently to treatment.

### Table 1: Metabolic Disorders detectable by MS/MS

<table>
<thead>
<tr>
<th>Amino Acid Metabolism</th>
<th>Fatty Acid Metabolism</th>
<th>Organic Acid Metabolism</th>
<th>Other Disorders</th>
</tr>
</thead>
<tbody>
<tr>
<td>Argininemia</td>
<td>Short-chain acyl-CoA dehydrogenase (SCAD) deficiency</td>
<td>Glutaric acidemia Type 1 (GA-1)</td>
<td>Galactosemia</td>
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<tr>
<td>Argininosuccinic aciduria</td>
<td>Medium-chain acyl-CoA dehydrogenase (MCAD) deficiency</td>
<td>Isovaleric acidemia</td>
<td>Congenital adrenal hyperplasia</td>
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<tr>
<td>Citrullinemia</td>
<td>Long-chain 3-hydroxy acyl-CoA dehydrogenase (LCHAD) deficiency</td>
<td>3-Ketothiolase deficiency</td>
<td>Cholestatic hepatobiliary disease</td>
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<tr>
<td>Homocystinuria</td>
<td>Very long-chain acyl-CoA dehydrogenase (VLCAD) deficiency</td>
<td>3-Methylcrotonyl-CoA hydratase deficiency</td>
<td></td>
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<tr>
<td>Maple syrup urine disease (MSUD)</td>
<td>Multiple acyl-CoA dehydrogenase deficiency (previously called glutaric aciduria type II)</td>
<td>3-Methylglutaconyl-CoA hydratase deficiency</td>
<td></td>
</tr>
<tr>
<td>Phenylketonuria</td>
<td>Carnitine palmitoyltransferase type II (CPT-2) deficiency</td>
<td>3-Hydroxy-3-methylglutaryl-CoA (HmG-CoA) lyase deficiency</td>
<td></td>
</tr>
<tr>
<td>Tyrosinemia type I and II</td>
<td>Carnitine-acylcarnitine translocase deficiency</td>
<td>Multiple CoA carboxylase deficiency</td>
<td></td>
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<tr>
<td></td>
<td>Mitochondrial Trifunctional protein (TFP) deficiency</td>
<td>Methylmalonic acidemias</td>
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<td></td>
<td>2,4 Dieneyl-CoA reductase deficiency</td>
<td>Propionic acidemia</td>
<td></td>
</tr>
</tbody>
</table>
News from Histology and Autopsy

The ABCs of Histology

First you get the tissue
Then you put it in stinky stuff
Then you wax it
slice it and dice it
stain it blue, stain it pink
get ’em under a looking glass
figure out what it is
Help, someone

Histology means the study of tissues. Every tissue removed from the body for analysis comes to the Histology lab. Our findings reflect the care given to that specimen from the moment of excision to the final diagnosis.

Fresh tissue for frozen section is done in the Histology lab for rapid diagnosis. A suspicious piece of tissue is rapidly frozen and cut in a chamber, which maintains a temperature of –20 degrees centigrade or below. This frozen section is mounted on a slide and rapidly stained with a Hematoxylin and Eosin stain. This stain provides the pathologist with a view of the tissue at the cellular level. The nuclear material stains blue-purple and the cytoplasm takes on the pink of the Eosin stain. A fast diagnosis guides the surgeon. Frozen sections can also be used for staining fat, which is destroyed in regularly processed tissue.

Unless tissue specimens are going to be used for frozen section-rapid diagnosis, microbiology, cytogenticities, or toxicology studies, they should be placed in the fixative of choice, usually 10% Neutral Buffered Formalin. The tissue should be placed in 15-20 times their volume of fixative, as soon as possible, in a container of adequate size. Failure to do so will result in the tissue being partially degenerated from the enzymatic and bacterial action from the dying tissue cells. Fresh tissues are sent to the lab for immediate attention and triage. (Never put specimens in water.)

The pathologist takes a representative section when cutting and examining the gross tissue. These sections should be approximately 1 cm. sq. and 2-3 mm. thick to ensure rapid fixation, dehydration, clearing, and infiltration of paraffin. This dehydration and paraffin infiltration is usually done on a tissue processor overnight. Water must be removed from the cells with increasing strengths of alcohol. Alcohol must be replaced in the tissues by a clearing agent, xylene. Clearing agents are used to remove the alcohol and dissolve the paraffin, with which the tissue must be infiltrated. This increases the refractile index making the tissues transparent.

Paraffin infiltration is the final step of the process, which enables the tissues to have support. Tissues must be firmly embedded in this paraffin, which will show the entire cut surface of the tissue. Tissues are then cut using very sharp knives and cool temperatures to ensure tissue ribbons of high quality. These cooled ribbons are floated on a warm waterbath and mounted on slides. There are many difficulties one may encounter in obtaining a good slide. This technique can only be learned with experience, and no attempt will be made to describe how this is done.

After a tissue is mounted on a slide, a paraffin solvent must remove the paraffin and the tissue rehydrated because most of the stains used are in a water medium. The most commonly used stain is the Hematoxylin and Eosin, usually referred to as an H&E. The pathologist examines this slide to help diagnose results. The pathologist may require a ‘special’ stain to help differentiate tissue elements not easily seen on the H&E. Special stains are routinely done for bacteria, fungi, pigments and minerals, carbohydrates, connective tissue, immunohistochemistry, and more.

Histology is a fun and fascinating field, which is enhanced by the care of the specimen from excision to the final diagnosis. Our slides help in the big picture that the entire laboratory provides for ultimate care of the patient.

The Perinatal Autopsy

The perinatal autopsy differs in many respects from that of an adult. In addition to documenting disease and, if possible, establishing the cause of death, the perinatal autopsy takes into consideration diseases that are unique to the maternal-fetal-placental unit, the evaluation of tissue maturity, and growth and development of the infant, and estimates the possibility of recurrent disease in future gestations. The perinatal autopsy also helps in recognizing unsuspected complications of the medical treatment, particularly in the neonate. The performance of the perinatal autopsy requires a pathologist interested in perinatal events and demands acceptable clinical review and interpretation of the anatomic findings in the context of clinical events.

To date, the autopsy rate for the perinatal period has not suffered the decline that has occurred in adults. The perinatal autopsy rate across the nation is approximately 60 percent compared to an average of 46 percent in the last six years in our hospital, 33 percent being the lowest rate, occurring in 2001. The Department of Pathology of Children’s Mercy Hospital is committed to constantly improve the quality of the medical information provided by the autopsy with the purpose of enhancing the explanation of perinatal events. All patients who expire at Children’s Mercy Hospital are offered an autopsy without cost.