Clinical Chemistry Trainee Council
Pearls of Laboratory Medicine
www.traineecouncil.org

Title: Newborn Screening

Presenter: Shannon Haymond, Ph.D., DABCC

Slide 1: Title Slide

Slide 2: Background

The goal of newborn screening is to detect congenital and inherited conditions in newborns that may otherwise appear normal at birth so they may be treated as soon as possible to prevent or ameliorate long-term consequences of the disease. The concept of newborn screening dates back to the early 1960s when Robert Guthrie developed a method to screen for phenylketonuria from blood spotted on filter paper. In the late 1990s, tandem mass spectrometry revolutionized the field by enabling detection of many inborn errors of metabolism as these could be included with minimal additional effort or cost.

Slide 3: Successful Public Health Program

Over 4 million infants are screened each year in the US alone and newborn screening is recognized as a highly successful public health program. As such, it extends beyond the screening aspect and includes coordination of follow-up, diagnostic testing, disease management, and program evaluation and quality assurance. It is important that clinicians and laboratorians know what is included in a particular state's menu, be familiar with any pre-analytical issues and recognize the limitations associated with results. Appropriate follow up requires timely communication of results and referral for diagnostic testing and clinical management. Diagnostic testing is performed by an independent method with improved sensitivity and specificity for the disorder. Disease management involves creation of the medical home and is often multi-disciplined care including clinicians specializing in metabolic, endocrine, or hematologic diseases, nutritionists, pediatricians, and genetic counselors. Evaluation and quality assurance strives to improve programs through efforts such as collection and review of follow up and outcome data, published technical standards and guidelines, proficiency testing and standardization of analytical methods and clinical cutoff ranges.

Slide 4: Regulatory Aspects

Newborn screening in the US is a state health department initiative. Menus are, therefore, regulated by state law and variation exists among states. The Secretary's Advisory Committee on Heritable Disorders and Genetic Diseases in Newborns and Children (SACHDNC), in collaboration with the American College of Medical Genetics (ACMG), has developed a system to evaluate the appropriateness of inclusion into a

'recommended uniform screening panel'. This expert panel utilizes a peer-reviewed evidence-based approach to identifying disorders recommended for universal newborn screening. The most recent recommendation (as of Sept 2011) includes 31 core disorders and 26 secondary disorders.

Secondary disorders are typically available by methods currently employed for newborn screening and are disorders likely to be part of the differential diagnosis for the core disorders. As of June 2010, all 50 states in the US screened for at least 29 of the uniform panel disorders. Severe combined immunodeficiency (SCID) testing was added in May 2010.

Slide 5: Classes of 'Uniform Panel' Disorders

The classes of disorders listed on the most recent recommended uniform screening panel include metabolic disorders, hemoglobin disorders, endocrine disorders, and others such as enzyme deficiencies, cystic fibrosis, severe combined immunodeficiency, and congenital heart disease.

Slide 6: Considering Tests for the Menu

Much consideration and debate goes into decisions related to the tests included in newborn screening menus. Some criteria for inclusion, adapted from Wilson and Junger, are related to the availability and characteristics of the screening test, the availability and complexity of the diagnosis, and the availability and efficacy of treatment. These state that a suitable test should be available. The condition should present an important health problem with a recognizable, symptomatic stage for which the natural history is understood and that evidence shows early detection benefits the infant. A comprehensive diagnostic and clinical management program, including efficacious therapy, should be available to the infant. Lastly, the cost of implementing screening as part of the newborn screening program (e.g., testing, follow-up, diagnosis, treatment) should be weighed against the overall cost of not screening for the condition.

Slide 7: Characteristics of a Suitable Test

In order for a test method to be considered suitable for newborn screening, there must be validated methods available for both screening and diagnostic purposes. It should be amenable to testing from dried blood spot on filter paper, and be high-throughput with low cost. Multiple analytes with secondary targets should be detected by the method and it should be a multiplex format such as that achievable with tandem mass spectrometry, HPLC, bead-based immunocapture assays, or microarray.

Slide 8: Specimen Collection

All newborns in the US are mandated to have a blood sample collected on a filter paper card. Parental refusal is accepted if documented and based on religious practices or beliefs. The initial specimen is typically collected within the first 24- 48 hours of life. Exceptions, including patients undergoing transfusion, transfers, early discharge, and those that are premature or acutely ill, may require initial collection at a later date (2-7 days of life). FDA now requires expiration date on all filter cards. The preferred method of collection per CLSI is by heelstick with direct application to the card. CLSI standard LA4-A5 also describes alternative methods for collection including capillary tubes, venous blood, umbilical catheters, and cords. Use of EDTA and heparin anticoagulants should be avoided as these may cause interference with methods commonly used in newborn screening programs.

Slide 9: Specimen Collection

The heelstick method involves puncture at the sites indicated in grey in the diagram. The blood is directly spotted onto the filter paper card, which is typically attached to the requisition and includes information on the baby and mother's identity, birthweight, gestational age, collection time, and feeding status. Cards are then air-dried at room temperature for several hours, preventing stacking or exposure to sunlight or direct heat. Improperly collected or stored specimens may impact results for newborn screening.

Slide 10: Reporting Results

Newborn screening is NOT diagnostic. The goal of screening is to be 100% sensitive but this often occurs at the expense of false positives. Newborn screening is also NOT comprehensive. The disorders detected by newborn screening only represent a small fraction of all inborn errors of metabolism. Menus and cutoff ranges vary from state-to-state and menus are modified over time. Therefore, a 'normal' result does not exclude all inborn errors of metabolism and limitations of 'normal' results must be understood. States vary in the way and the algorithms that are used to report results. Most report normal, tiered abnormal, and unsatisfactory specimen results. If specimen integrity or collection is compromised, testing is usually performed and results are appended with a comment indicating that the specimen was unsatisfactory and a repeat collection is required.

Slide 11: Reporting Abnormal Results

Abnormal results are often reported in tiers of severity. Presumptive positives indicate a high probability that the infant has the disorder. Results are communicated immediately with recommendation for referral to an appropriate medical specialist and confirmatory diagnostic testing. Suspected or borderline abnormal results indicate that the screening result was slightly abnormal and recommend the infant receive medical evaluation and a repeat collection for newborn screening. The communication and confirmation of abnormal results is a situation of urgency as most therapies are effective if implemented as soon as possible and parental anxiety is often high in cases of abnormal newborn screens.

Slide 12: Second Tier Testing

As the number of tests included in a panel increases, the chance for false positives also increases. Second tier testing has been implemented for a variety of diseases with either high false positive rate and/or requirement for rapid turn-around-time due to the seriousness of the condition. This has proven effective at reducing the number of false positive newborn screens, thus reducing the number of required repeats and the anxiety associated with an abnormal result. Some commonly used examples are listed here.

We will now go over each class of disorder included in the recommended uniform screening panel.

Slide 13: Amino Acid/Urea Cycle Disorders

The amino acid/urea cycle disorders are autosomal recessive diseases of amino acid or protein metabolism associated with a specific enzyme defect causing accumulation of neurotoxic amino acids and metabolites or ammonia. They are detected by tandem mass spectrometry as quantification of amino acids or metabolites with interpretation of disease-specific patterns. The most common amino acid disorder is phenylketonuria (PKU) with an estimated incidence of 1:10,000 births. The most

common cause of PKU is deficiency of phenylalanine hydroxylase and it is detected as increased phenylalanine and phenylalanine/tyrosine by LC-MS/MS. Results may be affected by feeding status, transfusions, and type of diet.

Slide 14: Amino Acid/Urea Cycle Disorders
This table illustrates the core amino acid/urea cycle disorders currently included on the recommended uniform screening panel.

Slide 15: Organic Acid Disorders
Organic acid disorders are autosomal recessive diseases associated with a specific enzyme defect in the organic acid metabolic pathway, causing accumulation of organic acids in the blood and/or urine. They are detected by tandem mass spectrometry as quantification of acylcarnitines with interpretation of disease-specific profiles. There is significant risk of death associated with accumulation of organic acids. Results may be affected by feeding status and type of diet.

Slide 16: Organic Acid Disorders
This table illustrates core organic acid disorders currently included on the recommended uniform screening panel.

Slide 17: Fatty Acid Oxidation Disorders
Disorders of fatty acid oxidation are autosomal recessive diseases associated with a specific enzyme defect in the fatty acid metabolic pathway, affecting utilization of dietary or stored fats. They are detected by tandem mass spectrometry as quantification of acylcarnitines with interpretation of disease-specific profiles. There is significant risk of death associated with illness or fasting episodes as the body is unable to appropriately utilize fatty acids, resulting in hypoglycemia.

Slide 18: Fatty Acid Oxidation Disorders
This table illustrates core fatty acid oxidation disorders currently included on the recommended uniform screening panel.

Slide 19: Hemoglobin Disorders
Hemoglobinopathies and thalassemias are autosomal recessive diseases affecting production of normal adult hemoglobin. Variants, such as HbS and HbC, are due to mutations in globin genes that result in production of abnormal protein. Thalassemias are due to deletions of globin genes so the protein produced is normal but at decreased levels. The core recommended uniform screening panel includes HbSS, HbSC and HbS/beta-thal. Sickle cell disease (HbSS) affects 1:375 African Americans and 1:10 are carriers (HbS trait). Newborn screening programs use isoelectric focusing or HPLC to determine the presence (or absence) of various types of hemoglobin. Results are affected by transfusions and extreme prematurity.

Slide 20: Congenital Adrenal Hyperplasia

Congenital adrenal hyperplasia (CAH) is an autosomal recessive disorder of steroid hormone synthesis with an incidence of approximately 1:20,000. The predominant cause of CAH is deficiency in the enzyme 21-hydroxylase. Early detection and treatment is necessary to prevent adrenal crisis, dehydration, and sudden death in severe salt-wasting forms of CAH. Newborn screening programs use elevated 17-hydroxyprogesterone to screen for CAH. Prematurity, low birth weight, and physiological stress elevate 17-hydroxyprogesterone and may cause false positives. Additionally there is a surge in 17-hydroxyprogesterone in the first 24 hours of life. Treatment with glucocorticoids prior to collection may cause false negative results.

Slide 21: Congenital Hypothyroidism

Congenital hypothyroidism is due to the inability of the thyroid to produce thyroxine (T4). It is most commonly the result of a failing thyroid gland. Early detection and supplementation with thyroid hormone is critical to prevent mental retardation. The estimated incidence is 1:2000 births. Newborn screening programs rely on elevated TSH, low T4, and/or both TSH and T4 for detection of congenital hypothyroidism. Timing of collection is important as TSH spikes within the first few hours of birth and declines to normal within 72 hours; therefore, collection prior to 24-48 hours of life is not recommended. However, many samples are collected prior to 48 hours so programs often rely on T4 rather than TSH as the primary screen to minimize the impact of this TSH surge. Premature infants tend to have low T4 and may cause false positives. Patients that are critically ill, premature, or post-transfusion may have false negative results.

Slide 22: Biotinidase Deficiency

Biotinidase deficiency is an autosomal recessive disorder of biotin (vitamin B6) recycling and processing that leads to multiple carboxylase deficiencies. Early detection and vitamin B6 supplementation is necessary to prevent permanent neurological damage. The estimated incidence is approximately 1:80,000 for profound and 1:40,000 for partial deficiency forms. Newborn screening programs detect biotinidase deficiency as decreased or absent enzyme activity. Specimen exposure to extreme heat and/or delayed submission may cause false positives. Prematurity may also cause false positives. The effect of transfusions on biotinidase activity is not well known.

Slide 23: Galactosemia

Galactosemia is an autosomal recessive disorder of galactose metabolism with an estimated incidence of 1:40,000 births. Affected individuals are unable to convert galactose (via dietary lactose) to glucose. It is most commonly due to a deficiency in the GALT (galactose-1-phosphate uridyl transferase) enzyme activity. Deficiencies in galactokinase (GALK) and uridine diphosphate galactose-4-epimerase (GALE) enzymes also cause galactosemia. Newborn screening programs may measure total galactose, galactose-1-phosphate, and/or decreased GALT enzyme to detect galactosemia. Results are affected by feeding status and diet (galactose and galactose-1-phosphate) and transfusions (GALT enzyme activity). False negatives may result from specimens exposed to extreme heat or delayed submission due to degradation.

Slide 24: Cystic Fibrosis

Cystic fibrosis is an autosomal recessive disorder that results in production of a defective form of the CFTR protein, preventing proper ion exchange across epithelial membranes. The incidence is highly variable among race/ethnicities. The most common disease-causing mutation is delta F508 in the CFTR gene. Cystic fibrosis is detected in newborn screening typically using a tiered approach with elevated immunoreactive trypsinogen (either single or repeat samples over time) and/or DNA mutation analysis. Collection within the first 24 hours of life increases the false positive rate.

Slide 25: Newest Addition and Expanded Panels

The SACHDNC regularly reviews requests for tests to be included on the recommended uniform screening panel. They consider each test based on the established criteria and make recommendations accordingly. One of the most recent additions is severe combined immunodeficiency (SCID). Very few states have adopted this new recommendation as programs to handle this testing must be established and state law must be proposed and passed to modify newborn screening menus. Decisions at the state level to include disorders on the newborn screening panel are driven by a variety of factors. Therefore, many states offer testing that extends beyond the uniform panel recommended by the SACHDNC. Programs may then use this data to support evidence gaps identified by the advisory committee review of available data at the time of the request. Among those disorders are rare metabolic disorders, lysosomal storage diseases, mucopolysaccharidoses, hemoglobin variants, and alpha thalassemia.

Slide 26: Severe Combined Immunodeficiency

Newborn screening for SCID was first piloted in the state of Wisconsin. SCID describes 7 disorders resulting from defects in genes. The associated disorders include IL-7Ra, CD45, JAK3, RAG1, RAG2, Artemis, and XL-SCID (X-linked). It has an accepted therapy, a known progression of disease, and a suitable method for detection. The method used for screening is PCR-based quantification of T-cell receptor excision circles (TRECs). Patients with SCID have no or decreased TRECs.

Slide 27: Points to Remember

Newborn screening is a successful public health program that extends beyond the testing phase. Tandem mass spectrometry revolutionized the field with its sensitivity, specificity, and ability to multiplex. It is important to remember that newborn screening is not comprehensive or diagnostic. The US federal government has a mechanism for recommending disorders for uniform screening but the menu used by each state is regulated by that state's government. This recommended uniform screening panel currently contains 31 disorders but the committee regularly reviews data associated with requests for additional tests. Specimens are collected on filter paper cards within the first 1-2 days of life. Specimen collection, handling, and submission are important factors in determining data quality. Gestational age, birth weight, transfusion, and feeding status should be noted with the specimen submission as these factors may impact results.

Slide 28: References