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NEWBORN SCREENING

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Abstract

Newborn screening is a public health program designed to screen infants shortly after birth for a list of conditions that are treatable, but not clinically evident in the newborn period. Most newborn screening tests are done by measuring metabolites and enzyme activity in whole blood samples collected on specialized filter paper, however many areas are starting to screen infants for hearing loss using automated auditory brainstem response and congenital heart defects using pulse oximetry. Infants who screen positive undergo further testing to determine if they are truly affected with a disease or if the test result was a false positive. Follow-up testing is typically coordinated between geneticists and the infant's pediatrician or primary care physician. There are several conditions observed in newborn such as organic acid metabolism disorder, fatty acid disorders, amino acid metabolic disorders, hemoglobinopathies etc. with early detection, and dietary management, the negative effects of different diseases can be largely eliminated. Reproductive benefits are assuming elevated prominence in expanded newborn screening panels. Rapid expansion of newborn screening is underway in many jurisdictions around the world, and even more accelerated expansion is anticipated in the future.[1, 2, 3]

Keywords

Pulse oximetry, otoacoustic emissions, Auditory brainstem response, Hemoglobinopathies, Parental anxiety.

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Introduction:

What is newborn screening?

An essential public health program that prevents catastrophic health consequences through early detection, diagnosis and treatment.

A complexsystem of testing, evaluation, and treatment that involves families, laboratory personnel, administrative and follow-up personnel, primary and specialty health care professionals, policy makers, sources of payments, manufacturers, and other interested persons or groups.[4]

Benefits of newborn screening [5, 6, 7]

- Identification
- Early intervention:
    - Start treatment before symptoms present
    - Diagnosis while newborn is in crisis – earlier, targeted treatment
    - Reduction in unnecessary investigations
    - Cost-saving
- Reduced morbidity and mortality:
    - Prevent metabolic crisis, metal retardation, SIDS, death
    - Not all developmental delay/symptoms can be prevented, but with early treatment, affected children can reach their full potential
- Family Planning:
    - Parents can be informed of diagnosis and management
    - Family members can be counselled about their own risk (if any) and the risk for future children
    - Resources and support groups for parents

Risks of newborn screening[8, 9]

- Parental anxiety:

- There are concerns that parents may become anxious when they are informed of their child's initial screen positive result. Some of these parents may have persisting concerns about their child's health in spite of the negative confirmatory test. Other parents may express relief that the initial screening result was false.

- Missed diagnosis:
    - False negatives. No screening test is perfect and some cases may be missed by NBS. The sensitivity and specificity of NBS for classic forms of inborn errors of metabolism are high; this may not be true for the variant or less severe forms of these conditions.

- The right not to know:
    - Some parents would prefer not to know this information at birth and would prefer to wait until their child manifests symptoms of the disease, though with most of these conditions, the goal is to prevent and reduce symptoms before irreversible damage occurs.

- Unanticipated outcomes:
    - Misattributed paternity: When an infant is identified with a condition on the NBS panel, sequential testing of the parents and other family members can identify previously unknown non-paternity.
    - The misattributed paternity rate is ~4% per a recent meta-analysis, the literature ranges from 2 to 10% depending on the study.

- Labelling:
    - There is concern that particularly when children are identified as carriers of disorders through NBS (i.e. hemoglobinopathy or cystic fibrosis) that they may

experience the negative consequences of "labelling". This may include discrimination or "different" treatment by family, teachers etc. [10]

Components of the newborn screening system[11, 12]

Management:

- Treatment
- Long-term follow-up
- Specimen storage

Screening:

- Sample collection
- Sample submission
- Laboratory testing

Diagnosis:

- Subspecialist Assessment
- Results shared with family
- Counseling if necessary

Evaluation:

- Quality assurance
- Outcome evaluation
- Cost effectiveness

Follow-up:

- Obtain test results
- Get results to family
- Repeat test(s) if needed
- Ensure diagnostic testing

Timing of testing

- Samples should be collected between 24 hours (one day) to seven days after birth
- Ideal time for sample is 24 hours (one day) to 72 hours (three days) after birth

- Sample cards should be sent to the NBS laboratory when the sample is dry, about four to six hours after collection, and no later than 24 hours after collection.

- *Why is timing important?* The levels of the various compounds in the blood spot change with the baby's age. Analysis takes the age of the infant into consideration. Individual interpretation must be made by the lab director when an infant is tested outside of the one to seven day time frame. If infants are screened before 24 hours, the recommendation is to repeat these samples within 5 days. The goal is to conduct NBS within the first 7 days of life so that infants with these disorders can be identified and treated before serious problems such as brain damage have occurred.[13,14,15]

Pulse oximetry screening

Pulse oximetry, or pulse ox, is a non-invasive test that measures how much oxygen is in the blood. Infants with heart problems may have low blood oxygen levels, and therefore, the pulse ox test can help identify babies that may have Critical Congenital Heart Disease (CCHD). The test is done using a machine called a pulse oximeter, using a painless sensor placed on the baby's skin. The pulse ox test only takes a couple of minutes and is performed after the baby is 24 hours old and before he or she leaves the newborn nursery.

Pulse oximetry screening is most likely to detect seven of the critical CHDs. These seven main screening targets are hypoplastic left heart syndrome, pulmonary atresia, tetralogy of Fallot, total anomalous pulmonary venous return, transposition of the great arteries, tricuspid atresia, and truncus arteriosus. Other heart defects can be just as severe as the main screening targets and also require treatment soon after birth. However, pulse oximetry screening may not detect these heart defects as consistently as the seven disorders listed as the main screening targets. [16]

How Newborn Screening for Critical CHDs is Done

Newborn screening for critical CHDs involves a simple bedside test called pulse oximetry. This test estimates the amount of oxygen in a baby's blood. Low levels of oxygen in the blood can be a sign of a critical CHD. The test is done using a machine called a pulse oximeter, with sensors placed on the baby's skin. The test is painless and takes only a few minutes.Pulse oximetry screening does not replace a complete history and physical examination, which sometimes can detect a critical CHD before oxygen levels in the blood become low. Pulse oximetry screening, therefore, should be used along with the physical examination.[17, 18]

Possible Physical Symptoms of Critical CHDs

- Problems breathing
- Pounding heart
- Weak pulse
- Very pale or blue skin color
- Poor feeding
- Very sleepy

- **Pass**

  If the baby passes the screen (also called "negative" or "in-range" result), it means that the baby's test results did not show signs of a low level of oxygen in the blood. A baby that passes the screen is unlikely to have a critical CHD. However, not all babies with a critical CHD will have a low level of oxygen in the blood that is detected during newborn screening. Thus, it is possible for a baby who passes the screen to still have a critical CHD or other CHD.

- **Fail**

  If the baby fails the screen (also known as "positive" or "out-of-range" result), it means that the baby's test results showed low levels of oxygen in the blood, which

could be a sign of a critical CHD. This does not always mean that the baby has a critical CHD but could mean that more testing is needed. There may be other causes, such as breathing problems, for low levels of oxygen in the blood. The baby's doctor might recommend that the baby get screened again or have more specific tests, like an echocardiogram (an ultrasound picture of the heart), to diagnose a critical CHD.[19]

NEWBORN HEARING SCREENING

Today, the vast majority of newborns receive a hearing screening before being discharged from the hospital. Two types of objective test technologies are used to screen for hearing loss in newborns: otoacoustic emissions and the auditory brainstem response (sometimes called ABR test or BAER test). These screening tests can detect 80-90% of infants with moderate degrees of hearing loss and greater. However, no screening test is perfect. Children with mild hearing loss may pass newborn hearing screening. Newborn hearing screening cannot identify children with late onset or progressive types of hearing loss.

Even when an infant passes a hearing screening test in the hospital, it is important to monitor developmental milestones for hearing, language and speech. If your child was born with visual, cognitive or motor disabilities, a comprehensive audio logical evaluation would be important to ensure your child's hearing is completely normal.[20]

How Does Newborn Hearing Screening Testing Work?

Currently there are two tests that hospitals and agencies use to screen babies for hearing loss. Both ofthese tests are safe and comfortable. They pose no risks for babies.

- **Otoacoustic Emissions**

One of the tests is called **otoacoustic emissions** or **OAEs**. Forthis test, a miniature earphone and microphone are placed inthe ear, sounds are played and a response is measured. If ababy hears normally, an echo is reflected back into the earcanal and this is picked up by the microphone. When a babyhas a hearing loss, no echo can be measured on the OAE test.

- **Auditory Brainstem Response**

The second test is called **auditory brainstem response** or**ABR**. For this test, sounds are played to the baby's ears. BandaidLike electrodes that are placed on the baby's head detectbrainwaves. This test actually measures the brain responding tosounds. This test also identifies babies who have a hearing loss.The two test methods may be used individually or in combination. In some hospitals, babies are firstscreened using OAEs, and the babies who do not pass this test are given the ABR test.Both tests are accurate and reliable. Your hospital has selected a method based on hospital resources,available personnel, cost, and the number of babies born in the hospital.[21]

THE HEEL TEST

***Acceptable samples*:**

- Heel prick
- Dorsal hand vein
- Peripheral or central line unless this line is used for hyperalimentation

Unacceptable samples:

- Cord blood
- Samples that have been in contact with EDTA and citrate anticoagulants – these can cause false negative results (CH, CAH, CF)
- Samples that have been contaminated with water, urine, feeding formulas, antiseptic solutions, powder from gloves
- Milking or squeezing puncture may cause hemolysis and an admixture of tissue fluids

***How to collect a specimen*:**

1. To prevent contamination, do not touch any part of the filter paper circles before, during or after collection. Disposable gloves and powder lactose residue can contaminate the sample.

2. Hold the infant's heel lower than the heart, warm with water or a towel if necessary. Heat pack not to exceed 42 degrees C.

3. Select puncture site. In the photograph, this is the shaded area.
4. Cleanse puncture site with alcohol and NOT iodine. Allow puncture site to air dry.
5. Puncture heel with sterile lancet, depth <2.0mm.
6. Wipe away first drop of blood to remove tissue fluids. Sufficient blood should collect on the heel to fill a single circle with one application.
7. Air-dry the blood specimens at room temperature for 2 to 4 hours in the horizontal position. Do not dry on heater or in microwave. Make sure the sample is completely dry before mailing. Mail within 24 hours of collection. If this not possible due to weekend or holiday, store in a cool room.[22,23]

What makes a good spot?[24]

- Blood should soak all the way through the filter paper. Complete saturation is necessary for accurate testing.
- Collect blood from one side of the filter paper only.
- It is important NOT to superimpose or layer the blood drops on top of each other, this can cause false results.
- Collect blood for each circle on the filter paper/collection card. It is better to properly collect 4 circles than inadequately fill all 5 circles.
- Let each drop touch the paper 3 mm from the previous drop.
- Avoid contamination

Invalid specimens

- Insufficient quantity for testing:
    - Removing filter paper before blood has completely filled the circle or soaked through
    - Touching filter paper before or after specimen collection or filter paper contaminated with gloves, hand lotion, antiseptic etc.
- Specimen appears scratched or abraded:

    - Applying blood with a capillary tube or other device
- Specimen not dry before mailing:
    - Allow specimen to dry for 2 to 4 hours before mailing
- Specimen appears supersaturated:
    - Applying excess blood to the filter paper, usually with a device
    - Applying blood to both sides of the filter paper
- Specimen appears diluted, discoloured or contaminated:
    - Squeezing or milking the puncture site
    - Contamination
    - Blood spots exposed to direct heat
- Specimen exhibits serum rings:
    - Puncture site was not cleaned with alcohol/antiseptic before making puncture
    - Contamination
    - Squeezing or milking the puncture site
    - Improper drying
    - Applying blood with a capillary tube or other device
- Specimen appears clotted or layered:
    - Touching the same circle to blood drop several times (layering)
    - Applying blood to both sides of the filter paper
- No blood:
    - Failure to obtain specimen[25,26]

Screening results

What does 'screen positive' mean?

- The infant is at increased risk for the disorder indicated on the report. Further testing is needed to confirm the diagnosis. This does not mean that the infant is affected.

- The Newborn Screening Laboratory will immediately notify the regional treatment centre and will arrange with the infant's health care provider or the infant's parent(s) for confirmatory testing.
- If a diagnosis of a disorder is confirmed, the treatment centre will provide management, counselling and follow up. A report is also issued by mail to the referring hospital and health care provider, and should be filed in the baby's medical records.

- Please note that in some cases, screening for one "core" disorder - those considered as having met acceptable criteria for NBS - will pick up "secondary target" or variant disorders. Secondary targets may not meet the accepted criteria; however they are often part of the differential diagnosis when evaluating an infant for one of the core disorders.

What does screen negative mean?

- The infant is not at increased risk for the disorder indicated on the report. In rare cases newborn screening can miss an affected infant. If an infant exhibits signs of a specific disorder, then diagnostic testing is indicated.
- *Repeat sample:*
- The initial sample is insufficient or unacceptable, or the results are equivocal.

The provider will be contacted and asked to obtain another sample from the infant as soon as possible and repeat the submission procedure.

Expanded 29 conditions

- 20 inborn errors of metabolism
  - 9 organic acid disorders
  - 5 fatty acid oxidation disorders
  - 6 amino acid disorders
- 3 hemoglobinopathies
  - Sickle cell and related disorders

- 2 endocrine disorders
- 3 other metabolic disorders
- 1 hearing loss

- **Inborn errors of metabolism**, also known as inherited metabolic diseases, comprise a large class of genetic diseases involving disorders of metabolism. The majority are due to genetic defects in genes that code for enzymes which facilitate conversion of various substances into other products. In the majority of these disorders, problems arise due to accumulation of substances which are toxic or interfere with normal function, or to the effects of reduced ability to synthesize essential compounds.
- **Hemoglobinopathies** refer to diseases resulting from genetic alterations in the amount of, and/or structure of the alpha and/or beta chain components of hemoglobin. The clinical picture of hemoglobinopathies varies; ranging from benign (carriers) to transfusion-dependent anemia or lethal in some cases.
- **Endocrine disorders** refer to diseases involving the production or metabolism of hormones.
- **Other conditions**: galactosemia, biotinidase deficiency, cystic fibrosis and hearing loss. Galactosemia and biotinidase deficiency are both metabolic disorders that are not amino acid, organic acid or fatty acid disorders.

  Hearing loss will not be discussed in this module.[27]

❖ **Inborn errors of metabolism**

- IEM are individually rare but are collectively more common.
- IEM (usually autosomal recessive inheritance) have a collective incidence ranging from 1 in 2,500 to 1 in 4,000 newborns.

- In these cases the parents of the affected child are more likely to be related by blood.
- With few exceptions, most infants with inborn errors of metabolism have normal facial appearance and no physical birth defects.
- Symptoms are a result of either too much or too little of a component in a metabolic pathway.

Early diagnosis is essential to reduce morbidity (i.e. mental retardation) and mortality.

Organic Acid Disorders

- ***What are organic acid disorders?***
  - Body cannot metabolize certain amino acids and fats
  - Accumulation of organic acids in blood and urine
  - Serious potentially preventable effects on health and development, including death
- ***Symptoms***
  - acute encephalopathy, vomiting, metabolic acidosis, ketosis, hyperammonemia, hypoglycemia, coma
  - dehydration, failure to thrive, hypotonia, GDD
  - sepsis, death
- ***Treatment***
  - Low protein diet / restrict amino acids,
  - Supplements: carnitine, biotin, riboflavin, glycine
  - Avoid fasting

The nine disorders listed are core disorders recommended for screening by the Ontario NBS Advisory Committee following review of several reports and NBS programs including the ACMG NBS report. Below is a list of the core organic acid disorders including the secondary organic acid disorders or variants which can be detected when screening for these core disorders:

- Isovaleric acidemia (IVA)

    - secondary target:
        - 2-Methylbutyryl-CoA dehydrogenase deficiency (2MBG)
- Glutaric acidemia type 1 (GA1)
- Hydrodroxymethylglutaric acidemia (HMG)
    - secondary targets:
        - 3-Methylcrotonyl-CoA carboxylase deficiency (3MCC)
        - 2-Methyl 3-hydroxy butyric aciduria (2M3HBA)
        - 3-Methylglutaconic aciduria (3MGA)
- B-ketothiolase deficiency (BKT)
- Multiple carboxylase deficiency (MCD)
- Methylmalonic acidemia (MUT)
- Methylmalonic acidemia (Cbl A, B)
    - secondary targets:
        - Methylmalonic acidemia (Cbl C, D)
- 3-methylcrotonyl glycinuria (3MCG)
- Propionic acidemia (PROP)

Fatty Acid Oxidation Disorders

- ***What are disorders of fatty acid oxidation?***
    - Breakdown of fatty acids in mitochondria is essential part of body's ability to produce energy
    - Disorder: inability to break down fatty acids
- ***Symptoms***
    - Decompensate with any catabolic stress
        - fever, fasting, intercurrent illness
    - Hypoketotic hypoglycemia, liver, muscle, heart disease
    - Lethargy, seizures, coma, sudden death (SIDS)
- ***Treatment***
    - Avoid fasting

- Frequent feeding
- IV glucose when ill to prevent hypoglycemia

The five disorders listed are core disorders recommended for screening by the Ontario NBS Advisory Committee following review of several reports and NBS programs including the ACMG NBS report. Below is a list of core fatty acid oxidation disorders and secondary fatty acid oxidation disorders which can be detected when screening for these core disorders:

- Medium-chain acyl-CoA dehydrogenase (MCAD) deficiency
  - secondary targets:
    - Glutaric acidemia type 2 (GA2)
    - Medium-chain ketoacyl-CoA thiolase deficiency (MCKAT)
    - Medium/short-chain (L-3-OH acyl) hydroxyacyl-CoA dehydrogenase deficiency (M/SCHAD)
- Very long-chain acyl-CoA dehydrogenase deficiency (VLCAD)
  - secondary targets:
    - Carnitine palmitoytransferase deficiency type 2 (CPT II)
    - Carnitine acylcarnitine translocase deficiency (CACAT)
- Long-chain L-3-OH acyl-CoA dehydrogenase deficiency (LCHAD)
- Trifunctional protein deficiency (TFP)
  - catalyzes 3 steps in mitochondrial beta-oxidation of fatty acids
- Carnitine uptake defect (CUD)
  - secondary targets:
    - Carnitine palmitoyltransferase deficiency type 1 (CPT I)

Amino Acid Disorders

- ***What are Amino acid disorders?***
  - Occur when the body cannot either metabolize or produce certain amino acids
  - Results in toxic accumulation of substances

  - Serious potentially preventable effects on health and development including death
- ***Symptoms*** -example PKU
  - Hyperphenylalaninemia (neurotoxic)
  - Microcephaly, epilepsy, MR, behavior problems
- ***Treatment***
  - Diet: reduce phenylalanine, low protein, supplement cofactors or essential amino acids

Avoid fasting

The five disorders listed are core disorders recommended for screening by the Ontario NBS Advisory Committee following review of several reports and NBS programs including the ACMG NBS report. Below is a list of core amino acid oxidation disorders and secondary or variant amino acid oxidation disorders which can be detected when screening for these core disorders:

- Phenylketonuria (PKU)
  - secondary targets:
    - Defects of Biopterin cofactor biosynthesis (BIOPT (BS))
    - Defects of Biopterin cofactor regeneration (BIOPT (Reg))
    - Benign hyperphenylalaninemia (H-PHE)
- Maple syrup urine disease (MSUD)
- Tyrosinemia type 1 (TYR 1)
  - secondary targets
    - Tyrosinemia type 2 (TYR 2)
    - Tyrosinemia type 3 (TYR 3)
- Homocystinuria (HCY)
  - secondary targets
    - Hypermetioninemia (MET)
- Citrullinemia (CIT)
  - secondary targets

- Citrullinemia type 2 (CIT II)

- Argininosuccinicaciduria (ASA)[28,29]

❖ **Hemoglobinopathies**

- Sickle cell disease (Hb SS)
- Hemoglobin SC disease
- Sickle-β thalassemia (Hb S/β-thal)
- Other hemoglobin variants may be picked up as variants

Sickle Cell Disease

- ***What is sickle cell disease? (Hb SS)***
  - Change in the shape of the betaglobin component of the hemoglobin molecule that interferes with hemoglobin's ability to carry oxygen
- ***Symptoms***
  - Painful vaso-occlusive crises, hemolytic anemia, frequent infections, tissue ischemia, chronic organ dysfunction
- ***Diagnosis***
  - Quantitative hemoglobin electrophoresis
  - Do not rely on solubility testing methods (Sickledex etc)
- ***Treatment***
  - Prophylactic penicillin (84% reduction in infection)
  - Vaccinations (pneumococcal, influenza)

Other Hemoglobinopathies

- Hemoglobin C disease (Hb-CC)
  - 'benign' hemoglobinopathy

  - mild hemolytic anemia, retinopathy & dental infarctions, gallstones, splenomegaly, joint pain
- Sickle cell and C trait (carriers) (Hb AS, Hb AC)
  - > 50% normal hemoglobin – generally asymptomatic no clinical symptoms
- Other hemoglobin variants
- Autosomal recessive inheritance[30]

❖ Endocrine disorders

- Congenital Hypothyroidism (CH)
- Congenital Adrenal Hyperplasia (CAH)

Congenital Hypothyroidism (CH)

- ***What is CH?***
  - inadequate thyroid hormone production
  - Anatomic defect in gland, IEM, iodine deficiency
- ***Symptoms***
  - MR, ↓ growth & bone maturation, neurologic problems: spasticity, gait abn, dysarthria, autistic behavior
- ***Treatment***
  - Thyroid hormone replacement
  - Diagnosis made before 13 days to prevent symptoms

Congenital Adrenal Hyperplasia (CAH)

- ***What is CAH?***
  - Impaired synthesis of cortisol by the adrenal cortex leads to ↑↑↑ androgen biosynthesis

- Inability to maintain adequate energy & blood glucose level to meet stress of injury & illness

- ***Symptoms***
  - Virilization (♀ ambiguous genitalia), precocious puberty, infertility, short stature
  - Renal salt wasting leads to FTT, vomiting, dehydration, hypotension, hyponatremia, & hyperkalemia
- ***Treatment***
  - Glucocorticoid replacement therapy[31]

❖ **Other disorders**

Galactosemia

- ***What is galactosemia?***
  - Lactose is main sugar in breast milk & infant formulas
  - Metabolized into glucose and galactose in the intestine
  - Unable to break down galactose
- ***Symptoms***
  - Feeding problems, FTT, bleeding, infection, liver failure, cataracts, MR
- ***Treatment***
  - Lactose-galactose-restricted diet
    - must be started in first 10 days of life to prevent symptoms
  - Even with treatment - ↑ developmental delay, speech problems, abn motor function, premature ovarian failure.

Cystic fibrosis

- ***What is cystic fibrosis?***

  - Due to mutations in the *CFTR* gene which is responsible for chloride regulation and other transport pathways.

- ***Symptoms***
  - Chronic sinopulmonary disease
  - Gastrointestinal/nutritional abnormalities
  - Azoospermia (males)
  - Salt loss syndrome
  - Shortened life span – but improving with treatment
- ***Treatment***
  - Pulmonary: oral, inhaled, or IV antibiotics, bronchodilators, anti-inflammatory agents, mucolytic agents, chest physiotherapy
  - Gastrointestinal: Nutritional therapy special formulas for weight gain via improved intestinal absorption, and additional fat-soluble vitamins & zinc to prevent deficiencies [32,33]

Conclusion

Newborn screening has been universally accepted for the past 3 decades. The term is used to refer to program that may have linkage with Traditional biochemical screening for inherited conditions like metabolic, endocrine, hematological, etc. Newborn screening is a public health program designed to screen infants through blood test, hearing test, heart test shortly after birth for a list of conditions that are treatable. It represented the first population based genetic screening program, signalled the integration of genetic testing into public health programs. Today, advanced technology are making possible new forms of newborn screening programs such as newborn hearing screening. . These technologies advances will continue to have a significant impact on the sensitivity, specificity, scope of newborn screening programs, including newborn heelstick screening.

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