PEDIATRIC ENDOCRINOLOGY AND INBORN ERRORS OF METABOLISM

SECOND EDITION

KYRIAKIE SARAFOGLOU
GEORG F. HOFFMANN KARL S. ROTH

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DEDICATION

Medicine is art, science, and method. Its subspecialities have been shaped by distinguished colleagues and friends who paved the way. This history can and should be fundamental for us.
The pioneering work of our predecessors gives us knowledge, guidance, and perseverance.
Illustrious examples of such pioneering clinical scientists we commemorate are
Horst Bickel (Heidelberg), Ivar Asbjørn Følling (Oslo), Robert Guthrie (Buffalo),
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PREFACE

This book is designed as a source of practical information for the diagnosis and management of pediatric patients with endocrine diseases and inborn errors of metabolism. From its conception, Pediatric Endocrinology and Inborn Errors of Metabolism (PEIEM) was created with this dual purpose: to be both a comprehensive, clinically-focused medical reference for a broad audience from specialist nurses and general physicians to specialists in each discipline, and to be an information bridge providing inroads into the fundamental concepts of the two interrelated disciplines. The contributors and editorial team strove to make the chapters on inborn errors approachable by endocrinologists and the endocrine chapters approachable by metabolic specialists through what became the underlying precept of the textbook—explanation, not simplification. Following this paradigm, chapters first elucidate the mechanisms underlying a disorder and how they relate to the corresponding phenotypes through clinically relevant discussions of genetics and pathophysiology, thus framing the basis of disease; and second, provide complete and detailed discussions of clinical features, laboratory evaluations, treatment modalities, and follow-up management. Rather than simply listing signs and symptoms under the assumption that their occurrence within a disorder is always self-explanatory, PEIEM explains through the pathophysiology why and how these manifestations occur and how they can be approached, modified, or prevented. As a result of this step-wise approach, we hope that medical professionals at any level involved in caring for endocrine and metabolic patients will find this textbook a useful and comprehensive resource.

We are very grateful for the wide acceptance the first edition of PEIEM quickly achieved and are proud of recognition such as the Medical Book Award of the British Medical Association in 2010. In 2009, the New England Journal of Medicine review stated "it is a unique book that is pleasing to the eye, nurturing for the mind, and instructive for a broad readership." It has quickly become an in-depth clinical reference resource for inborn errors of metabolism and pediatric endocrinology.

Since PEIEM's release in 2009, huge advances of knowledge and important improvements in diagnostics as well as therapeutic approaches necessitated a second edition. This allowed the corrections of some errors of the first edition that maybe only authors and editors spotted, as well as the inclusion of additional disorders not covered previously and those that were recently identified. Following a stringent concept, it was still possible to provide even more detailed and clinically relevant information concerning presentation, diagnosis, and treatment of more than 700 disorders within a single volume. To achieve this goal, we had to address the question "What is the most pertinent information needed for the practicing physician to fully understand the etiology and pathophysiology of a disease in order to make informed decisions concerning the diagnosis and management of a patient?" To remain a single volume, we focused on describing disease pathogenesis, clinical presentation, and therapy, and where relevant, the most frequently recurring mutations in relation to phenotype, rather than lengthy discussions of a disorder's historical background and itemized accounts of the discovery of each mutation, both of which can be found in many textbooks and established internet databases.

What is unique to this book and not easily found in other textbooks or on the internet is a single organized source that provides detailed information for the practicing physician concerning the pathophysiology, diagnosis, and management of both inborn errors of metabolism and endocrine disorders. By combining the two disciplines, a physician contemplating the differential diagnosis of a patient with hypoglycemia, for example, will need only one textbook to find full coverage of the potential underlying disorders (ie, hyperinsulinism, glycogen storage diseases, fatty acid oxidation disorders, adrenal insufficiency, and disorders of growth). As there can be many subtypes of a disorder, to assist in identifying the information you need quickly, disease-oriented chapters begin with the At-A-Glance page, a quick reference summary for easy access to the biochemical profile, presentation, occurrence rate, locus, etc., of the disorders covered in the chapter. Another important feature of this textbook that aids in the differential diagnosis is that many subtypes of disorders—even rare ones—covered within a chapter are individually discussed following a specific format. In most cases, each subtype of a disorder is structured in the following format: Etiology/Pathophysiology, Presentation, Diagnosis, and Treatment. Full descriptions of the etiology/pathophysiology of the overall disorder are aided by multiple graphics to show how the different enzymatic defects affect a pathway, rather than a single graphic with a multitude of defect markers. Thus, with PEIEM, the reader can readily and consistently find the information (s)he seeks. The structured format also has the added benefit of addressing the heterogeneity of contributors and writing styles created by any multi-author textbook.

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PART I

Newborn Screening, Emergency Treatment, and Molecular Testing

PART I CONTENTS

CHAPTER 1

Newborn Screening

Andreas Schulze, MD, PhD, FRCPC; Dietrich Matern, MD, PhD, FACMG; and Georg F. Hoffmann, MD

Protecting children from the burden of inherited diseases is the aim of newborn screening. Selection of candidate disorders depends upon the following prerequisites: 1) feasible means of disease detection in a presymptomatic/early stage of the disease; 2) treatability of the disease; 3) ability to start of treatment in the presymptomatic/early stage.

Screening of neonates for signs of disease or distress has several components, with the perinatal clinical evaluation being of first and foremost importance. The clinical approach to screening is limited to the detection of symptoms, which in many disorders have been proven to be irreversible if not treated. For example, classic phenylketonuria (PKU) due to phenylalanine (Phe) hydroxylase deficiency is characterized by the insidious development of irreversible neurological damage unless treatment is initiated within the first few weeks of life. Newborn screening was first developed for the identification of this inborn error of amino acid metabolism, which was typically not diagnosed before 6 months of life and mostly even much later when developmental delay or other nonspecific neurologic symptoms become apparent. Treatment based on a Phe-restricted diet was developed by Horst Bickel in the 1950s, but it was quickly realized that therapy only improved the patient's symptoms but was inadequate to reverse neurologic damage.[1] Furthermore, it was recognized that a limited intake of the essential amino acid Phe requires the regular monitoring of its concentration in blood. A simple method for Phe determination was developed by Robert Guthrie, a scientist initially working in cancer research and the father of a child with mental retardation.[2] This test was a bacterial inhibition assay (BIA) performed on serum dried on filter paper. Guthrie then began to apply his BIA to the analysis of Phe in small blood samples also dried on filter paper with the aim of allowing the presymptomatic identification of PKU in patients and facilitating the timely initiation of dietary intervention.[3] Once the efficacy of this assay was established, newborn screening began 50 years ago in several regions of the United States and Germany and rapidly spread around the world using the Guthrie test.[4,5] Over the ensuing 30 years, a few additional disorders such as congenital hypothyroidism, galactosemia, and sickle cell disease were gradually added to many newborn screening programs, usually one new assay for each additional disorder.

The BIA was initially modified to detect other disease markers and eventually more sophisticated technologies were applied, such as fluorometric, colorimetric, and immunoassays to determine either disease-related metabolites or specific enzyme activities. Over the last two decades, the introduction of tandem mass spectrometry (MS/MS) into newborn screening laboratories has dramatically expanded the number of disorders that can be detected in a single blood spot. More than 30 additional conditions can be detected by simultaneous acylcarnitine and amino acid analyses, including inborn errors of amino acid, organic acid, and fatty acid metabolism.

DISORDERS INCLUDED IN NEWBORN SCREENING PROGRAMS

To aid in the selection of diseases to be included into screening programs, screening principles were developed by Wilson and Jungner on behalf of the World Health Organization in 1968.[6] Although these principles were not developed specifically for newborn screening, with some adaptation they remain the most commonly used selection criteria for newborn screening in almost all countries. However, despite seemingly agreed-upon criteria, diseases included vary widely between countries—for example, in Europe from 1 to 30 conditions.[7] In general there is an increase in the number of conditions screened for in many countries over the last years. Screening raises concerns about privacy and autonomy, highlighting the importance of the evaluation of ethical, legal, and societal aspects. As most screened conditions are inherited disorders, consequences for family members often exist. Furthermore, healthcare expenses need to be balanced: if screening programs are funded, other activities may not be possible. When deciding which diseases to include in any newborn screening program, careful consideration must be given to weighing the impact for affected individuals against the burden for unaffected individuals. Detailed recommendations for screening policy will vary from country to country and region to region, depending on local economic, political, and medical factors and public health organizations.

In 2002, the American College of Medical Genetics (ACMG) was commissioned by the Maternal and Child Health Bureau of the Health Resources and Services Administration of the United States Department of Health and Human Services to review the scientific basis of newborn screening and develop recommendations for which disorders should be included in newborn screening programs. The impetus for a comprehensive review of the status of newborn screening was the scattered implementation of MS/MS in screening laboratories in the United States, which led to marked discrepancies in the number of conditions included in the various screening programs. Several states provided newborn screening for only three diseases, whereas those that implemented amino acid and acylcarnitine profiling by MS/MS were screening for more than 30 conditions. (A regularly updated list of conditions screened for in each state is available at: http://genes-r-us.uthscsa.edu/.) In 2006, the ACMG reported their conclusions,[8,9] recommending screening for 29 diseases by all programs, and three additional conditions have been added since then (core conditions; Table 1-1). These conditions were considered to fulfill three basic principles that were developed to update and replace the original Wilson and Jungner criteria: 1) each condition is identifiable in a period of time

AT-A-GLANCE

Newborn Screening

Newborn screening is an important and widely established program of preventive medicine. It typically is a public health program and represents a population-based method to identify newborns with inherited or congenital, metabolic, endocrine, and other disorders. Detection of affected children in the pre-symptomatic state of the disease is the prerequisite for early initiation of treatment, to prevent most if not all disease manifestations and complications.

Newborn screening was implemented for phenylketonuria (PKU) more than half a century ago and today more than 50 different conditions can be tested for. However, agreement on and interpretation of criteria such as those outlined by Wilson and Jungner[6] (Table 1-1) for inclusion of a condition into a screening program is not universal. Although there is consensus that a careful balance between benefit and harm is important, the number of conditions included in newborn screening programs is variable between and also within countries.

Blood from newborns is taken during the first 1 to 4 days of life by a heel prick, spotted onto filter paper, and sent to a screening laboratory. The laboratory investigates the dried blood spots for the diseases of the respective screening panel. In addition to testing of dried blood spots, bedside testing for hearing loss and critical congenital heart disease are increasingly added to newborn screening programs. In the majority of newborns, the presence of disease can be ruled out as result of the first investigation. In the case of a positive result in the screening test, most screening programs request a repeated blood spot sample (recall); some refer positive-tested newborns to treatment and follow-up centers for recall testing and counseling. Of those newborns who underwent a recall investigation, up to 90% are not affected. Distinct abnormalities in the primary test or confirmation of abnormal primary results in the recall investigation raise strong suspicion for a disease and necessitate the initiation of confirmatory testing. Because the conditions screened for are rare and confirmation and treatment are complex, care of the presumptively affected newborns should occur in close consultation and collaboration with a pediatric specialist.

DISORDER	PREVALENCE[a]	KEY METABOLITE	COMMENTS/CONFIRMATION ANALYSIS
Phenylketonuria (PKU)	1:~10,000 (1:16,500)[b]	↑ Phe ↓ Tyr ↑ Phe/Tyr ratio	Confirmation: Plasma Phe, Tyr; pterins in urine, dihydropteridine reductase activity in DBS, molecular genetic analysis.[d]
Galactosemia	1:~70,000 (1:53,500)[b]	↑ Total galactose ↓ Galactose-1-phosphate uridyltransferase (GALT)	Galactokinase def. and UDP-gal-epimerase def. are also detected by total galactose screening. Total galactose screening gives false negatives when baby has not yet received lactose-containing milk. Confirmation: Enzyme in erythrocytes, molecular genetic analysis.[d] Galactokinase def. and UDP-gal-epimerase def. are not detected by GALT screening. GALT screening is independent from feeding.
Biotinidase deficiency	1:~60,000 (1:68,000)[b]	↓ Biotinidase activity	Confirmation: Enzyme in serum, molecular genetic analysis.[d]
Congenital hypothyroidism (CH)	1:~3500	↑ Thyroid-stimulating hormone (TSH)	T_4 may also be measured as part of newborn screening. Hypothalamo-hypophyseal forms of hypothyroidism are not detected by TSH screening. Citrate and EDTA blood causes false positives. Confirmation: Plasma thyroid hormones.
Congenital adrenal hyperplasia (CAH)	1:~13,000	↑ 17-OH-Progesterone (17-OHP)	Screening does not reliably detect 11- and 17-hydroxylase/17,20-lyase, and 3β-OH-steroid dehydrogenase deficiency. For preterm newborns cut-offs adjusted for gestational age, birth weight, and/or age at sample collection are necessary. Confirmation: Plasma steroids, molecular genetic analysis.[d]
Glucose-6-phosphate dehydrogenase (G6PD) deficiency	1:~3000	↓ G6PD activity	Enzyme in EDTA blood, molecular genetic analysis.
Maple syrup urine disease (MSUD)	1:160,000 (1:198,000)[b]	↑ Leucine + Isoleucine + Allo-Isoleucine ↑ Valine ↑ (Leu+Ile+Allo-Ile)/Phe -ratio	Screening during the first 24 hours might miss cases. Confirmation: Plasma amino acids and urine organic acids, molecular genetic analysis.[d]
Hepatorenal tyrosinemia type 1 (TYR-1)	1:100,000 (1:781,000)[b]	(↑) Tyr ↑ Succinylacetone	Confirmation: Plasma amino acids, α-fetoprotein, succinylacetone, enzyme in fibroblasts, molecular genetic analysis.[d]
Homocystinuria (HCY)	< 1:200,000 (1:457,000)[b]	↑ Methionine ↑ Homocysteine	Confirmation: Total homocysteine in plasma, molecular genetic analysis,[d] enzyme in fibroblasts.

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DISORDER	PREVALENCE[a]	KEY METABOLITE	COMMENTS/CONFIRMATION ANALYSIS
Citrullinemia (ASS)	1:~250,000 (1:156,000)[b]	↑ Citrulline ↓ Arginine	Confirmation: Plasma amino acids, orotic acid in urine, molecular genetic analysis.[d]
Argininosuccinate lyase deficiency (ASL)	1:220,000 (1:305,000)[b]	↑ Arginino-succinate ↑ Citrulline ↓ Arginine	Confirmation: Plasma and urine amino acids, orotic acid in urine, enzyme in erythrocytes/fibroblasts, molecular genetic analysis.[d]
Medium-chain acyl-CoA dehydrogenase deficiency (MCAD)	1:~15,000 (1:18,000)[b]	↑ C8 carnitine ↑ C8/C2 ratio ↑ C8/C10 ratio ↑ C8/C12 ratio	CDmay cause a non-informative acylcarnitine profile; C_8 may be also elevated in glutaric type II (along with other acylcarnitines). Confirmation: Acylcarnitines in DBS/blood, urine organic acids, molecular genetic analysis[d], enzyme in lymphocytes/fibroblasts.
(Very) long-chain acyl-CoA dehydrogenase deficiency (VLCAD)	1:85,000 (1:63,500)[b]	↑ C14:1 carnitine ↑ C14 carnitine	CDor glucose infusion may cause a non-informative acylcarnitine profile. Blood for acylcarnitine profiling has to be taken prior to (not after) regular meal. Confirmation: Acylcarnitines in DBS/blood, urine organic acids, molecular genetic analysis[d], enzyme in lymphocytes/fibroblasts.
Long-chain 3-OH-acyl-CoA dehydrogenase deficiency (LCHAD) and trifunctional protein deficiency (TFP)	1:250,000 (1:300,000)[b]	↑ C16OH carnitine ↑ C18OH carnitine	CDmay cause a non-informative acylcarnitine profile. Fat infusion may cause false positives. C16OH may be the only abnormal finding in LCHAD/TFP deficiency even with normal C2. Confirmation: Acylcarnitines in DBS/blood, molecular genetic analysis,[d] enzyme in lymphocytes/fibroblasts.
Carnitine-palmitoyl transferase I deficiency (CPTI)	< 1:750,000	↑ C0 (free) carnitine ↓ C16 carnitine ↓ C18 carnitine ↑ C0/(C16+C18) ratio	Carnitine supplementation (prematures) may cause false positives. Confirmation: Acylcarnitines in DBS/blood, free carnitine in blood, enzyme in lymphocytes/fibroblasts, molecular genetic analysis.[d]
Carnitine-palmitoyl transferase I deficiency (CPTII) and carnitine-acylcarnitine-translocase deficiency (CAT)	< 1:750,000	↑ C14 carnitine ↑ C16 carnitine ↑ C18 carnitine ↑ C18:1 carnitine	CDmay cause a non-informative acylcarnitine profile. Special premature formula gives false positives. Confirmation: Acylcarnitines in DBS/blood, enzyme in lymphocytes/fibroblasts, molecular genetic analysis.[d]
Carnitine uptake deficiency (CUD)	1:77,000 (1:142,000)[b]	↓ ↓ C0 (free) carnitine	Organic acid disorders, prematurity, and maternal carnitine deficiency may also cause low free carnitine. Maternal carnitine supplementation can cause false negatives. Confirmation: Determine free carnitine in plasma and urine, determine fractional tubular reabsorption of free carnitine (normal > 98%) in child and mother; carnitine uptake studies in fibroblasts and/or molecular genetic analysis.[d]
Isovaleric aciduria (IVA)	1: 100,000 (1:159,000)[b]	↑ C5 carnitine	CDmay cause a non-informative acylcarnitine profile. Treatment with pivalic acid containing antibiotics may cause false positive results. C5 carnitine is also elevated in SBCAD deficiency. Confirmation: Urine organic acids and acylglycines, acylcarnitines in DBS/blood, molecular genetic analysis.[d]
Glutaric aciduria type 1 (GA-1)	1:100,000 (1:92,000)[b]	↑ C5DC ↑ C_5DC/C_8 ↑ C_5DC/C_{16}	CDmay cause a non-informative acylcarnitine profile. Confirmation: Urine organic acids (glutaric and 3-hydroxyglutaric acid by a sensitive stable isotope dilution method), acylcarnitines in DBS/blood, enzyme in lymphocytes/fibroblasts, molecular genetic analysis.[d]
Propionic aciduria (PA)	1:200,000 (1:238,000)[b]	↑ C3 carnitine ↑ C3/C0 ratio ↑ Methylcitrate	CDmay cause a non-informative acylcarnitine profile. Confirmation: Urine organic acids, acylcarnitines in DBS/blood, ammonia, enzyme in fibroblasts, molecular genetic analysis.[d]
Methylmalonic aciduria (MMA)	1:150,000 (1:160,000)[b]	↑ C3 carnitine ↑ C3/C0 ratio ↑ Methylmalonic acid ↑ Methylcitrate	CDmay cause a non-informative acylcarnitine profile. Confirmation: Urine organic acids, plasma methylmalonic acid, plasma amino acids (methionine) and total homocysteine, ammonia, enzyme in fibroblasts, molecular genetic analysis.[d]

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DISORDER	PREVALENCE[a]	KEY METABOLITE	COMMENTS/CONFIRMATION ANALYSIS
Cobalamin deficiency (CBLA, B, C, D, F, J, X, TC-II) and succinyl-CoA synthetase (SUCLA2) deficiency	1:100,000	↑ C3 carnitine ↑ C3/C0 ratio ↑ Methylmalonic acid ↑ Methylcitrate	CD may cause a non-informative acylcarnitine profile. Confirmation: Urine organic acids, plasma methylmalonic acid and acylcarnitines, plasma amino acids (methionine) and total homocysteine, ammonia, molecular genetic analysis[d] complementation analysis in fibroblasts; vitamin B_{12} levels in the mother.
3-Methylcrotonyl-CoA carboxylase deficiency (3-MCC)	1:60,000 (1:39,000)[b]	↑ C5OH carnitine	CD may cause a non-informative acylcarnitine profile. C5OH carnitine can also be elevated in 3-methylglutaconic aciduria type I, multiple carboxylase deficiency including biotinidase and holocarboxylase deficiency, biotin deficiency, β-ketothiolase deficiency, 2-methyl 3-hydroxy butyryl-CoA dehydrogenase deficiency and 3-hydroxy-methylglutaryl (HMG)-CoA lyase deficiency. Confirmation: Urine organic acids, blood ammonia, enzyme in fibroblasts, molecular genetic analysis;[d] acylcarnitines and urine organic acids in the mother.
3-Hydroxy-methylgutaryl-CoA lysase deficiency (HMG)	< 1:200,000 (1:1,528,000)[b]		
Cystic fibrosis (CF)	1:5000	↑ Immune reactive trypsinogen (IRT) ↑ Pancreatitis associated protein (PAP) Presence of common CFTR mutations	Confirmation: Comprehensive molecular genetic analysis (if not part of newborn screening); sweat chloride.
Severe combined immuno-deficiency syndrome (SCID) Severe B cell deficiency	1:~ 70,000 1:100,000	↑ T-cell receptor excision circles (TREC) ↓ Kappa-deleting recombination excision circles (KREC)	In addition to SCID, 22q11.2 deletion syndrome is detected by TREC screening but not B cell defects. X-linked agammaglobulinemia, Ataxia Teleangiectasia and Njimegen-Breakage Syndrome are detected in a Duplex PCR assay (TREC/KREC). Confirmation: CBC with differential and lymphocyte enumeration, antibody levels, lymphocyte proliferation to mitogens, and molecular genetic testing.[d]
Sickle cell disease	< 1:200,000 – 1:300[c]	Hemoglobin analysis by electrophoresis, HPLC or MS/MS	Confirmation: Hemoglobin electrophoresis with other than screening method.

[a]Prevalence as estimated from newborn screening in a Caucasian population, it may vary among screening populations of different ethnic background.
[b]Newborn screening data US 2001–2010.[106]
[c]In black population.
[d]Molecular genetic analysis is typically not required to establish a diagnosis and its value should be evaluated on a case-by-case basis.
CD, carnitine deficiency; DBS, dried blood specimen; Phe, phenylalanine; Tyr, tyrosine.
Note: Information and suggestions for follow up of abnormal newborn screening results is also available at: http://www.ncbi.nlm.nih.gov/books/NBK55832/.

(24 to 48 hours after birth) at which it would not ordinarily be clinically detected; 2) a test with appropriate sensitivity and specificity is available; and, 3) benefits of early detection, timely intervention, and efficacious treatment have been demonstrated. Because screening tests do not primarily determine disease status, but measure analytes that in most cases are not specific for a particular disease, the ACMG report also included 25 conditions (secondary targets) that did not meet all three selection criteria but are identified nevertheless because most of them are included in the differential diagnosis of screening results observed in core conditions (Table 1-1). Most of these secondary conditions are identified through metabolite profiling by MS/MS, which enables the determination of more than 50 analytes and analyte ratios in a small newborn screening blood spot punch. This also increases the responsibility of newborn screening laboratories to provide testing with the highest sensitivity and specificity to allow identification of affected patients while minimizing the false-positive rate.

The current state of newborn screening programs in Europe was evaluated through a comprehensive survey in the European Union (EU) program of Community Action in Public Health 2010/2011 among 27 EU member states, four candidate countries, three potential candidates, and two European Free Trade Association (EFTA) countries. The comprehensive overview addressed all aspects of screening, spanning from the supporting legislation to confirmation diagnostics and start of treatment. For each step it evaluated existing guidelines, actual practices, quality assurance, and training schemes and resulted in an agreed-upon Expert Opinion document with recommendations to the EU Commission for improvement. Ethical aspects and the systematic evaluation of the screening programs were investigated. The survey documented large discrepancies concerning 1) education of parents, including informed consent; 2) which conditions are screened for, ranging from 2 to more than 30; 3) age at sample collection; 4) screening methodology; 5) storage of residual specimens, varying from 1 to 1000 years. Confirmatory diagnostics, treatment, and follow-up displayed similar differences.[7,10,11] All reports are available at: http://www.iss.it/cnmr/prog/cont.php?id=1621&lang=1&tipo=64.

PERFORMANCE OF A NEWBORN SCREENING PROGRAM

An ideal screening test would detect all newborns in a population affected with the disease with 100% sensitivity and the unaffected newborns would have normal results (100% specificity). In practice, every screening test fails under certain circumstances and to a different extent. Metabolites or hormones are used as marker(s) for most of the screening conditions (eg, Phe in screening for PKU, thyroid stimulating hormone [TSH] in congenital hypothyroidism [CH]). These metabolites reveal usually a bimodal distribution, where the "disease range" is ideally separated from the "normal range" (Figure 1-1). However, based on the (patho)physiologic distribution of the metabolite(s) and the characteristics of the screening test the ranges often overlap. Diagnostic sensitivity and specificity then depends on how the decision limit ("cutoff" point) for the marker is set. In the example given in Figure 1-1, the cutoff set at point A yields 100% sensitivity but many false positives; the cutoff set at point B yields 100% specificity but many false negatives, and at point C yields some false positives and false negatives. Note that sensitivity and specificity vary reciprocally to the setting of the cutoff.

Setting the cutoff at the 99.5th percentile of healthy newborns often results in 100% sensitivity while keeping the specificity in an acceptable range. A higher cutoff is feasible when missing of disease variants (mildly affected subjects not needing treatment) is acceptable or when there is a gap between "normal" and "disease."

Applying a screening test to a population will produce four categories of results (true positives, false positives, false negatives, and true negatives; Table 1-2). If the number of cases for each category is known, the false-positive rate and positive predictive value (PPV) can be calculated. The false-positive rate should be low and the PPV, which is a measure of the proportion of persons with positive test results who are truly affected, should be high. These measures provide insight into the performance of a screening program and physicians receiving newborn screening results should be able to obtain this information from their respective screening programs. Physicians must be aware, however, that screening programs have different definitions of what constitutes a positive result. Some programs count any abnormal result as positive, whereas others consider only a confirmed abnormal result on a repeated blood spot test as positive.[12,13]

To illustrate how indices help in the assessment of a screening test, consider the situation

TABLE 1-1 Recommended Uniform Screening Panel (US) and Key Analytes

Analyte	Core Condition	Secondary Targets	Other Identifiable Conditions
Phe	PKU	BS HPA RFLG	
Leu/Ile/AlloIle, Val	MSUD		BCKDK (if branched-chain amino acids below reference range)
Met	HCY	MET	RMD (if Met is below reference range)
Cit, Arg, Asa	ASA CIT	ARG CTLN2	
Tyr	TYR-I	TYR-II TYR-III	TIN
C0	CUD	CPT-I (when elevated)	Maternal CUD or conditions associated with secondary C0 deficiency
C3	CBLA, CBLB MUT PA	CBLC, CBLD	CBLF CBLJ CBLX TG-II SUCLA2
C4		IBDH SCAD	RCHLU
C5	IVA	SBCAD	Medication artifact Ethylmalonic encephalopathy
C5-OH	BKT HMG MCC MCD	MGA-I MHBD	Maternal MCC
C8	MCAD	GA-II MCKAT M/SCHAD	
C3-DC		MAL	
C10:2		DR	
C5-DC	GA-I		
C14:1, C16, C18:1	VLCAD	CACT CPT-II CPT-I (when below reference range)	
C16-OH	LCHAD TFP		
Biotinidase	BIOT		
17-OHP	CAH		
TSH and/or fT_4	CH		
Total galactose and/or GALT	GALT	GALE GALK	
IRT+/- PAP+/-CFTR mutation panel	CF		
TREC	SCID	Secondary immunodeficiencies (eg, DiGeorge syndrome, trisomy 21, CHARGE syndrome)	

(Continued)

TABLE 1-1 Recommended Uniform Screening Panel (US) and Key Analytes (Continued)

Analyte	Core Condition	Secondary Targets	Other Identifiable Conditions
Hemoglobin electrophoresis	Sickle cell anemia; S, β-thalassemia; S,C disease	Other hemoglobinopathies	
Audiometry	Hearing loss		
Pulse oximetry	Critical congenital heart disease		

Phe, phenylalanine; PKU, phenylketonuria; BS, defects of biopterin cofactor biosynthesis; HPA, benign hyperphenylalaninemia; REG, defects of biopterin cofactor regeneration; Leu, leucine; Ile, isoleucine; Alloile, Allo-isoleucine; Val, valine; MSUD, maple syrup urine disease; BCKDK, branched-chain ketoacid dehydrogenase kinase deficiency; Met, methionine; HCY, homocystinuria (due to cystathionine β synthase deficiency); MET, hypermethioninemia; RMD, remethylation disorders; TIN, transient tyrosinemia of the neonate; Cit, citrulline; Arg, arginine; Asa, argininosuccinate; ASA, argininosuccinic acidemia; CIT, citrullinemia; ARG, argininemia; CIT-II, citrullinemia type II (citrin deficiency); Tyr, tyrosine; TYR-I, tyrosinemia type I; TYR-II, tyrosinemia type II; TYR-III, tyrosinemia type III; TIN, transient hypertyrosinemia of the newborn; CUD, carnitine uptake defect; CBL, methylmalonic acidemia due to a cobalamin deficiency; MUT, methylmalonic acidemia (mutase deficiency); PA, propionic acidemia; TC-II, transcobalamin II deficiency, SUCLA2, succinyl-CoA synthetase deficiency, IBDH, isobutyryl-CoA dehydrogenase deficiency, SCAD, short-chain acyl-CoA dehydrogenase deficiency, FIGLU, glutamate formiminotransferase deficiency, IVA, isovaleric acidemia; SBCAD, short branched-chain acyl-CoA dehydrogenase deficiency, BKT, β-ketothiolase deficiency, HMG, 3-hydroxy 3-methyl glutaric aciduria (HMG-CoA lyase deficiency); MCC, 3-methylcrotonyl-CoA carboxylase deficiency, MCD, multiple carboxylase deficiency, MGA-I, methylglutaconic aciduria type I; MHBD, 2-methyl 3-hydroxybutyryl-CoA dehydrogenase deficiency, MCAD, medium-chain acyl-CoA dehydrogenase deficiency, GA-II, glutaric aciduria type II (multiple acyl-CoA dehydrogenase deficiency); MCKAT, medium-chain ketoacyl-CoA thiolase deficiency, M/SCHAD, medium/short-chain 3-hydroxy acyl-CoA dehydrogenase deficiency, MAL, malonic aciduria; DR, dienoyl-CoA reductase deficiency, GA-I, glutaric acidemia type I; VLCAD, very long-chain acyl-CoA dehydrogenase deficiency, CACT, carnitine:acylcarnitine translocase deficiency, CPT-I, carnitine palmitoyltransferase I deficiency, CPT-II, carnitine palmitoyltransferase II deficiency, LCHAD, long-chain 3-hydroxy acyl-CoA dehydrogenase deficiency, TFP, trifunctional protein deficiency, BIOT, biotinidase deficiency, 17-OHP, 17-OH-Progesterone; CAH, congenital adrenal hyperplasia (21-hydroxylase deficiency); TSH, thyroid-stimulating hormone; CH, congenital hypothyroidism; GALT, classical galactosemia; GALE, galactose epimerase deficiency, GALK, galactokinase deficiency, IRT, immune reactive trypsinogen; PAP, pancreatitis associated protein; CF, cystic fibrosis; TREC, T-cell receptor excision circles; SCID, severe combined immune deficiency syndrome. (http://www.hrsa.gov/advisorycommittees/mchbadvisory/heritabledisorders/recommendedpanel/index.html).

of screening for tyrosinemia type I (TYR-I). Traditionally, the primary marker used to identify patients (with TYR-I is tyrosine Tyr); however, Tyr levels in newborns with TYR-I can be in the normal range. Furthermore, Tyr elevation is most often associated with other disorders or benign transient hypertyrosinemia of the newborn. Assuming two patients with TYR-I were born among a screened population of 200,000 newborns and Tyr was 160 and 240 μmol/L in the patients' respective screening samples, a cutoff for Tyr chosen at the 99.5th percentile corresponding to a Tyr concentration of 180 μmol/L would yield an insufficient sensitivity of only 50%, a false-positive rate of 0.5%, and a PPV of 0.1% (Table 1-3). Lowering the cutoff to the 97th percentile (150 μmol/L) raises the sensitivity to 100% but has its drawback in an increased false-positive rate (3%), and further reduction of the PPV (0.03%) (Table 1-3). To overcome this untenable situation, some screening programs have stopped screening for TYR-I and others have implemented testing for succinylacetone, a specific marker for TYR-I[14-16] The latter can be performed as a primary screening[16] test or in a second-tier approach in which any sample yielding an elevated Tyr value will be analyzed for succinylacetone.[14,15] Using the two-tier approach, which is not 100% sensitive, only samples containing both elevated analytes would be reported as abnormal.[17]

A second-tier approach has also been introduced for several other conditions associated with high false-positive rates and poor PPV in which biochemical or molecular genetic approaches are used.[18-22] For example, screening for cystic fibrosis (CF) is performed by determining immunoreactive trypsinogen. If trypsinogen is abnormally elevated, DNA is extracted from the existing blood spot to determine the presence of at least the most common CFTR mutations. A screening report is not issued until both tests have been completed.[21,22] Despite this approach, however, the false-positive rate for CF screening remains high because any elevated immunoreactive trypsinogen (IRT) concentration associated with at least carrier status for one of the evaluated CFTR mutations requires follow-up, thereby identifying mostly CF carriers. As an alternative, IRT/PAP (pancreatitis-associated protein) protocols have been shown to have similar sensitivity with respect to detection of CF patients when compared to an IRT/DNA–based protocol.[23-25] One advantage of using PAP as second tier test is that in contrast to genetic CF newborn screening the majority of carriers are not detected. This strategy can be combined with DNA testing as third tier.

In addition to second-tier tests, an increasing number of screening programs have improved their performance at least for the conditions identified through amino acid and acylcarnitine analysis by departing from the use of strict and arbitrary cutoffs to determine whether a result can be considered normal or abnormal.[26] The Region 4 Collaborative's Laboratory Performance Database (freely available by registering at: https://www.nbstrn.org/research-tools/lab-performance-database) has established a web-based system that allows laboratories to determine within seconds the relevance of amino acid and acylcarnitine results obtained by MS/MS analysis. This system establishes risk factors for a result being presumptive positive for relevant conditions by determining and weighing the degree of penetration into the disease range of multiple analytes and ratios of analytes. The disease ranges are based on a minimum of 50 true positive result sets obtained from a continuously growing number of collaborating newborn screening programs worldwide.[27] Application of these tools in routine newborn screening leads to significant performance improvements over the static use of cutoffs

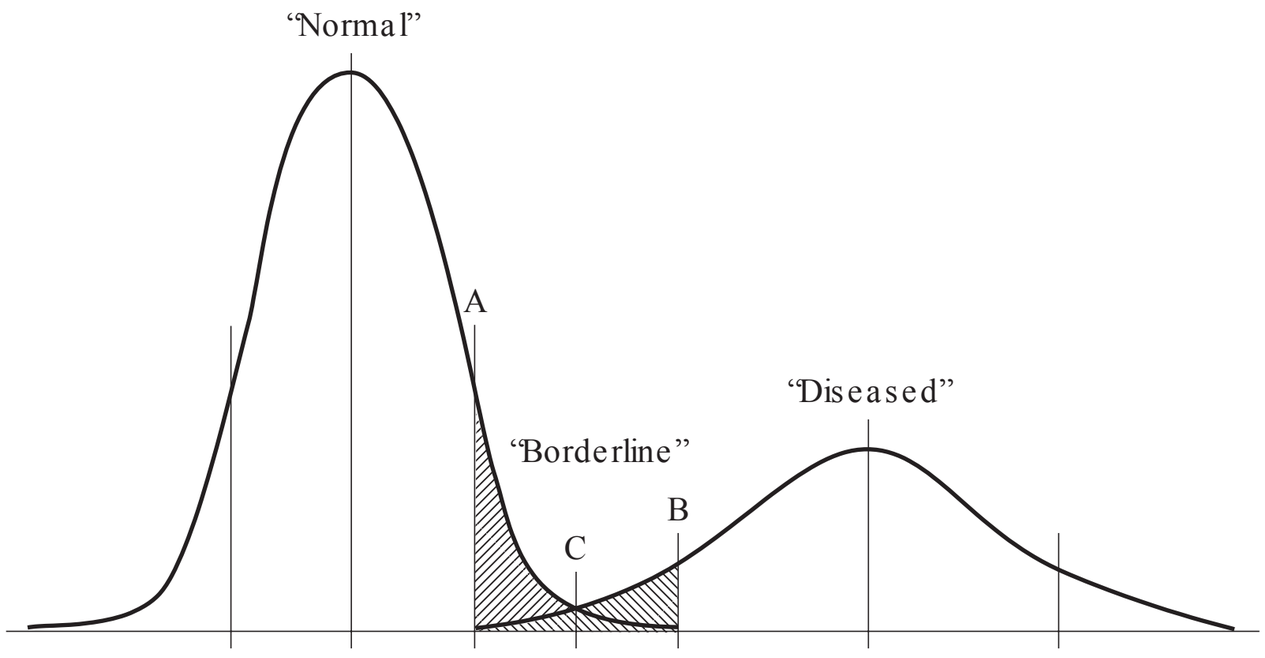

FIGURE 1-1. Bimodal distribution of a variable (eg, the marker metabolite for a screening disease) in a population.

TABLE 1-2 The Performance of a Screening Test

	No. of Newborns w/ Positive Screening Result	No. of Newborns w/ Negative Screening Result	Total
No. of newborns w/ disease	True positives (TP)	False negatives (FN)	TP + FN
No. of newborns w/o disease	False positives (FP)	True negatives (TN)	FP + TN
Total	TP + FP	FN + TN	All newborns screened

$$\text{Sensitivity} = \frac{\text{Affected newborns with positive test (TP)}}{\text{All affected newborns in tested population (TP + FN)}}$$

= Proportion of affected patients that have a positive test result

$$\text{Specificity} = \frac{\text{Healthy newborns with negative test (TN)}}{\text{All healthy newborns in tested population (FP + TN)}}$$

= Proportion of unaffected newborns having a negative test result

$$\text{False-positive rate} = \frac{\text{Healthy newborns with positive test (FP)}}{\text{All newborns with positive test (TP + FP)}}$$

$$\text{PPV}^{a} = \frac{\text{Affected newborns with positive test (TP)}}{\text{All positive tests (TP + FP)}}$$

= Proportion of newborns with positive test results who are truly affected

[a]Positive predictive value.

for single analytes while avoiding unnecessary costs for repeat analyses and follow-up investigations.[28]

Additional performance improvements in the interpretation of metabolite profiles such as those obtained for amino acids and acylcarnitines can be achieved when any available information provided on the newborn screening card is considered. Such result interpretation requires knowledge of and experience with not only detectable diseases but also with typical clinical situations encountered in neonates. For example, C5 acylcarnitine is a marker for isovaleric aciduria (IVA), a classic organic aciduria that can result in a devastating outcome unless metabolic decompensation is prevented. IVA is therefore included in most screening panels; however, C5 acylcarnitine is also elevated in 2-methylbutyrylglycinuria and in a milder variant of IVA, both of which are of uncertain clinical significance.[29-31] To further complicate the differential diagnosis of C5 acylcarnitine elevations, this analyte is also present at abnormal levels in patients treated with pivalic acid–containing medications.[32] Simple notification of the referring birthplace about any C5 acylcarnitine elevation will therefore increase the number of false-positive results, in particular when it is encountered in premature neonates exposed to particular medications.[32,33]

A screening program's performance is also determined through ongoing assessment of the outcome or consequences of abnormal results. The impact of false-positive results was documented through an objective and quantitative assessment by Waisbren et al.[34,35]

TABLE 1-3 Effect on Screening Performance of Variable Cut-off Levels of Tyrosine in Detecting Two Cases of Tyr-I in a Population of 200,000 Newborns

Case #1

Tyr Cut-off Set at 99.5% (180 μmol/L)	No. of Newborns w/ Positive Screening Result	No. of Newborns w/ Negative Screening Result	Total
No. of newborns w/ disease	1 (Tyr 240 μM)	1 (Tyr 160 μM)	2
No. of newborns w/o disease	999	198,999	199,998
Total	1000	199,000	200,000

Sensitivity, 50%; specificity, 99.5%; false-positive rate, 0.5%; PPV, 0.1%

Case #2

Tyr Cut-off Set at 95% (150 μmol/L)	No. of Newborns w/ Positive Screening Result	No. of Newborns w/ Negative Screening Result	Total
No. of newborns w/ disease	2 (Tyr 160 and 240 μM respectively)	0	2
No. of newborns w/o disease	5998	194,000	199,998
Total	6000	194,000	200,000

Sensitivity, 100%; specificity, 97%; false-positive rate, 3%; PPV, 0.03%

Although it was found that expanded screening provides better long-term outcome for those patients subjected to early initiation of treatment because of early identification of their condition, infants with false-positive screening results were more often hospitalized than healthy children with normal screening results. Families who received false-positive newborn screening results were at higher risk of developing dysfunctional parent–child relationships.[35] Furthermore, with the ability to identify newborns with conditions of either uncertain clinical significance (ie, short chain acyl-CoA dehydrogenase [SCAD] deficiency) or for which there is no effective long-term treatment (ie, carnitine-acylcarnitine translocase deficiency), the impact of these conditions on the newborns, their families, and the healthcare system must be continuously evaluated to obtain evidence that can be used to determine whether to continue screening for specific conditions. Despite the major advantages of newborn screening for physical and cognitive outcome, living with a metabolic disorder causes considerable stress on patients and their families. Although in a German study more than 90% of families expected that their child's future development will be normal and that their child will lead an independent adult life, the majority of families also reported a significant strain posed on the family (child) by the disorder.[36] For some disorders the perceived burden was highly variable between families, and disorders grouped as potentially very burdensome according to expert rating were not necessarily perceived as such by parents. This emphasizes the need for comprehensive

multidisciplinary care including psychological and social support for these patients and their families.

Traditional newborn screening programs such as screening for CH or PKU are highly cost-effective.[37] There would also always be a major benefit in transitionary countries as documented for Libya.[38] Nevertheless, worldwide many countries still lack a complete newborn population screening including China (87%) and India (<1%).[39]

Surprisingly, there are as yet only scarce data regarding the cost-effectiveness of extended newborn screening programs. Superb cost-effectiveness could be documented for newborn screening strategies for CF, medium-chain acyl-CoA dehydrogenase deficiency (MCAD), and glutaric academia type 1 (GA-I).[40-42] Two studies have analyzed the overall cost-effectiveness of introducing expanded MS/MS based newborn screening programs.[43-45]

The Newborn Screening Process

Newborn screening programs are typically state-mandated and administered. Responsibility for a successful program, however, lies with all parties involved to ensure the fundamental objectives of newborn screening are met. Therefore, newborn screening not only includes laboratory testing, but the complete process from parent education and sample collection to confirmation and initiation of treatment of identified patients as well as registry-based evaluation of long-term outcome.[10] Aside from the actual laboratory analysis, interpretation, and reporting, which must be undertaken in a timely fashion, the healthcare provider is responsible for ensuring that screening is performed and that blood spots are properly collected at the appropriate time and sent to the screening laboratory expeditiously. Once results become available, the healthcare provider must inform the families of the results and initiate follow-up as indicated. Finally, feedback of confirmed or rejected diagnoses to the screening laboratory helps to adjust screening algorithms. To aid the primary care providers who receive abnormal screening results, the ACMG developed brief clinical descriptions and recommendations for clinical and laboratory follow-up for each condition detectable by newborn screening (available online at: http://www.ncbi.nlm.nih.gov/books/NBK55832/).

Preanalytical Phase The preanalytical phase of the screening process consists of counseling and sampling. Healthcare providers (obstetricians, midwifes, and pediatricians) bear responsibility for this phase.

Counseling Newborn screening is mandated in many countries and regions to ensure that all babies benefit from this preventive health measure. Written informed parental consent is required by some screening programs but voluntary opt-out is more common.

Sampling The appropriate time for blood sampling is between 24 and 72 hours post partum. The outer or inner side of the baby's heel is pricked, and blood dripped on a filter paper card ("Guthrie card") so that the marked circles on the card are completely soaked by blood (Figure 1-2). Care must be taken to ensure that blood is free-flowing and not obtained by tissue compression, which will dilute the sample and create artefactual results. The filter paper is kept at ambient temperature for 2 to 3 hours until the blood is completely dried.

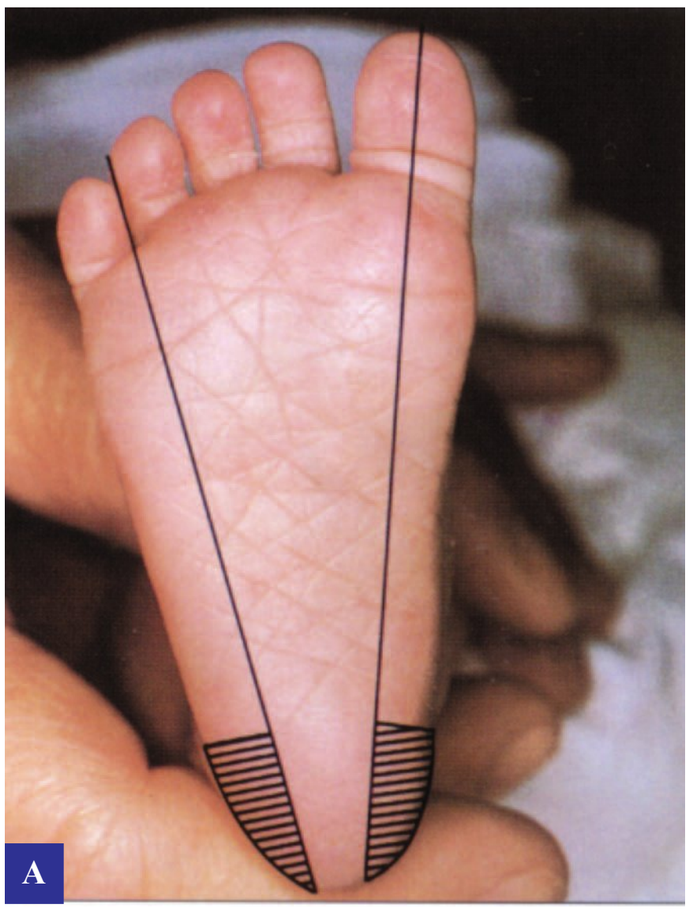

FIGURE 1-2. Technique of blood sampling in newborn screening. The heel-prick is made on the inner or outer side of the heel (hatched area) and should not exceed a depth of 2.4 mm (1.9 mm in premature infants). Blood is applied to the filter paper by completely filling the circles on both front and back sides. (Photographs with permission from Whatman GmbH)

In some circumstances (significant prematurity based on gestational age or birth weight, early discharge, blood transfusion, parenteral nutrition, treatment with corticosteroids or dopamine), an initial screening must be followed by a second screening, performed from 1 week to 3 months later depending on the circumstance. Not repeating the newborn screening in the above circumstances has resulted in a number a documented screening failures.[46]

Analytical Phase The analytical phase of the screening process consists of data entry, analysis, assessment, recalling, and reporting. The screening laboratory bears the responsibility.

Data Entry Data on the newborn and the mother is provided as written information on the Guthrie card and is usually recorded by automated scanning systems. Information such as date of birth, time of blood sampling, birth weight, gestational age, feeding, medication, and so forth, is necessary for interpretation of results.

Analysis Small dots are punched out of the dried blood spot specimen (DBS) into microtiter or filtration plates for extraction of blood. The different methods require specific extractions. Each extract is then used for the individual assay. In addition to the specimens, quality controls, calibrators, and external or internal standards are analyzed depending on the assay. In the majority of assays, data transfer and processing for analysis is performed by computer systems. A schematic application flow is shown for MS/MS analysis (Figure 1-3).

Assessment Data assessment involves flagging all samples that exceed or fall below the established cutoff levels and interpretation of the results in consideration of the information provided on the screening card.

Recalling In the case of a presumptive positive screening result, the primary healthcare provider is informed of the findings and advised regarding the appropriate follow-up investigations (http://www.ncbi.nlm.nih.gov/books/NBK55832/). In some jurisdictions, for example, Ontario, Canada, the positive screening test gets reported to one of the designated newborn screening follow-up centers which will then arrange for all further action and will report outcomes back to the screening program.

Reporting Apart from the direct contact in urgent cases, screening results are sent to the provider of the sample who then informs the parents of the newborn screening results.

Postanalytical Phase In the postanalytical phase, the responsibility is clearly defined for the different necessary steps for all parties involved in newborn screening.

Confirmation A presumptive positive screening result should be followed up by

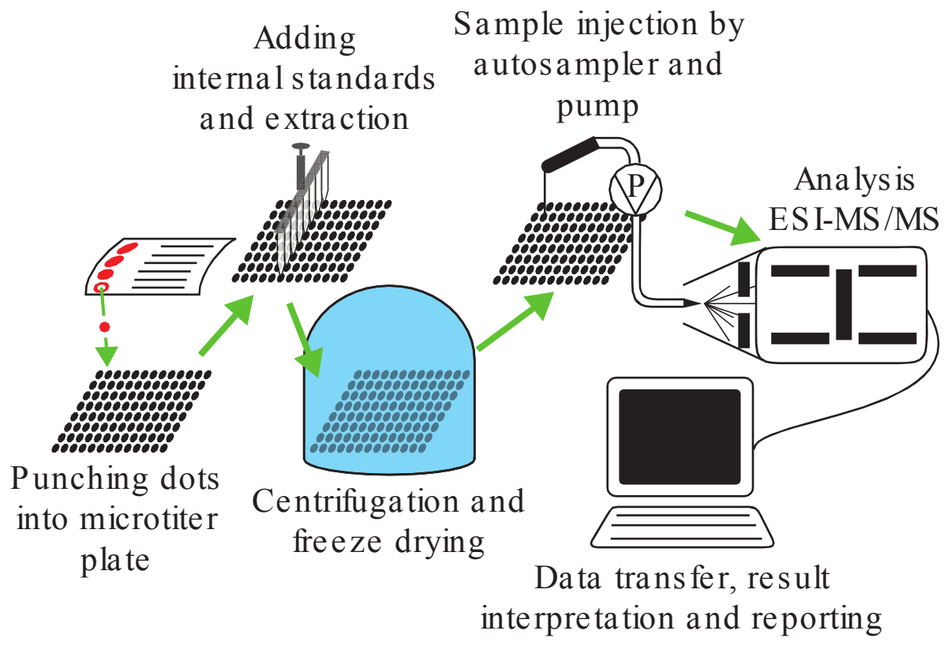

FIGURE 1-3. Schematic work flow in newborn screening laboratories exemplified for MS/MS screening.

confirmatory testing that, with few exceptions, requires specific tests in addition to repeating the screening assay. For example, if an elevated TSH has been detected in the initial screening, confirmatory testing would require a repeat determination of TSH (<20 mU/L), or in the case of higher values, serum determinations of TSH, T_4, fT_4 and T_3. Depending on these results an ultrasound of the thyroid could become necessary as well as additional determinations of thyroglobulin and several antibodies (thyroid antiperoxidase antibodies, antithyroglobulin antibodies, thyroid receptor-blocking antibodies). The screening laboratory has to be informed about the results of confirmatory studies.

Treatment Initiation In some conditions and under some circumstances, treatment should be initiated even before confirmatory testing of a positive screening result becomes available. For example, in a female newborn with ambiguous genitalia an abnormal newborn screen for congenital adrenal hyperplasia treatment with hydrocortisone and fludrocortisone should be initiated even before the serum 17-hydroxyprogesterone (17-OHP) results become available to prevent adrenal crisis.[47] Laboratory diagnosis and treatment initiation as endpoints are integral parts of the newborn screening process and therefore both should be registered.

Epidemiological Evaluation Epidemiological evaluation is important for screening programs. It helps to adjust screening algorithms, and feedback of results of confirmation to a central registry will allow calculation of prevalence data.

Monitoring Long-Term Outcome Good long-term outcome is the ultimate goal of screening programs and its monitoring is necessary to evaluate the whole program. Data on long-term outcome should be evaluated with the emphasis of evaluation and optimization of therapeutic as well as of screening practices.[48]

Laboratory Screening Methodology

The Bacterial Inhibition Assay (BIA)

For the BIA or original "Guthrie" test, the dried blood spots are placed on agar plates containing a strain of Bacillus subtilis that requires Phe for growth.[49] The agar also contains β-2-thienylalanine, a Phe analog that inhibits bacterial growth. When excessive Phe is present in the blood spot, the analog's action is overcome and bacterial growth occurs, which is easily detectable. Calibrator spots allow for a rough estimate of Phe concentrations in the patient sample. Based on the success with PKU, BIAs were adapted for other inborn errors of metabolism and were used for screening of galactosemia ("Paigen test," see Figure 1-4), maple syrup urine disease (MSUD), and homocystinuria.

These assays were simple, inexpensive, and suited to screening large numbers of individual specimens; however, they represent semiquantitative methods with limited sensitivity (eg, antibiotic treatment can cause false negative results), and the results must be manually entered into the laboratory information system. Therefore different, more automated analytical techniques for newborn screening have slowly replaced the BIAs.

Fluorometric and Photometric Tests In the 1990s, progress toward more automated screening assays was achieved by the development and implementation of fluorometric and photometric microassays for PKU and galactosemia screening. These assays can be applied to quantify analytes and enzyme activities. For example, galactosemia screening can be accomplished by determination of total galactose and galactose-1-phosphate uridyltransferase (GALT) activity. Total galactose represents the sum of free galactose and galactose fixed in galactose-1-phosphate and is measured utilizing the enzymes alkaline phosphatase and galactose dehydrogenase, both included in the commercially available assay kit. Additional estimation of the galactose-1-phosphate concentration is helpful for differentiation between galactosemia and galactokinase deficiency and for therapy monitoring. It is feasible by running the assay twice: once without and once with alkaline phosphatase in the assay. The difference in galactose concentration between the two assays allows an estimate of the concentration of galactose-1-phosphate. The assay for determination of GALT activity is a modification of the Beutler–Baluda test.[50]

Glucose-6-phosphate dehydrogenase (G6PD) activity is measured by a fluorescent spot test giving semiquantitative results of the activity of G6PD. Positive results are followed up by quantitative determination of enzyme acitivity by spectrophotometric assay.[51]

For screening of biotinidase deficiency a semiquantitative colorimetric assessment of biotinidase activity in DBS is used.[52] Samples with biotinidase activity show a characteristic purple color upon addition of developing reagents after incubation with biotinyl p-aminobenzoate, whereas those with little or no activity remain straw colored. Assessment is feasible by visual interpretation but more accurate by photometric measurement.

In the early 2000s, the late Chamoles developed fluorometric methods for the screening of several lysosomal storage disorders (LSD).[53] These methods have first been applied in Taiwan for screening of Pompe disease and Fabry disease.[54] Sista and colleagues multiplexed the fluorometric method using digital microfluidics to measure four LSDs in a single DBS.[55] This assay is currently used in Missouri to screen for Pompe disease, Fabry disease, Gaucher disease, and mucopolysaccharidosis I.[56,57]

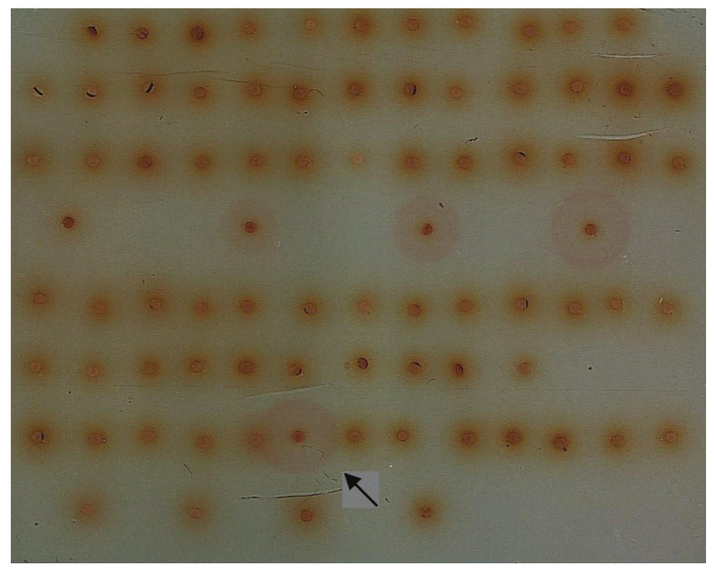

FIGURE 1-4. The bacterial inhibition assay (BIA) agar plate of a microbiological inhibition assay. The figure represents an example for adoption of the aboriginal "Guthrie test" for galactose measurement ("Paigen Test"). Control discs to which known increasing quantities of galactose were added are seen in the middle of the plate. In the lower part a positive screening sample can be found (arrow).

Immunoassays Fluoroimmunometric assays are traditionally used to screen for congenital adrenal hyperplasia and congenital hypothyroidism. The assays use mono- or polyclonal antibodies directed against distinct antigens on the analyte of interest (eg, TSH, 17-OHP). Either the antibody or the analyte is labeled with a fluorochrome, for example, europium. The antigen–antibody complexes are fixed to a solid phase in a microtiter plate before an enhancing reagent dissociates the fluorochrome. The fluorescence of the resulting chelate solution is proportional to the analyte's concentration in the blood spot (Figure 1-5). The third generation assays are fully automated and their sensitivity and specificity is above 99%. The disadvantages of immunoassays derive from cross-reacting substances in blood, which is of special importance in congenital adrenal hyperplasia (CAH) screening in which placental and fetal adrenal steroids can cross-react with the antibody directed against 17-OHP.

Newborn Screening and Tandem Mass Spectrometry (MS/MS) Mass spectrometers are instruments measuring the weight of ions derived from a neutral compound following ionization. The mass spectrometer separates the ions based on their mass-to-charge (m/z) ratio after which they are recorded by a detector that generates a plot of m/z values over intensities, that is, a mass spectrum. The abundance of each ion in the original sample correlates to the height of each ion peak in the mass spectrum, allowing for qualitative interpretation of the ion profile. Quantitative determination of the concentration of a specific ion is possible by the addition of defined concentrations of isotopically labeled internal standards early during sample preparation (Figure 1-3). In the 1980s, MS/MS was introduced into clinical laboratories because of this technology's ability to analyze acylcarnitines efficiently in complex biological samples.

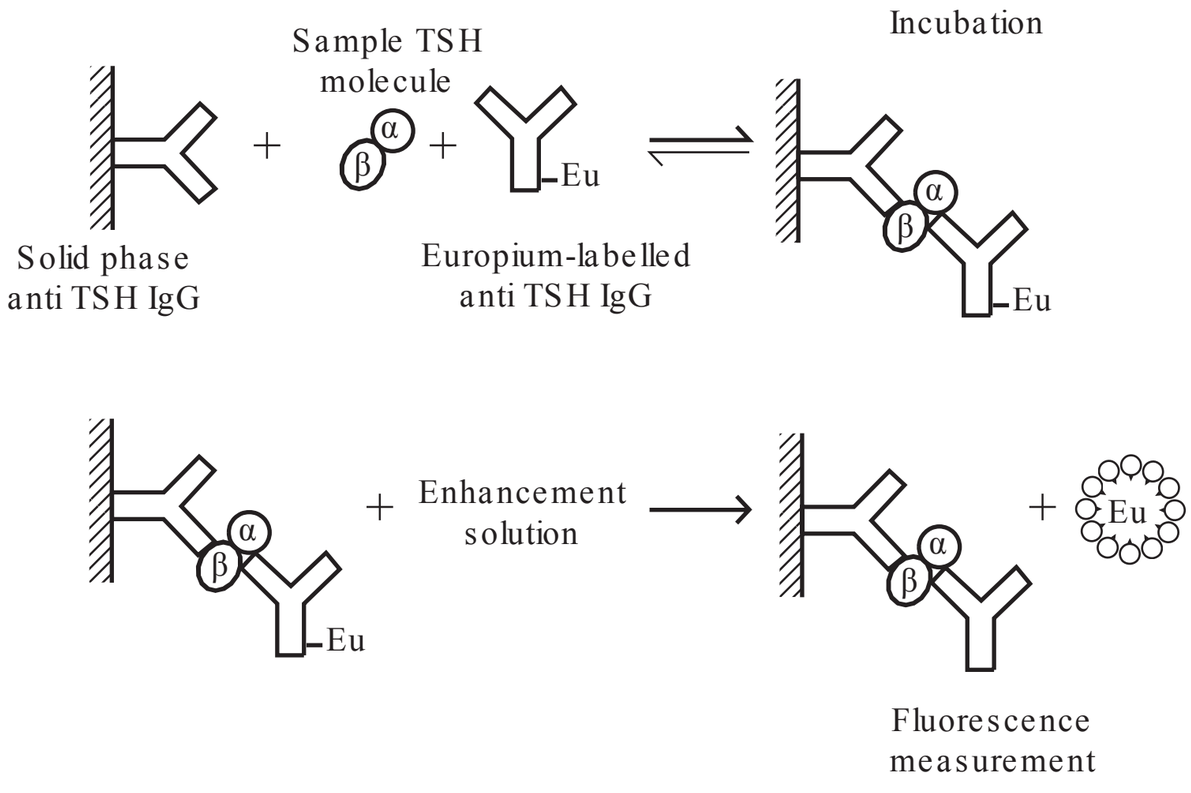

FIGURE 1-5. Principle of a fluoroimmunometric assay for measurement of thyroid-stimulating hormone (TSH).

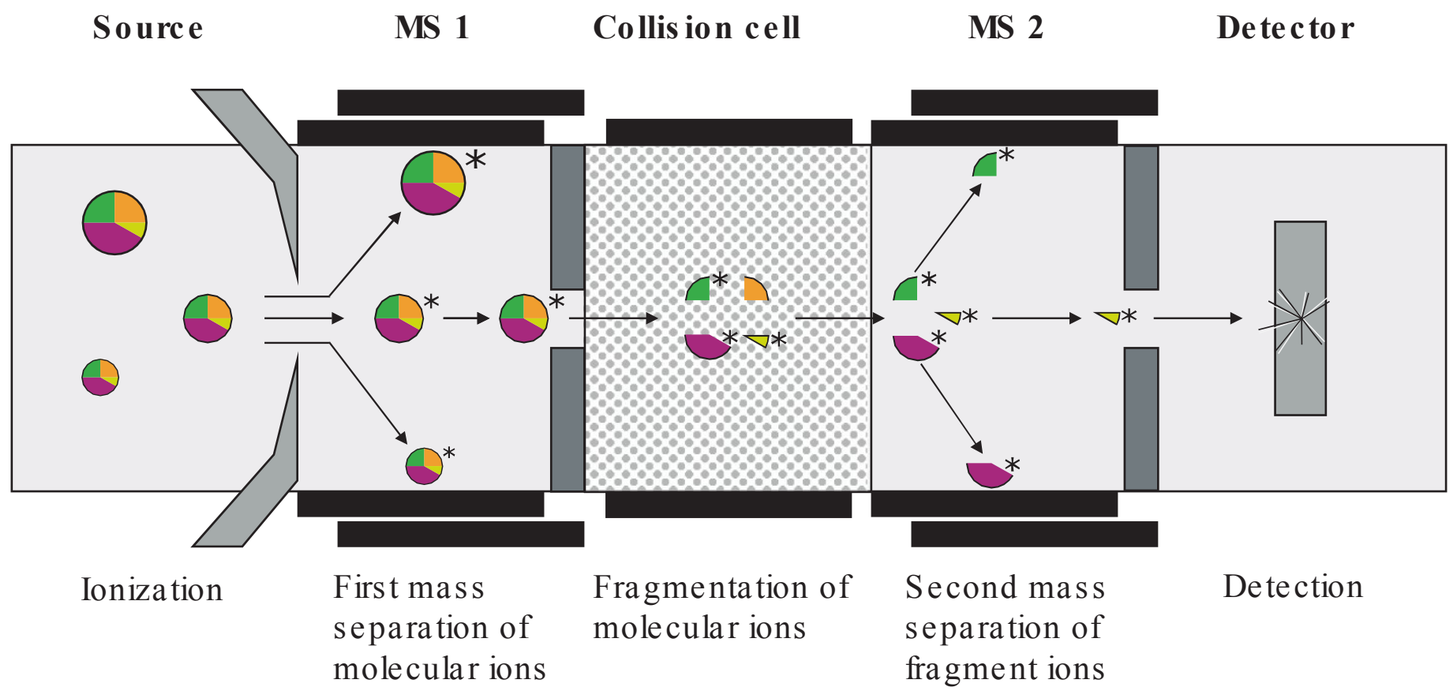

FIGURE 1-6. Principle of tandem mass spectrometry (MS/MS).

Tandem mass spectrometers consist of two mass spectrometers coupled in series and separated by a "collision cell" (Figure 1-6). The first MS (MS_1) analyzes the ions in a sample (precursor ions), which are then fragmented in the collision cell by using an inert gas. The product ions resulting from fragmentation are further analyzed by the second MS (MS_2). Either MS_1 or MS_2 can be set to scan a mass range or to select one or more individual ions.[58,59]

When it was recognized that acylcarnitine and amino acid analyses can be performed simultaneously on the same sample, the use of this technology for newborn screening was developed.[60] Since the early 1990s, it has been proven that this technology can be applied to newborn screening because it is amenable to high-throughput, population-wide testing for a large number of disorders of fatty acid, organic acid, and amino acid metabolism.[61-65] For some of these conditions effective treatment is currently not available, which has raised many questions and concerns; however, even when treatment is not available, it can be argued that early identification is still of benefit.[66]

Additional applications of MS/MS in newborn screening were developed for CAH,[67] for propionic and methylmalonic aciduria,[68] and for lysosomal storage disorders. Based on Chamoles' fluorometric methods for the measurement of lysosomal enzyme activities, Gelb and Scott developed reagents and methods to measure multiple lysosomal enzyme activities where several substrates are incubated with a DBS in buffer systems facilitating lysosomal enzyme reactions followed by simultaneous quantitation of the respective enzyme products by MS/MS. Based on the concentration of enzyme product, enzyme activities are calculated. This approach has been applied to newborn screening studies in various programs worldwide and while only Pompe disease was added in 2015 to the Recommended Uniform Screening Panel (RUSP) in the United States, the MS/MS assay has been applied to routine newborn screening for Krabbe disease in New York State since 2006.[57] While the MS/MS method to determine galactocerebrosidase (GALC) activity appears to be accurate, the overlap of GALC activities between cases of Krabbe disease, GALC pseudodeficiency, and GALC mutation carrier status has caused significant concern and led the US Secretary of Health to reject proposals to include Krabbe disease for inclusion in newborn screening programs.[69]

Molecular Genetic Analyses Mutation analysis is not (yet) feasible as a primary test in newborn screening but is being applied in a two-tier approach to increase diagnostic sensitivity and specificity of screening. Examples are screening for cystic fibrosis and—in New York state—Krabbe disease. Since severe

combined immunodeficiency (SCID) was added to the RUSP in the United States, molecular genetic analysis is being applied as a first-tier method to determine the quantity of T-cell receptor excision circles (TRECs) by PCR. TRECs are DNA biomarkers of normal T-cell development and significantly reduced in SCID.[70] Pilot studies to investigate the feasibility of massive parallel sequencing for conditions that lack reliable biochemical screening biomarkers are underway, ie for proximal urea cycle disorders.

SCREENED METABOLIC AND ENDOCRINE CONDITIONS

Phenylketonuria (PKU)

PKU was the first disease for which a screening test was developed. Since the implementation of newborn screening for PKU in many countries, early detection and initiation of a Phe-restricted diet has dramatically improved the prognosis of affected children.

PKU, an inborn error of amino acid metabolism (see Chapter 15), is caused by deficiency of the enzyme Phe hydroxylase, which catalyzes conversion from Phe to tyrosine (Tyr). In PKU, the Phe level is elevated in blood, whereas the Tyr level is low. Screening for Phe allows recognition of the disease. Additional assessment of the Phe/Tyr ratio lowers the rate of false positives. In addition to PKU other forms of impaired Phe hydroxylase function, such as non-PKU hyperphenylalaninemia (HPA) and deficiency of enzymes involved in the metabolism of the cofactor tetrahydrobiopterin, can be detected by Phe (and Tyr) measurement. Detection of HPA in females, even if it does not require dietary treatment, is of importance because in adulthood their offspring may suffer from maternal PKU.

Screening Method BIA, fluorometric, and photometric microassays have been replaced by MS/MS in most newborn screening laboratories.

Key Metabolite Phe and Phe/Tyr ratio.

Differential Diagnosis HPA, biopterin cofactor deficiencies, transient benign hyperphenylalaninemia of the newborn, total parenteral nutrition, severe liver disease.

What to Do If Test is Positive Contact family immediately about result and report findings to newborn screening program. Consult with metabolic specialist.

Confirmatory Studies Phe and Tyr in plasma (or again in DBS); pterins in urine or in DBS,[71] dihydropteridine reductase activity in DBS, tetrahydrobiopterin loading test.

Prevalence 1:10,000 (Germany); 1:20,000 (US).[72-75]

Galactosemia

Classic galactosemia (see Chapter 6) is caused by virtually complete absence of GALT activity. Two different approaches exist to screen for galactosemia. 1) Measurement of total galactose, which is the sum of free galactose and galactose from galactose-1-phosphate. The sensitivity of the test increases when the baby receives small amounts of breast milk or lactose-containing formula, which is crucial in the case of early discharge, especially if screening is done prior to the first feeding. 2) Measurement of GALT activity in DBS by a fluorometric screening test (Beutler test) has high sensitivity and is independent of diet; however, GALT testing is associated with a higher false-positive rate (up to 1%) when the sample has been exposed to higher temperatures (eg, during transport in the summer) due to enzyme denaturation. Patients who have had blood transfusions may have false-negative Beutler test results for as long as 2 to 3 months because of the contribution of normal GALT in the donor cells. The combined approach with measurement of total galactose and GALT has proven to be most effective. Symptoms may occur before the results of newborn screening are available.

While confirmation of the screening result is pending, patients in whom there is a high index of suspicion and the urine is positive for reducing substances should be put on a galactose-free diet and monitored for hypoglycemia, liver failure, Escherichia coli sepsis, and coagulopathy. Early recognition and treatment of galactosemia prevents severe liver failure and death, which otherwise might occur in the first month of life, as well as formation of cataracts. Despite strict treatment, the long-term outcome is still complicated in many patients by the development of neuropsychological and cerebellar symptoms, such as verbal dyspraxia, mental retardation, ataxia, and tremor. Females frequently develop ovarian failure. In males cryptorchism and delayed puberty is observed.

In addition to classic galactosemia with GALT activities less than 1%, milder forms of galactosemia are identified by newborn screening as well. The most common variants are the Duarte variant and compound heterozygotes with one Duarte allele and one classic galactosemia allele. Uncertainty remains as to whether the latter represents a non-disease.[76] Uridine diphosphate-galactose-4′-epimerase (GALE) deficiency can also cause high total galactose at birth. The majority of patients with GALE deficiency have been asymptomatic (peripheral form), except for a few children who have had dramatic neurologic involvement. A severe generalized form of GALE deficiency exists but appears to be very rare, with only five patients from two families described to date.[77]

Galactokinase deficiency (GALK), another rare condition, is characterized by extraordinarily high screening levels of free galactose, and treatment with a galactose-free diet prevents development of cataracts, the only consistent pathology in this disease.

Screening Method Fluorometric and photometric microassays.

Key Metabolite Total galactose and/or GALT activity.

Differential Diagnosis GALE deficiency, galactokinase deficiency, and severe liver disease (in the case of total galactose measurement); phosphoglucomutase, glucose-6-phosphate dehydrogenase, and 6-phosphoglycerate dehydrogenase deficiency (in the case of GALT measurement).

What to Do If Test is Positive Contact family immediately about result and report findings to newborn screening program. Consult with metabolic specialist. If total galactose is above 30 mg/dL stop lactose-containing milk feeding immediately until the confirmatory studies are completed.

Confirmatory Studies GALT, GALE, and galactokinase activities in erythrocytes (lysates), galactose-1-phosphate in erythrocytes (or in DBS). Mutation analysis.

Pitfalls/Attention DBS contaminated with breast milk or formula will cause false-positive results due to high total galactose. False-positive results for GALT activity often occur during summer months when samples are not protected from heat.

Prevalence 1:70,000 (Germany and US).[72-75,78]

Biotinidase Deficiency

The vitamin biotin is a cofactor of several carboxylases. Biotinidase deficiency (see Chapter 14) leads to disturbed recycling of biotin, thus causing multiple carboxylase deficiency. Biotinidase deficiency is a model screening disease. Severe symptoms occurring weeks to months after birth are completely preventable by inexpensive treatment with oral biotin. The disease can be detected by a simple enzyme assay amenable to newborn screening.

Differential Diagnosis None.

What to Do If Test is Positive Contact family immediately about result and report findings to newborn screening program.

Consult with metabolic specialist. If biotinidase activity is markedly reduced, biotin supplementation (5–10 mg biotin per day po) should be started before confirmatory studies are completed.

Confirmatory Studies Biotinidase activity in serum. Mutation analysis.

Pitfalls/Attention Acylcarnitine analysis is not a reliable newborn screening method for biotinidase deficiency since maternal biotin supply to the fetus prevents the accumulation of abnormal metabolites.

Prevalence 1:60,000 (Germany and US).[72-75,78]

Glucose-6-phosphate Dehydrogenase (G6PD) Deficiency

G6PD deficiency is an X-linked recessive condition with variable phenotype ranging from chronic hemolytic anemia to intermittent hemolysis or no disease expression based on the degree of residual enzyme activity. It is the most common enzymopathy worldwide, affecting about 500 million people in the world. The highest gene frequencies of the X-linked disorder are found in tropical Africa, the Middle East, in some areas of the Mediterranean as well as in tropical and subtropical Asia. Severe hemolytic episodes are caused by exposure to exogenous agents (eg, medications, infections, ingestion of fava beans) leading directly or indirectly to oxidative stress within the erythrocytes where G6PD is expressed. In newborns, the most significant complication is the development of Kernicterus due to hyperbilirubinemia. Therefore, newborn screening for G6PD has been proposed but because the geographic occurrence of G6PD deficiency is similar to that of malaria, newborn screening has been implemented mostly in relevant regions. Countries such as the United States or Germany have not yet included G6PD into their screening panels; however, given the evolving ethnic background of northern populations, more consideration is given to this possibility.[79] The goal of early identification is primarily to prevent exposure to triggering agents, as specific treatments are neither available nor required.

Screening Method Fluorometric enzymatic spot test

Key Metabolite G6PD activity.

What to Do If Test is Positive Contact family immediately about result and report findings to newborn screening program. Consult with obstetrician, pediatrician, or family doctor about possible neonatal jaundice, acute hemolytic crises, and long-term counseling.

Confirmatory Studies Enzyme assay in red blood cells.

Pitfalls/Attention False-negative results may occur if the blood is collected during an acute hemolytic episode and in female carriers when applying biochemical assays. False-positive results are possible when the dried blood spot is exposed to heat during transport.

Prevalence Three percent to 70% of people native to areas endemic to malaria are affected by G6PD deficiency. In the United States, 10% of African Americans are affected.

Congenital Hypothyroidism (CH)

CH was the first nonmetabolic disease included in newborn screening programs and in most regions it is the most prevalent disease among those for which screening is performed. CH is caused by inadequate production of thyroid hormone due to agenesis or an ectopic thyroid gland, dyshormonogenesis, endemic cretinism, and defects of the pituitary or hypothalamus (see Chapter 27). Except for central hypothyroidism all cases of CH are characterized by low thyroxine (T_4) and an elevated TSH level. Most North American screening programs measure T_4 followed by TSH when T_4 values are at the lowest 5% to 10%. In Europe and Asia primary TSH screening is routine. CH represents an ideal screening disease because of its relatively easy treatment, which prevents the development of severe cognitive delays.

Screening Method Immunoassay.

Key Metabolite T_4, TSH.

Differential Diagnosis Iodine deficiency.

What to Do If Test is Positive Contact family immediately about result and report findings to newborn screening program. Consult with pediatric endocrinologist about initiating treatment.

Confirmatory Studies Free T_4, TSH, radioisotope scanning (needs to be performed within 2 days of initiation of therapy), and thyroid ultrasound. Thyroid-binding globulin (TBG) in plasma may be measured if congenital TBG deficiency or TBG deficiency secondary to congenital nephrotic syndrome is suspected. Elevated reverse triiodothyronine (rT_3), low T_4 and normal TSH are characteristic of sick euthyroid syndrome.

Pitfalls/Attention Hypothalamo-hypophyseal forms of hypothyroidism are not detected by TSH screening.

In practice, overtreatment and overestimation of CH may occur if hormone supplementation is started without prior initiation of adequate confirmatory studies. Blood collected in citrate or EDTA tubes and then spotted onto a Guthrie card gives false-negative results. Specimens collected in the first 24 to 48 hours of life may lead to false-positive TSH elevations when using any screening approach.

Prevalence 1:3500 (almost uniform worldwide).[72-75,78]

Congenital Adrenal Hyperplasia (CAH)

CAH, the second endocrinopathy included in newborn screening programs, is caused by a defect in cortisol synthesis and in 90% of cases is due to a deficiency of the enzyme 21α-hydroxylase (21-OHD). Measurement of 17-OHP is used as the screening marker for CAH due to 21-hydroxylase deficiency. However, CAH due to 17α-hydroxylase/17,20 lyase and StAR deficiencies are not detected by 17-OHP screening as 17-OHP levels are not elevated in these disorders. CAH due to 11β-hydroxylase deficiency results in elevated 17-OHP levels but the associated elevated 11-deoxycorticosterone (DOC) levels allow differentiation from 21-OHD (for details see Chapter 27). Screening assays for 17-OHP with antibodies have problems in the specificity of the test because of the occurrence of crossreactivity of steroid compounds related to 17-OHP. In premature newborns in particular, significantly higher 17-OHP levels are measured. Therefore in this group, reference values adjusted either for gestational age or for birth weight have to be applied.

Screening Method Immunoassay and LC-MS/MS as a second-tier test in some programs.

Key Metabolite 17-OHP.

Differential Diagnosis None.

What to Do If Test is Positive Contact family immediately about result and report findings to newborn screening program. Consult with pediatric endocrinologist. If patient is symptomatic with hypoglycemia, vomiting, and/or electrolyte imbalance, admit immediately, consider 25 mg IM hydrocortisone en route to hospital, and refer to pediatric endocrinologist. If patient has ambiguous genitalia and is asymptomatic with normal electrolytes and glucose, refer immediately to pediatric endocrinologist.

Confirmatory Studies 17-OHP and other adrenal steroids in plasma and molecular genetic analysis.

Pitfalls/Attention The biggest pitfall in newborn screening for CAH is that the accuracy of current steroid assays is confounded by the timing of the newborn screening sample. In most programs, especially in the United States, newborn screening samples are obtained at 24–48 hours of life which can lead to false-positive 17-OHP results in premature babies[80,81] and can lead to false negatives in affected infants with the classic forms of CAH, salt wasting, and salt virilizing.[82,83] Missed cases of the classic forms occur because of either decreased inactivation of maternal cortisol by the placental 11β-hydroxysteroid dehydrogenase type 1 or increased sensitivity of the fetal hypothalamic-pituitary-adrenal axis to maternal cortisol which can result in a delayed rise of 17-OHP levels during the first 24–48 hours of life. For preterm newborns cutoffs adjusted for gestational age and/or birth weight and/or age at collection are necessary. Of note, preterm infants may have falsely low levels if they are on cortisol therapy or if their mother received multiple courses of bethamethasone before delivery.

Prevalence 1:13,000 (Germany); 1:15,000 (US).[72-75,78]

Maple Syrup Urine Disease (MSUD)

MSUD is a disorder of branched-chain amino acid (leucine, isoleucine, valine) metabolism caused by deficiency of the enzyme branched-chain ketoacid dehydrogenase. Classic MSUD presents clinically as a progressive encephalopathy within the first week of life (see Chapter 14). Immediate action must be taken if branched-chain amino acids are found distinctly elevated. Immediate hospitalization in a metabolic center is urgent. The affected children benefit greatly from lifelong dietary treatment, even if completely normal psychomotor development will not always be achieved. Screening for MSUD by leucine determination, as in former BIAs, lacks specificity. A relatively high number of newborns with benign transient hyperleucinemia or hyperhydroxyprolinemia test false positive (PPV <<1%). Additional assessment of valine by MS/MS allows for more specific and sensitive screening for MSUD.

Screening Method MS/MS.

Key Metabolites Leucine + isoleucine, valine, (leucine + isoleucine)/Phe ratio; some programs use a second-tier test to measure allo-isoleucine to increase the specificity of screening.[84]

Differential Diagnosis Intermittent and intermediary forms of MSUD, E_3 subunit (combined for dehydrogenase complexes) deficiency, thiamine deficiency.

What to Do If Test is Positive Immediately contact the family to ascertain the baby's clinical status. If any concern (ie, poor feeding, vomiting, lethargy, tachypnea), immediately initiate transport to hospital, preferably a metabolic center.

Confirmatory Studies Branched-chain amino acids including alloisoleucine in plasma, organic acids in urine.

Pitfalls/Attention Parenteral nutrition causes false-positive results. Hyperhydroxyprolinemia causes false-positive results for MSUD because standard MS/MS analysis cannot distinguish hydroxyproline from the isomers leucine and isoleucine. Second-tier measurement of allo-isoleucine can prevent these pitfalls. Intermittent MSUD may not be detected in all affected infants by newborn screening.

Prevalence 1:160,000 (Germany); 1:160,000 (US).[63-66,68]

Tyrosinemia Type I (TYR-I)

TYR-1 is caused by deficiency of the enzyme fumarylacetoacetase, which catalyzes the last step in Tyr degradation (see Chapter 17). Untreated TYR-I can lead to liver and renal failure, rickets, porphyric and neurologic crises, and hepatocarcinoma. Some infants with severe neonatal presentation of TYR-I die within the first months of life. Since treatment with the synthetic drug 2-(2-nitro-4-trifluoromethylbenzoyl) cyclohexane-1,3-dione (NTBC) became available, the disease has a favorable prognosis when treatment is initiated early. In TYR-I, blood concentrations of Tyr can be normal but succinylacetone, which is a specific marker for TYR-I, is elevated. Screening solely for Tyr also lacks specificity because some other and more common conditions cause elevation of Tyr. The issue of specificity and sensitivity in Tyr screening is exemplified in Table 1-3. Only when succinylacetone is part of the primary newborn screen does TYR-I screening become feasible.[16]

Key Metabolite Succinylacetone and Tyr.

Differential Diagnosis None, if succinylacetone is part of the screen; liver impairment, tyrosinemia types 2 and 3, and benign transient hypertyrosinemia of the newborn, in the case of exclusive screening for Tyr.

What to Do If Test is Positive Contact family immediately about result and report findings to newborn screening program. Consult with metabolic specialist to initiate confirmatory diagnostic testing.

Confirmatory Studies Plasma amino acids, serum α-fetoprotein, succinylacetone in urine and plasma, genetic testing.

Pitfalls/Attention EDTA blood or exposure of the DBS to heat will give false-positive results for some second tier assays.[15]

Prevalence <1:100,000; 1:20,000 (Saguenay-Lac St. Jean Region, Quebec, Canada).[63-66,68]

Homocystinuria (HCY)

HCY caused by cystathionine β-synthase deficiency is an inborn error of the transsulfuration pathway (see Chapter 18). Evidence from observations in patients with HCY in whom the disease was detected early and who were treated, indicates that presymptomatic initiation of treatment can prevent cognitive impairment, lens dislocation, and thromboembolic events.[85] The results are especially good in pyridoxine-responsive forms. The disorder is biochemically characterized by accumulation of homocysteine, methionine, and a variety of other metabolites of homocysteine in the body and, ultimately, excretion in the urine. Screening is done by measuring methionine, previously by BIA and today by MS/MS. In the majority, figures on the frequency of HCY are derived from the number of patients in whom methionine is detected. The estimated frequencies range from 1 in 1800 in Qatar[86] to 1:50,000 in Ireland to 1:1,000,000 in Japan; the overall frequency has been reported to be between 1:200,000 and 1:335,000.[87] Several lines of evidence indicate that some of these frequencies are very likely to be highly underestimated. Particularly the pyridoxine-responsive form of HCY, representing the most readily treatable form, is mostly if not always missed by newborn screening. This is corroborated by some studies on allele frequencies that reveal estimates for homozygote frequency of approximately 1:20,000,[88] a significantly higher prevalence than the aforementioned figure of detection by newborn screening for hypermethioninemia. In addition to insufficient sensitivity for HCY screening, methionine measurement has low specificity because several conditions other than HCY can cause increased methionine at birth. This lack of specificity for homocystinuria alone is reflected by PPVs that may be below 1% when screening relies on methionine alone. More recently, direct screening for homocysteine in blood spots using a liquid chromatography MS/MS method has been applied in Qatar (ca. 20,000 births per year), where cystathionine β-synthase deficiency has an incidence of 1 in 1800 live births.[86] In less homogenous and larger populations, this approach cannot be justified as it would require dedicated MS/MS equipment. However, second-tier MS/MS methods have been developed that measure total homocysteine, and also other specific disease

markers such as methylmalonic acid (MMA), 3-hydroxy propionic acid, and 2-methylcitric acid (MCA), thereby also improving the newborn screening performance for conditions associated with elevated propionylcarnitine and low methionine.[68]

Screening Method MS/MS.

Key Metabolite Methionine and total homocysteine.

Differential Diagnosis Other hypermethioninemias: S-adenosylhomocysteine hydrolase deficiency, glycine N-methyltransferase deficiency, methionine adenosyltransferase deficiency, impaired liver function.

What to Do If Test is Positive Contact family immediately about result and report findings to newborn screening program. Consult with metabolic specialist to initiate confirmatory diagnostic testing.

Confirmatory Studies Total homocysteine in plasma, amino acids in plasma, organic (methylmalonic) acids in urine, mutation analysis, enzyme study in fibroblasts.

Prevalence <1:250,000 (Germany), 1:270,000 (US).[72-75]

Citrullinemia

Citrullinemia is an urea cycle defect caused by deficiency of the enzyme argininosuccinate synthase (see Chapter 13). Citrullinemia may present neonatally with hyperammonemia and coma or it may have a milder course manifesting clinically after the postnatal period. Before screening for citrullinemia became available, the clinical outcome was generally poor. Most of the patients suffered serious brain damage with global developmental delay. Treatment implemented after the first manifestation of the disease could not prevent significant neurologic sequelae. Promising evidence that timely implementation of treatment has an obvious impact on the natural course of the disease arises from the first patients detected by newborn screening.[48,72] The disease is reliably detected in newborn screening by citrulline measurement using MS/MS. Surprisingly, the number of affected newborns disclosed in newborn screening markedly exceeded expectation. That fact turned out to be due to presumably milder variants of the disease or through citrin deficiency.[89] The impact of detection and treatment in children with such milder variants has still to be elucidated.

Screening Method MS/MS.

Key Metabolites Citrulline (high), arginine (low).

Differential Diagnosis Milder, variant form of citrullinemia, citrullinemia type II (citrin deficiency), argininosuccinate lyase deficiency, pyruvate carboxylase deficiency.

What to Do If Test is Positive Immediately contact the family to ascertain the baby's clinical status. If any concern (ie, poor feeding, vomiting, lethargy, tachypnea, seizures), immediately initiate emergency treatment with IV glucose and transport to a hospital, preferably a metabolic center that can facilitate extracorporeal detoxification.

Confirmatory Studies Amino acids in plasma, blood ammonia, orotic acid in urine.

Prevalence 1:50,000 (Germany).

Argininosuccinate Lyase Deficiency (ASL)

ASL is a urea cycle defect caused by deficiency of the enzyme argininosuccinate lyase (see Chapter 13). Affected newborns benefit from early recognition and treatment initiation; however, the long-term outcome remains to be elucidated. The condition is reliably detected in newborn screening by argininosuccinate and citrulline measurement by MS/MS.

Screening Method MS/MS.

Key Metabolites Argininosuccinate (high), citrulline (high), arginine (low).

Differential Diagnosis None.

What to Do If Test is Positive Immediately contact the family to ascertain the baby's clinical status. If any concern (ie, poor feeding, vomiting, lethargy, tachypnea, seizures), immediately initiate transport to a hospital, preferably a metabolic center that can facilitate extracorporeal detoxification.

Confirmatory Studies Amino acids in plasma and urine, blood ammonia, orotic acid in urine, enzyme studies in erythrocytes/fibroblasts.

Prevalence 1:180,000 (Germany, US, Australia).[72-75]

Medium-chain Acyl-CoA Dehydrogenase Deficiency (MCAD)

In Caucasians MCAD is the most frequent inborn error of fatty acid mitochondrial β-oxidation (see Chapter 9). Enzyme deficiency causes hypoglycemia, Reye-like episodes, and sudden unexpected death. In the prescreening era, approximately 5% of "sudden infant death syndrome" cases were thought to be caused by MCAD. The high prevalence of the disease, the simple treatment, and the reliable MS/MS-based-acylcarnitine analysis make MCAD a model screening disease. Parental awareness that the child is affected and ensuring that the child avoids fasting are effective in the prevention of any sequelae of the disease. In the past, a timely diagnosis was impossible to achieve because of the lack of initial symptoms, and thereafter because of often non-informative metabolic routine analysis during compensated state. Since MS/MS became available, diagnosis, even in the compensated state, is achievable.

Screening Method MS/MS.

Key Metabolite C8 (octanoyl carnitine), ratios C8/C2, C8/C10, C8/C12.

Differential Diagnosis Multiple acyl-CoA dehydrogenase deficiency.

What to Do If Test is Positive Immediately contact the family to ascertain the baby's clinical status. If any concern (ie, poor feeding, vomiting, lethargy) immediately initiate emergency treatment with IV glucose and transport to a hospital, preferably a metabolic center. Asymptomatic infants do not require emergency follow-up but should be evaluated and counseled by a metabolic specialist within days.

Confirmatory Studies Acylcarnitine profile in DBS/blood, free and total carnitine in blood, organic (dicarboxylic) acids and acylglycines in urine, enzyme studies in lymphocytes/fibroblasts, molecular genetic analysis.

Pitfalls/Attention Carnitine deficiency may cause a noninformative acylcarnitine profile. Maternal MCAD can be uncovered by newborn screening of offspring.[90-91]

Prevalence 1:10,000 (Germany), 15,000 (US), 1:20,000 (Australia).[72-75]

Very Long-Chain Acyl-CoA Dehydrogenase Deficiency (VLCAD)

VLCAD is an inborn error of mitochondrial fatty acid β-oxidation causing hypoglycemia, sudden death, cardiomyopathy, liver disease, and later on recurrent rhabdomyolysis (see Chapter 9). Diagnosis is feasible by MS/MS-based acylcarnitine analysis; however, key metabolites are often only slightly increased and may become normal after a carbohydrate-rich meal.

Screening Method MS/MS.

Key Metabolites C14:1 (myristoleyl-), C14 (myristoyl carnitine).

Differential Diagnosis Multiple acyl-CoA dehydrogenase deficiency.

What to Do If Test is Positive Immediately contact the family to ascertain the baby's clinical status. If any clinical abnormalities are reported (ie, poor feeding, vomiting, lethargy) immediately initiate emergency treatment with IV glucose and transport to a hospital, preferably a metabolic center. Asymptomatic infants with mildly abnormal screening results do not require emergency follow-up but should be evaluated and counseled by a metabolic specialist within days.

Confirmatory Studies Acylcarnitine profile in DBS/blood collected prior to a regular meal, free and total carnitine in blood, serum creatine kinase, organic acids in urine (elevated dicarboxylic acids), enzyme studies in lymphocytes/fibroblasts-based acylcarnitine analysis, molecular genetic analysis.

Pitfalls/Attention When infant is carnitine deficient or well fed, acylcarnitine profile may be uninformative, particularly in the milder variants of the disease. Recall testing in milder enzyme deficiency may produce false negative results and should therefore be avoided. More appropriate follow up includes enzyme assay or molecular genetic analysis of the VLCAD gene.[92]

Prevalence 1:85,000.[93]

Carnitine Uptake Deficiency (CUD)

Deficiency of the plasmalemmal carnitine transporter leads to carnitine uptake deficiency (CUD, see Chapter 9). The defect can present in infancy with Reye-like liver failure or hypoglycemia, in childhood with cardiomyopathy, and later on with muscle pain or recurrent rhabdomyolysis. Treatment with carnitine supplementation prevents these symptoms. Diagnosis can be achieved by assessing the fractional tubular reabsorption of free carnitine (normal >98%). Since introduction of newborn screening for CUD many mothers have been detected and confirmed to have CUD or other inherited conditions associated with secondary carnitine deficiency, some of them being asymptomatic.[90,94,95]

Screening Method MS/MS.

Key Metabolites C0 (free carnitine).

Differential Diagnosis CPT II, CACT, organic acidurias (secondary carnitine deficiency).

What to Do If Test is Positive Contact family immediately about result and report findings to newborn screening program. Consult with metabolic specialist to initiate confirmatory diagnostic testing.

Confirmatory Studies Total and free carnitine in plasma and urine, determine fractional tubular reabsorption of free carnitine (in mother and child), carnitine uptake studies in fibroblasts, molecular genetic analysis.

Pitfalls/Attention Carnitine supplementation during pregnancy can lead to false-negative newborn screening result. Maternal CUD and other inherited conditions associated with secondary carnitine deficiency can be uncovered by newborn screening of offspring.[90,94,95]

Prevalence 1:77,000.

Other Disorders of Mitochondrial Fatty Acid β-Oxidation and the Carnitine Cycle

This group comprises conditions such as short-chain acyl-CoA dehydrogenase (SCAD), medium/short-chain and long-chain hydroxyl-CoA dehydrogenases (M/SCHAD, LCHAD), multiple acyl-CoA dehydrogenase or glutaric aciduria type II (MADD or GA-II), and carnitine cycle defects (see Chapter 9), for which a final assessment on screening properties is not yet available. Most of these have been included among the primary newborn screening targets recommended in the United States, others as secondary targets. Inclusion into screening panels, however, is not universal. Long-chain 3-OH-acyl-CoA dehydrogenase (LCHAD) deficiency and complete mitochondrial trifunctional protein (TFP) deficiency are β-oxidation defects that can be screened with high sensitivity and specificity. Carnitine-palmitoyl transferase I (CPT1) deficiency, carnitine-palmitoyl transferase II (CPT2) deficiency, and carnitine-acylcarnitine-translocase deficiency (CACT) represent inborn errors in the carnitine cycle. CPT1 is the only condition in which free carnitine is elevated, whereas it is severely reduced in carnitine uptake defect (CUD). CPT2 and CACT are indistinguishable by acylcarnitine analysis and outcome for CPT2 deficiency appears promising after early initiation of treatment afforded by newborn screening. A benefit of early detection of CPT1, CACT, and glutaric aciduria type II deficiencies may be limited to early counseling of the affected family while successful treatment of the infant is not yet available. SCAD deficiency is of special concern as some regard it as a serious and treatable condition while others consider it merely a biochemical variant.

Screening Method MS/MS.

Key Metabolites (See Newborn Screening At-A-Glance).

What to Do If Test is Positive Contact family immediately about result and report findings to newborn screening program. Consult with metabolic specialist.

Confirmatory Studies Acylcarnitine profile in DBS/blood, free and total carnitine in blood (CPT1), serum creatine kinase, organic (dicarboxylic or hydroxy-dicarboxylic acids) acids in urine, urine acylcarnitines and acylglycines, enzyme studies in lymphocytes/fibroblasts, molecular genetic analysis.

Pitfalls/Attention Carnitine deficiency may cause a noninformative acylcarnitine profile (LCHAD, CPT2, CACT); false-positive results can be caused by fat infusion (LCHAD), carnitine supplementation in premature infants (CPT1), premature infants with special formula and screened after the first week of life[72,74,75] (CPT2, CACT).

Combined Prevalence App. 1:20,000.[72-75,93]

Isovaleric Aciduria (IVA)

IVA is a disorder of leucine degradation that is caused by deficiency of the enzyme isovaleryl-CoA dehydrogenase (see Chapter 14). The disease is characterized clinically by severe metabolic encephalopathy presenting mostly in the neonatal period with acute, overwhelming illness, vomiting, characteristic odor of "sweaty feet," seizures, coma, intraventricular/cerebellar hemorrhage, and hyperammonemia. Fifty percent of patients die during the first episode of decompensation. Experience with affected newborns since implementation of IVA screening indicates a favorable outcome with early diagnosis and treatment. Patients with a mild, possibly benign phenotype are also being identified by newborn screening, which has led to the identification of affected but asymptomatic family members.[30]

Screening Method MS/MS.

Key Metabolite C5 (isovalerylcarnitine).

Differential Diagnosis 2-methylbutyryl-CoA dehydrogenase (2-MBG or SBCAD), ethylmalonic encephalopathy (increase of C4 and C5).

What to Do If Test is Positive Immediately contact the family to ascertain the baby's clinical status, preferably by a pediatric examination. If any clinical abnormalities are

reported or observed (ie, poor feeding, vomiting, lethargy, tachypnea, odor of sweaty feet) immediately transport to a hospital, preferably a metabolic center. Asymptomatic infants with mildly abnormal screening results do not require emergency follow-up but should be evaluated and counseled by a metabolic specialist within days.

Confirmatory Studies Organic acids in urine, free and total carnitine in blood, enzyme studies in lymphocytes/fibroblasts, mutation analysis. Urine acylglycine and acylcarnitine analysis may also be informative.

Pitfalls/Attention Carnitine deficiency might cause an uninformative acylcarnitine profile; exposure to several medications can cause false-positive results.[32,33]

Prevalence 1:100,000.[72-75,78]

Glutaric Aciduria Type I (GA-I)

GA-I is a disorder of lysine and tryptophan metabolism caused by deficiency of the enzyme glutaryl-CoA dehydrogenase (GCDH) (see Chapter 14). An encephalitis-like decompensation followed by severe dystonic–dyskinetic movement disorder represents the most common presentation of infants with GA-I. After a presymptomatic period with only minor symptoms but frequently with progressive macrocephaly, the encephalopathic crisis occurs mostly before the first birthday, often triggered by febrile illness/immunizations. Screening and treatment may prevent this course. Newborn screening by MS/MS-based acylcarnitine analysis requires high analytical sensitivity because the key metabolite, glutarylcarnitine, in GA-I occurs physiologically in low concentrations and is often only slightly increased in patients. In a subgroup of children with GA-I that is characterized by very low excretion of the pathognomonic organic acids glutaric and 3-hydroxyglutaric acid, MS/MS newborn screening may even be negative.

Screening Method MS/MS.

Key Metabolite C5DC (glutarylcarnitine). Some laboratories are also using ratios to other acylcarnitines.

Differential Diagnosis GA-II/MADD.

What to Do If Test is Positive Contact family immediately about result and report findings to newborn screening program. Consult with metabolic specialist.

Confirmatory Studies A diagnostic algorithm has been recently optimized by an international expert group.[96] Most important are quantitative determinations of organic acids in urine (glutaric and 3-hydroxyglutaric acid). If strongly consistent with GA-I, treatment should be initiated immediately and molecular genetic analysis of the GCDH gene pursued. If the characteristic urine organic acid profile is not observed, exact quantification of 3-hydroxyglutaric acid should be performed by a sensitive stable isotope dilution method (if available). A normal excretion of 3-hydroxyglutaric acid will exclude the diagnosis of GA-I (false positive). Otherwise, treatment and molecular genetic confirmation should be initiated. If only one known disease-causing mutation or no mutation is detected, GCDH activity should be determined in isolated peripheral leukocytes or cultured fibroblasts. Low enzyme activity will confirm the diagnosis of GCDH deficiency whereas normal activity will exclude the diagnosis (false positive).

Pitfalls/Attention Carnitine deficiency might cause a uninformative acylcarnitine profile.

Prevalence 1:100,000.[72-75,78]

Propionic Aciduria (PA)

Among the organic acidurias, PA has the most severe clinical phenotype. PA is caused by deficiency of propionyl-CoA carboxylase, an enzyme in the metabolism of several amino acids and in the degradation of odd-chain fatty acids. Already in the first postnatal days, many affected newborns suffer from severe hyperammonemia requiring extracorporeal detoxification. Outcome is strongly connected with brain injury, which depends on duration, extent, and frequency of metabolic decompensation/hyperammonemia. Despite aggressive treatment, recurrent metabolic decompensation is not always avoidable. The potential benefit of newborn screening for PA depends on whether the positive result becomes available prior to the first decompensation and clinical diagnosis—sometimes not an achievable objective. Increased propionylcarnitine is a sensitive parameter for PA screening; however, it is not specific. Some newborns not suffering from an inborn error of metabolism have high propionylcarnitine levels of so far unknown origin. However, these cases can be excluded by application of first- or second-tier screening test for methylmalonic and methylcitric acid.[68]

Screening Method MS/MS.

Key Metabolite C3 (propionylcarnitine), C3/C2 and C3/C0 ratio, methylcitric acid.

Differential Diagnosis Methylmalonic aciduria (MMA), cobalamin (CBL) A, B, C, D, or F deficiencies; (maternal) vitamin B_{12} deficiency.

What to Do If Test is Positive Immediately contact the family to ascertain the baby's clinical status, preferably by a pediatric examination. If any clinical abnormalities are present (ie, poor feeding, vomiting, lethargy, tachypnea) immediately transport to a hospital, preferably a metabolic center. Asymptomatic patients with mildly abnormal screening results do not require emergency follow-up but should be evaluated and counseled by a metabolic specialist within days.

Confirmatory Studies Organic acids in urine, free and total carnitine in blood, plasma acylcarnitines, amino acids (methionine) and total homocysteine, blood ammonia, enzyme studies in fibroblasts, molecular genetic analysis.

Pitfalls/Attention Carnitine deficiency might cause an uninformative acylcarnitine profile; carnitine supplementation, sometimes applied in preterm babies, may cause a C3/C0 ratio that is not informative.

Prevalence 1:125,000 (Germany), 1:80,000 (US), <1:300,000 (Australia).[48,72-75,78]

Methylmalonic Aciduria (MMA) and Cobalamin Deficiencies (CblA, B, C, D, F)

MMA can be due to defects of the enzyme methylmalonyl-CoA mutase ($mut^{-/0}$) (see Chapter 14) or can be caused by defects in cobalamin (Cbl) metabolism (see Chapter 19). In the latter the disturbed synthesis of adenosylcobalamin, the cofactor of methylmalonyl-CoA mutase, leads to MMA. MMA can occur isolated ($mut^{-/0}$, CblA, CblB, CblD) or combined with homocystinuria (CblD, CblF). Maternal cobalamin deficiency can also result in MMA and homocystinuria.[97] Clinical manifestations of these diseases occur somewhat later than in propionic aciduria. Newborn screening is of benefit for affected individuals. False-positive propionylcarnitine elevations can be excluded by application of a second-tier screening test for methylmalonic acid and methylcitric acid.[68]

Screening Method MS/MS.

Key Metabolite C3 (propionylcarnitine), C3/C2 and C3/C0 ratio, methylcitric acid.

Differential Diagnosis PA, transcobalamin II deficiency, transcobalamin receptor deficiency, (maternal) vitamin B_{12} deficiency, succinyl-CoA synthetase (SUCLA2) deficiency.

What to Do If Test is Positive Immediately contact the family to ascertain the baby's clinical status, preferably by a pediatric

examination. If any concern (ie, poor feeding, vomiting, lethargy, tachypnea), immediately transport to a hospital, preferably a metabolic center. Asymptomatic infants with mildly abnormal screening results do not require emergency follow-up but should be evaluated and counseled by a metabolic specialist within days.

Confirmatory Studies Organic acids in urine, free and total carnitine in blood, acylcarnitines, amino acids (methionine) and total homocysteine in plasma, blood ammonia, enzyme studies in fibroblasts, molecular genetic analysis.

Pitfalls/Attention Carnitine deficiency might cause an uninformative acylcarnitine profile; carnitine supplementation, sometimes applied in preterm babies, may cause a C3/C0 ratio that is not informative.

Prevalence 1:125,000 (Germany), <1:300,000 (Australia).[48,72-75,78]

3-Methylcrotonyl-CoA Carboxylase Deficiency (3-MCC)

A deficiency of 3-MCC is caused by a defect in leucine degradation (see Chapter 14). Since introduction of screening for 3-MCC a great number of affected newborns have been found. Several concerns have to be addressed in screening for this condition. It appears that 3-MCC is a common, mostly benign condition. Whether treatment with a low-protein diet, carnitine, and glycine supplementation has the potential to change the clinical course in severely affected patients remains to be elucidated. No evidence has so far emerged of a benefit from presymptomatic treatment. Increases of C5OH carnitine are not specific for 3-MCC deficiency because it can represent either elevations of 3-hydroxyisovalerylcarnitine or 2-methyl-3-hydroxybutyrylcarnitine. Maternal 3-MCC was initially a surprising finding of MS/MS screening programs. Distinct increases in the baby's blood were caused by the mother who turned out to have this condition. Concerns were raised as to how to handle the mother because her 3-MCC deficiency did not always have apparent clinical consequences. In addition to confirmed cases, a substantial number of positive C5OH results persist during recalls, but cannot be assigned to an underlying disease.

Screening Method MS/MS.

Key Metabolite C5OH (3-hydroxyisovalerylcarnitine).

Differential Diagnosis Multiple carboxylase deficiency (including biotinidase and holocarboxylase deficiencies), HMG-CoA lyase deficiency, β-ketothiolase, 2-methyl-3-hydroxy butyric acidemia, 3-methylglutaconic aciduria type I, and maternal 3-MCC deficiency.

What to Do If Test is Positive Immediately contact the family to ascertain the baby's clinical status. If any concern (ie, poor feeding, vomiting, lethargy, tachypnea) immediately transport to a hospital, preferably a metabolic center. Asymptomatic infants with mildly abnormal screening results do not require emergency follow-up but should be evaluated and counseled by a metabolic specialist within days.

Confirmatory Studies Organic acids in urine, free and total carnitine in blood, blood ammonia, enzyme studies in leukocytes or fibroblasts, mutation analysis; urine organic acids and blood acylcarnitines in the mother.

Pitfalls/Attention Carnitine deficiency might cause a noninformative acylcarnitine profile.

Prevalence 1:40,000 (Germany, US), 1:120,000 (Australia).[72-75,78]

3-Hydroxy-3-Methylglutaryl-CoA (HMG-CoA) Lyase Deficiency

HMG-CoA lyase catalyzes the last step in leucine degradation and plays an important role in ketogenesis (see Chapter 10). Patients usually present with sudden hypoketotic hypoglycemia after an unremarkable development. Hypoglycemia combined with lack of ketones cause severe brain impairment. Preventing or quickly reversing catabolic states is efficient. Thus, HMG-CoA lyase deficiency represents a favorable screening disease; however, increases of the key metabolite C5OH may be small or even absent. Affected patients might therefore be missed by screening.

Screening Method MS/MS.

Key Metabolites C5OH (3-hydroxyisovalerylcarnitine), MeGlu (methylglutarylcarnitine).

Differential Diagnosis 3-MCC, multiple carboxylase deficiency (including biotinidase and holocarboxylase deficiencies), β-ketothiolase, 2-methyl-3-hydroxybutyric acidemia, 3-methylglutaconic aciduria type I, and maternal 3-MCC deficiency.

What to Do If Test is Positive Immediately contact the family to ascertain the baby's clinical status. If any concern (ie, poor feeding, vomiting, lethargy, tachypnea), immediately transport to a hospital, preferably a metabolic center. Asymptomatic infants with mildly abnormal screening results do not require emergency follow-up but should be evaluated and counseled by a metabolic specialist within days.

Confirmatory Studies Organic acids in urine, acylcarnitines in plasma, enzyme studies in fibroblasts, molecular genetic analysis.

Pitfalls/Attention Carnitine deficiency might cause a noninformative acylcarnitine profile. Affected, well-fed newborns may be missed.

Prevalence <1:200,000.[72-75,78]

Pompe Disease

Pompe disease (glycogen storage disease type II) is an autosomal recessive disorder caused by deficiency of the lysosomal enzyme acid α-glucosidase (GAA; see Chapter 5). Pompe disease causes muscle hypotonia, weakness, cardiomyopathy, and eventually leads to cardiorespiratory or respiratory failure and death. The phenotype is dependent on residual enzyme activity, with complete loss of activity causing infantile onset Pompe disease which is fatal within the first year of life. Milder variants are characterized by later onset and longer survival. Symptomatic patients have elevated muscle enzymes in blood (creatine kinase, transaminases, and lactate dehydrogenase) and increased urinary glucotetrasaccharides.[98] Enzyme replacement therapy is beneficial and enzyme assay analysis from dried blood spots is available. Therefore, Pompe disease was included in the United States in the RUSP in 2015.[57]

Screening Method MS/MS or fluorometry.

Differential Diagnosis GAA pseudodeficiency.

What to Do If Test is Positive Contact the family to inform them of the newborn screening result and evaluate the newborn with attention to hypotonia, feeding difficulties, and clinical evidence of heart disease. If clinical symptoms are present immediately transport to a hospital with metabolic expertise. If asymptomatic refer to metabolic specialist.

Confirmatory Studies α-Glucosidase enzyme assay in DBS or leukocytes, creatine kinase in serum, electrocardiogram and echocardiogram to determine presence of cardiomyopathy; molecular genetic analysis.

Pitfalls/Attention Several pseudodeficiency alleles cause low in vitro enzyme activity in otherwise healthy individuals.