Review

Journal of Inborn Errors of Metabolism & Screening
2018, Volume 6: 1–6
© The Author(s) 2018
DOI: 10.1177/2326409818792065
journals.sagepub.com/home/iem
SAGE

High-Risk Screening and Diagnosis of Inborn Errors of Metabolism: A Practical Guide for Laboratories

Gabriel Civallero, PhD[1,2], Raquel de Kremer, MD, PhD[3], and Roberto Giugliani, MD, PhD[1,2]

Abstract

Inborn errors of metabolism (IEM) are a large and heterogeneous group of genetic diseases. In most of these conditions, the presence of variants in specific genes leads to enzyme deficiencies that affect a particular metabolic step. The number of laboratories dedicated to the study of IEM is very limited worldwide, and its multiplication is urgently required for a more effective diagnosis. With the scarcity of specialized centers, the diagnosis of affected individuals comes too late or does not happen at all. Moreover, the biological samples have to travel long distances, compromising its quality and delaying still more the diagnosis. In this work, we suggest a practical guide for a basic biochemical laboratory to get involved in the study of IEM. This proposal was based on already described metabolic tests and involves the need of just a few, simple, and affordable instruments that can give an enormous quantity of information about the possible metabolic defect faced, such as a spectrophotofluorometer and a gas chromatography/mass spectrometry (GC/MS) instrument. The procedures proposed can be customized and adapted to particular needs and situations, which make it especially useful for developing countries.

Keywords

inborn errors of metabolism, biochemical diagnosis, gas chromatography/mass spectrometry, spectrophotofluorometry, screening

Introduction

Inborn errors of metabolism (IEM) are a large and heterogeneous group of genetic diseases. In most of these conditions, the presence of variants in specific genes leads to enzyme deficiencies that affect a particular metabolic step. Depending on the metabolic error involved, the clinical presentation can be severe or lethal with neurodegenerative process, skeletal abnormalities, and/or physiologic dysfunctions.[1]

The global incidence of each particular IEM is low, but considered all together they represent more than 1 affected individual each 1000 births. The occurrence of each disease can be significantly different in particular regions. Based on clinical suspicions, the biochemical study of IEM generally begins with qualitative or quantitative tests involving metabolites related to sugars, organic acids, amino acids, sterols, fatty acids, complex lipids, porphyirins, pterines, purines and pyrimidines, and so on. These studies can be definitive or presumptive to define a specific IEM, and they are complemented with enzyme assays and/or molecular genetic testing.[2,3]

The analytical technology used by sophisticated laboratories for the diagnosis of IEM mainly includes gas chromatography/mass spectrometry (GC/MS), tandem mass spectrometry (MS/MS), amino acid analyzer, high-pressure liquid chromatography (HPLC), thin-layer chromatography, electrophoresis, and specific spectrophotometric or fluorometric enzyme assays.

In the last few years, an important strategy for early identification of IEM was initiated with the study of acylcarnitines and

[1] Medical Genetics Service, HCPA, Porto Alegre, Brazil
[2] Department of Genetics, UFRGS, Porto Alegre, Brazil
[3] Centro de Estudios de las Metabolopatías Congénitas, CEMECO, Facultad de Ciencias Médicas, Universidad Nacional de Córdoba, Argentina

Received May 24, 2018, and in revised form July 4, 2018. Accepted for publication July 9, 2018.

Corresponding Author:
Gabriel Civallero, PhD, Medical Genetics Service, Hospital de Clinicas de Porto Alegre, Rua Ramiro Barcelos 2350, Porto Alegre, RS 90035-903, Brazil.
Email: gecivallero@yahoo.com

amino acids in newborn infants. With this simple analysis, made in dried blood spot (DBS) samples, it is possible to prevent the death or severe damage in affected babies, allowing immediate complementary studies to define the diagnosis and begin due therapy. Year after year, new countries or regions are joining to this scheme and also new metabolites are added to the screening.[4-6] The incorporation of DBS samples was also extended to perform diverse biochemical assays, especially focused on the study of lysosomal storage diseases (LSDs). The use of this type of samples facilitates its transportation from remote areas to specialized centers and could be extended to other metabolite studies, since their effectiveness was truly demonstrated.[7-20]

Despite these advances, an important deficiency is still evident. The number of laboratories dedicated to the study of IEM is very limited worldwide, and more laboratories performing IEM screening and diagnosis are required for a more efficient detection and therapy of patients. In the absence of specialized centers, the affected individuals are frequently undiagnosed, or their identification comes too late. Moreover, the biological samples have to travel long distances to reach a suitable laboratory, being exposed to deterioration during shipment and delaying still more the diagnosis.

Here, we present a practical guide for a basic biochemical laboratory to become involved in the study of IEM. This proposal was based on already described metabolic tests involving a few, simple, and affordable instruments that can give an enormous quantity of information about the possible metabolic defect faced, such as a spectrophotofluorometer and a GC/MS system. The next section describes the scope of tests that can be performed and the possible or definitive IEM involved.

Basic Biochemical Laboratory for IEM Study

Detailed biochemical procedures and its limitations can be found in the references cited in this section.

Screening Tests

Screening tests can be performed with a reactive strip or simple qualitative procedures as indicated subsequently. Despite being less used in the present days, these preliminary tests can be useful to guide the metabolic study, especially in emergency situations.[2,3]

Reactive Strips. Different reagent strips are commercially available for quick and qualitative analysis of several intermediates in urine, including protein, blood, leukocytes, nitrite, glucose, ketones (acetoacetic acid), pH, specific gravity, creatinine, bilirubin, and urobilinogen. Other strips allow the quick determination of reducing substances (glucose, lactose, fructose, galactose, pentoses, sulfite, etc). The interpretation of the results can be made by visual observation.

Qualitative tests. Complementing the reactive strip tests, there are simple qualitative assays that could bring useful information about the possible metabolic error present. The interpretation of the results can be made with simple sight, except the Ehrlich test and Bratton-Marshall reaction where a spectrophotometer is required.[2,3] The most common tests are as follows:

- Benedict test for reducing substances,
- 2,4-Dinitrophenylhydrazine) for α-keto acids,
- Nitroprusside test for cystine and homocystine,
- Nitrosonaphthol test for tyrosine metabolites,
- Ferric chloride test for PKU, Histidemia, MSUD, Tyrosinemia, Alkaptonuria, etc,
- Ehrlich test for porphyrins,
- Urine thiosulfate determination for defect of sulfite metabolism,
- Toluidine blue spot test for glycosaminoglycans (GAGs),
- Electrophoresis and thin-layer chromatography (TLC) of GAGs, oligosaccharides, sialyloligosaccharides, and
- Bratton-Marshall reaction.[21]

Spectrophotofluorometric Studies

These studies aim to determine total concentration of specific metabolites and to measure specific enzyme activities.[2,3,7-18,22-24] Chitotriosidase activity can be used for screening, as it is increased in plasma and DBS in several LSDs. Creatinine and protein determinations are generally performed as parameters to normalize other biochemical assays that refer its results to creatinine or protein concentrations, such as organic acids, GAGs, sialic acid, enzyme assays in tissues or leucocytes, and so on. Creatinine concentration is also an important parameter for the investigation of defects in the creatine metabolism. A summary of the most common spectrophotofluorometric measurements is presented in Table 1.

Gas Chromatography and MS

Organic acid assay (total ion chromatogram, TIC). This is one of the most informative assays that can be easily performed to investigate many IEM. Through a simple organic acid profile, it is possible to identify particular products related to a specific enzyme deficiency or to visualize "flags" that would help guide new studies. It is important to note that the organic acid profiles can be different in clinically sick and asymptomatic patients. As the number of new pathologies is growing fast, new key metabolites are being identified. Thus, it is not unusual reviewing old profiles, even those obtained years before. The organic acid profile also offers interesting information about enriched diet, drug intake, bacterial or plastic contamination, and so on.[2,3,25-28] A list of IEM that can be studied by organic assays is presented in Table 2.

Additional specific organic acid detection by GC and MS (simple ion monitoring, SIM). The extracted organic acids can also be analyzed by programming the mass spectrometer to detect only one or few isolated ions, allowing the identification and

Table 1. Details of Spectrophotoflurometric Assays and the IEM Studied.

Spectrophotoflurometric Assays	IEM
Carbohydrates and glycogen	
Total galactose test (P); Total fructose test (P);	Galactosemia
Total glycogen test and glycogen structure assay (P)[2,3]	Hereditary fructose intolerance
Enzyme assays[3]	Glycogenosis type Ia, II, IV, V, VI, VII, and IX
Galactose-1-phosphate, UDP-galactose-4-epimerase (P); fructose-bisphosphate aldolase, fructose-1,6-bisphosphatase (P); glucose-6-phosphatase (P); α-glucosidase (P); 1.4-α-glucan branching enzyme (P); phosphorylase (P); 6-hosphofructokinase (P); phosphorylase b-kinase (P)	
Pyruvate and ketone bodies	
Total Lactate, Pyruvate, and Ketone bodies tests (P)[2,3]	Pyruvate metabolism defects
Porphyrins	
Porphobilinogen test (P)[2,3]	Porphyrin metabolism defects
Pterins	
Dihydropteridine reductase assay (P)[2,3]	Pterin metabolism defects
Purine	
Bratton-Marshall reaction (P)[21]	Purine metabolism defect
Biotin	
Biotinidase enzyme assay (P)[2,3]	Biotinidase Deficiency
Lysosomal storage diseases (GAGs)	
Total urinary glycosaminoglycans (GAGs; P); plasma and urine free and total sialic acid test (P)[2,3,19,20]	Mucopolysaccharidoses (MPSs) Sialidosis, Galactosialidosis
Lysosomal Enzyme assays[2,3,7-18,22,24] β-galactosidose (F); β-glucosaminidase A (F); β-glucosaminidase A/ B (F); Arylsulfatase A (P); Galactocerebrosidase (F); α-galactosidose (F); β-glucosidase (F); Chitotriosidase (F); α-L-iduronidase (F); Iduro-2-sulfatase (F); Heparan-N-sulfatase (F); α-N-acetylglucosaminidase (F); Acetyl CoA:α-glucosaminide-N-acetyltransferase (F); N-acetylglucosamine-6-sulfate (F); N-acetylgalactosamine-6-sulfate (F); Arylsulatase B (P); β-glucuronidase (F); α-L-fucosidase (F); α-Mannosidase (F); β-Mannosidase (F); α-neuraminidase (F); Lipase (F); Ceramidase (F); α-N-acetylgalactosaminidase (F)	GM1 gangliosidoses; Mucolipidosis II/ III; B variant Tay-Sachs, B1 variant, Sandhoff disease; Metachromatic leukodystrophy; Multiple sulfatase Deficiency; Krabbe's disease; Fabry's disease; Gaucher's disease; Niemann-Pick disease types A/B/C; MPS types I, II, III-A, III-B, III-C, III-D, IV-A, VI, VII; Fucosidosis; α-Mannosidase; β-Mannosidase; Sialidosis; Galactosialidosis; Wolman disease. Cholesterol ester storage disease; Farber disease; Shindler Disease

Abbreviations: F, fluorometric assay; IEM, Inborn errors of metabolism; P, spectrophotometric assay.

quantification of metabolites at very low concentrations. The most important ions checked are usually succinylacetone (tyrosinemia type 1), orotic acid (urea cycle disorders), guanidinoacetoacetate (defects of creatine metabolism), methylmalonic acid (methylmalonic acidemia and disorder of cobalamin metabolism), mevalonic acid (mevalonic aciduria), and acetyl-N-aspartate (Canavan disease).[29-34]

Very long-chain fatty acids assay. Saturated and nonsaturated fatty acids and plasmalogens can be easily methylated and analyzed by GC/MS. The range of fatty acids to be screened include acids with medium-, long-, and very long-chain plasmalogens, along with pristanate and phytanate.[2,3] The fatty acid profile can give important information about possible peroxisomal disorder as described in Table 2.

Sterol profile assay. Sterols can be separated and identified by GC-MS (TIC and SIM modes). The range of pathologies studied is detailed subsequently. Similar to the organic acid profile, many "unknown metabolites" could be the future markers for novel enzyme deficiencies identified in the future, in the pre- or postsqualene steps of cholesterol synthesis.[2,3,35,36] A list of IEM that can be studied by the sterol profile is presented in Table 2.

Minimal Requirements for the Basic IEM Laboratory

Human Resources

Basically, the laboratory should count with qualified technicians for sample preparation, performance of the assays, and instrument operation. Inclusion of microtechniques with the use of multiwheel plate systems and/or microfluid-based enzyme assays[22] is recommended. Autosamplers for GC/MS or other robotic implements are also helpful. This means an enormous saving of expensive artificial substrates, buffers, reading times, and so on. Well-organized laboratories are more efficient, saving time, human effort, and chemical products.

Table 2. Details of GC/MS assays and the IEM studied.

GC/MS ASSAYS	IEM
Urine organic acid assay	Defects of aromatic amino acid metabolism
GC/MS, TIC[2,3,25–28]	Phenylketonuria, tyrosinemia, hawkinsinuria, alkaptonuria
GC/MS, SIM[29–34]	Defects of branched chain amino acids metabolism
	Maple urine syrup disease, dihydrolipoyl dehydrogenase (E3) deficiency, isovaleric acidemia, 3-methylcrotonyl-CoA carboxilase deficiency, multiple carboxylase deficiency, 3-methylglutaconic aciduria, 3-OH-3-methylglutaric aciduria, 3-oxothiolase deficiency, propionic acidemia, methylmalonic acidemia, malonyl-CoA decarboxylase deficiency
	Defects of dibasic amino acids metabolism
	Glutaric aciduria type I, 2-Ketoadipic aciduria
	Defects of fatty acid oxidation/pyruvate/Krebbs cycle/ mitochondrial respiratory chain
	Long-, medium-, and short-chain acyl-coA dehydrogenase deficiency, multiple acyl-CoA dehydrogenase deficiency
	Lactic acidemia (pyruvate carboxylase deficiency, pyruvate dehydrogenase deficiency, multiple carboxylase deficiency, defects in oxidative phosphorylation), 2-ketoglutarate dehydrogenase deficiency, fumarase deficiency, Barth syndrome
	Other organic acidurias:
	5-Oxo-prolinuria, D-2-OH-glutaric aciduria, 4-OH-butiric aciduria, Canavan disease, mevalonic aciduria, n-glyceric aciduria, glyceroluria, hyperoxaluria, ketosis, Reye's syndrome, thymine and uracil acidurias. Urea cycle disorders and defects of creatine metabolism
Plasma very long-chain fatty acid assay GC/MS, TIC/SIM[2,3]	X-ALD, defects of phytanic/pristanic acid metabolism, infantile Refsum disease, RCDP, Zellweger and Zellweger-like syndromes, dihydroxyacetone phosphate acyltransferase deficiency
Plasma Sterol profile assay GC/MS, TIC/SIM[2,3,35,36]	Mevalonate kinase deficiency, CHILD syndrome, CDPX2. Greenberg dysplasia, Smith-Lemli-Opitz syndrome, lathosterolosis, desmosterolosis, sitosterolemia, CTX.

Abbreviations: CDPX2, X linked dominant Conradi-Hünermann chondrodysplasia; CTX, cerebrotendineos xanthomatosis; GC/MS, gas chromatography/mass spectrometry system; IEM, inborn errors of metabolism; RCDP, rhizomelic chondrodysplasia punctata; SIM, simple ion chromatogram mode; TIC, total ion chromatogram mode; X-ALD, X linked adrenoleukodystrphy.

The technicians should be backed by professionals who have a background in inherited metabolic diseases to evaluate the results and to take the appropriate actions to confirm positive cases and direct the patients to quick management and therapy.

For the assessment of laboratory performance, it is very important to adhere to international schemes of quality control, such as ERNDIM, CDC, and others.

Installations and Instruments

The laboratory should have an appropriate size (around 100 m^2 is recommended), with adequate ventilation and temperature control. This last point is especially important due to the sensitive instrumentation required. Work benches, hermetic hood for manipulation of toxic gases, nitrogen and helium tanks, and a safe system for reagent storage and for discarding dangerous substances are also necessary.

A spectrophotoflurometer and a GC/MS are the major instruments required for the basic IEM laboratory. It is important to subscribe to maintenance programs. The first instrument is a single machine with UV-visible and fluorescence detectors. In the case that a spectrophotometer is already available, only a spectrofluorometer needs to be added.

Minor instruments also are required, and these are similar to the ones available in a basic biochemical laboratory, including vortex, pH meter, digital balance, water bath, plate incubator, TLC and electrophoresis systems, and so on.

Final Considerations

Figure 1 shows a flowchart for the biochemical study of IEM. It is clear that a vast number of IEM can be explored with this basic laboratory. Evidently, not all IEM will be covered, but the most important areas will be studied. The relatively low cost of the major equipment required to run this laboratory makes them quite affordable, especially when compared to the high cost of an MS/MS or other sophisticated equipment. This is essentially important in countries or regions where there is a lack of specialized centers to perform these studies. Once the basic laboratory is running, the range of the pathologies investigated can be expanded, incorporating new equipment, such as an amino acid analyzer, HPLC, MS/MS, and instruments for molecular genetic tests and for cell culture. Local or regional incidence of IEM and the availability of therapies should be considered for such expansion. If the resources are very limited, a GC/MS instrument should be the first option because of the large scope of IEM that can be studied through a simple chromatography profiling of organic acids, very long chain fatty acids, and sterols.

Finally, we should emphasize that the IEM are "rare diseases" when considered individually, but the entire group has a

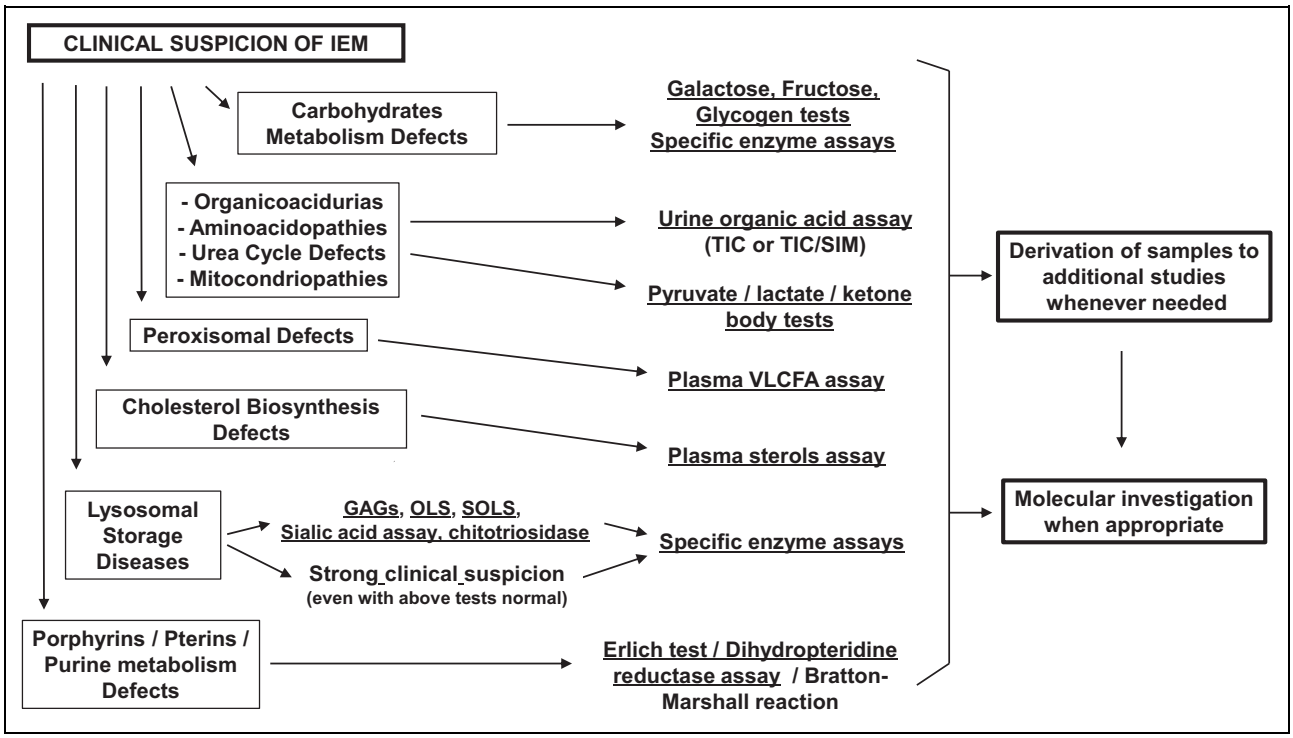

Figure 1. Proposed flow chart for the biochemical study of IEM.

frequency higher than 1 per 1000 births. Moreover, the number of patients detected and the definition of new IEM are continually expanding worldwide.[37-40] Many centers dedicated to the study of IEM are offering regular courses and training to physicians, biochemists, biologists, and related professionals, helping to extend the knowledge of these diseases and to encourage the health authorities and other international institutions to take appropriate actions to address these diseases.

Acknowledgments

This review was possible due to the continuous help and support provided along for many years by professionals from: (1) the Center for Study of Inherited Metabolic Diseases (CEMECO), Cordoba, Argentina; (2) Hugo and Ann Moser, Richard Kelley, Lisa Kratz, and professionals from the Kennedy Krieger Institute, Baltimore, USA; (3) professionals from the Medical Genetics Service of the Hospital de Clínicas de Porto Alegre, Brazil.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

References

1. Scriver CR, Beaudet AL, Sly WS. *Metabolic and Molecular Basis of Inherited Disease, Eighth ed*. New York: McGraw Hill; 2001.
2. Hommes FA. *Techniques in Diagnostic Human Biochemical Genetics*. New York, NY: Wiley-Liss John Wiley & son; 1991.
3. Blau N, Duran M, Gibson KM. *Laboratory Guide to the Methods in Biochemical Genetics*. Heidelberg, Germany: Springer-Verlag Berlin; 2008.
4. Chace DH, Kalas TA. A biochemical perspective on the use of tandem mass spectrometry for newborn screening and clinical testing. *Clin Biochem*. 2005;38(4):296-309.
5. Haynes CA, De Jesús VR. Simultaneous quantitation of hexacosanoyl lysophosphatidylcholine, amino acids, acylcarnitines, and succinylacetone during FIA–ESI–MS/MS analysis of dried blood spot extracts for newborn screening. *Clin Chem*. 2016; 49(1-2):161-165.
6. Tortorelli S, Turgeon CT, Gavrilov DK, et al. Simultaneous testing for 6 lysosomal storage disorders and X-adrenoleukodystrophy in dried blood spots by tandem mass spectrometry. *Clin Chem*. 2016; 62(9):1248-1254.
7. Chamoles NA, Blanco MB, Gaggioli D, Casentini C. Hurler-like phenotype: enzymatic diagnosis in dried blood spots on filter paper. *Clin Chem*. 2001;47(12):2098-2102.
8. Chamoles NA, Blanco M, Gaggioli D, Casentini C. Gaucher and Niemann-Pick diseases-enzymatic diagnosis in dried blood spots on filter paper: retrospective diagnoses in newborn-screening cards. *Clin Chim Acta*. 2002;317(1-2):191-197.
9. Civallero G, Michelin K, de Mari J, et al. Twelve different enzyme assays on dried-blood filter paper samples to detection of patients with selected inherited lysosomal storage diseases. *Clin Chim Acta*. 2006;372(1-2):98-102.
10. Camelier MV, Burin MG, De Mari J, Vieira TA, Marasca G, Giugliani R. Practical and reliable enzyme test for the detection

of Mucopolysaccharidosis IVA (Morquio Syndrome type A) in dried blood samples. *Clin Chim Acta*. 2011;412(19-20): 1805-1808.
11. Oemardien LF, Boer AM, Ruijter GJ, et al. Hemoglobin precipitation greatly improves 4-methylumbelliferone-based diagnostic assays for lysosomal storage diseases in dried blood spots. *Mol Genet Metab*. 2011;102(1):44-48.
12. Tolun AA, Graham C, Shi Q, et al. A novel fluorometric enzyme analysis method for Hunter syndrome using dried blood spots. *Mol Genet Metab*. 2012;105(3):519-521.
13. Ullal AJ, Millington DS, Bali DS. Development of a fluorometric microtiter plate based enzyme assay for MPS IVA (Morquio type A) using dried blood spots. *Mol Genet Metab Rep*. 2014;22(1):461-464.
14. Hamilton J, Jones I, Srivastava R, Galloway P. A new method for the measurement of lysosomal acid lipase in dried blood spots using the inhibitor Lalistat 2. *Clin Chim Acta*. 2012;413(15-16): 1207-1210.
15. Civallero G, De Mari J, Bittar C, Burin M, Giugliani R. Extended use of a selective inhibitor of acid lipase for the diagnosis of Wolman disease and cholesteryl ester storage disease. *Gene*. 2014;539(1):154-156.
16. Civallero G, De Mari J, Viapiana Camelier M, Burin M, Giugliani R. Assay of heparan-N-sulfamidase in dried leukocytes impregnated in filter paper: a new tool for the identification of mucopolysaccharidosis IIIA and potentially other lysosomal diseases. *Mol Gen Metab*. 2013;108(4):267-268.
17. Camelier M, Civallero G, De Mari J, Burin M, Giugliani R. Galactocerebrosidase assay on dried-leukocytes impregnated in filter paper for the detection of Krabbe disease. *Clin Chim Acta*. 2015;438:178-180.
18. Camelier M, De Mari J, Burin M, Civallero G, Giugliani R. Extended use of dried-leukocytes impregnated in filter paper samples for detection of Pompe, Gaucher, and Morquio A diseases. *Clin Chim Acta*. 2015;446:218-220.
19. Whitley CB, Draper KA, Dutton CM, Brown PA, Severson SL, France LA. Diagnostic test for mucopolysaccharidosis. II. Rapid quantification of glycosaminoglycan in urine samples collected on a paper matrix. *Clin Chem*. 1989;35(10):2074-2081.
20. Civallero G, Bender F, Gomes A, et al. Reliable detection of mucopolysacchariduria in dried-urine filter paper samples. *Clin Chim Acta*. 2013;415:334-336.
21. Laikind PK, Seegmiller JE, Gruber HE. Detection of 5'-phosphoribosyl-4-(N-succinylcarboxamide)-5-aminoimidazole in urine by use of the Bratton-Marshall reaction: identification of patients deficient in adenylosuccinate lyase activity. *Anal Biochem*. 1986;156(1):81-90.
22. Sista RA, Wang T, Wu N, et al. Multiplex newborn screening for Pompe, Fabry, Hunter, Gaucher, and Hurler diseases using a digital microfluidic platform. *Clin Chim Acta*. 2013;424:12-18.
23. Bedia C, Camacho L, Abad JL, Fabriàs G, Levade T. A simple fluorogenic method for determination of acid ceramidase activity and diagnosis of Farber disease. *J Lipid Res*. 2010;51(12): 3542-3547.
24. Vines DJ, Warburton MJ. Classical late infantile neuronal ceroid lipofuscinosis fibroblasts are deficient in lysosomal tripeptidyl peptidase I. *FEBS Lett*. 1999;443(2):131-135.
25. Tanaka K, West-Dull A, Hine DG, Lynn TB, Lowe T. Gas-chromatographic method of analysis for urinary organic acids. II. Description of the procedure, and its application to diagnosis of patient with organic acidurias. *Clin Chem*. 1980;26(13):1847-1853.
26. Tanaka K, Hine DG, West-Dull A, Lynn TB. Gas-chromatographic methods of analysis for urinary organic acids. I- Retention indices of 155 metabolically important compounds. *Clin Chem*. 1980;26(13):1839-1846.
27. Chalmers RA, Lawson AM. *Organic Acids in Man: Analytical Chemistry, Biochemistry and Diagnosis of the Organic Acidurias*. London, UK: Chapman and Hall; 1982.
28. Thompson JA, Miles BS, Fennessey PV. Urinary organic acids quantitated by age groups in a healthy pediatric population. *Clin Chem*. 1977;23(9):1734-1738.
29. Cyr D, Giguère R, Villain G, Lemieux B, Drouin R. A GC/MS validated method for the nanomolar range determination of succinylacetone in amniotic fluid and plasma: an analytical tool for tyrosinemia type I. *J Chromatogr B Analyt Technol Biomed Life Sci*. 2006;832(1):24-29.
30. McCann MT, Thompson MM, Gueron IC, Tuchman M. Quantification of orotic acid in dried filter-paper urine samples by stable isotope dilution. *Clin Chem*. 1995;41(5):739-743.
31. Struys EA, Jansen EE, ten Brink HJ, Verhoeven NM, van der Knaap MS, Jakobs C. An accurate stable isotope dilution gas chromatographic-mass spectrometric approach to the diagnosis of guanidinoacetate methyltransferase deficiency. *J Pharm Biomed Anal*. 1998;18(4-5):659-665.
32. Straczek J, Felden F, Dousset B, Gueant JL, Belleville F. Quantification of methylmalonic acid in serum measured by gas chromatography-mass spectrometry as tert.-butyldimethylsilyl derivatives. *J Chromatogr*. 1993;620(1):1-7.
33. Lindenthal B, von Bergmann K. Determination of urinary mevalonic acid using isotope dilution technique. *Biol Mass Spectrom*. 1994;23(7):445-450.
34. Kelley RI, Stamas JN. Quantification of N-acetyl-L-aspartic acid in urine by isotope-dilution gas chromatography-mass spectrometry. *J Inher Metab Dis*. 1991;15(1):97-104.
35. Kelley RI. Diagnosis of Smith-Lemli-Opitz syndrome by gas chromatography/mass spectrometry of 7-dehydrocholesterol in plasma, amniotic fluid and cultured skin fibroblasts. *Clin Chim Acta*. 1995;236(1):45-58.
36. He M, Kratz LE, Michel JJ, et al. Mutations in the human SC4MOL gene encoding a methyl sterol oxidase cause psoriasiform dermatitis, microcephaly, and developmental delay. *J Clin Invest*. 2010;121(3):976-984.
37. Ferreira CR, Gahl WA. Lysosomal storage diseases. *Transl Sci Rare Dis*. 2017;2(1-2):1-71.
38. Gilbert-Barness E, Farrell PM. Approach to diagnosis of metabolic diseases. *Transl Sci Rare Dis*. 2016;1(1):3-22.
39. Wanders RJ, Waterham HR, Ferdinandusse S. Metabolic interplay between peroxisomes and other subcellular organelles including mitochondria and the endoplasmic reticulum. *Front Cell Dev Biol*. 2016;3:83. https://doi.org/10.3389/fcell.2015.00083.
40. Corso G, Dello Russo A, Gelzo M. Liver and the defects of cholesterol and bile acids biosynthesis: rare disorders many diagnostic pitfalls. *World J Gastroenterol*. 2017;23(29):5257-5265.