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Biomarker testing for lysosomal diseases: A technical standard of the American College of Medical Genetics and Genomics (ACMG)

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ABSTRACT

Measurement of lysosomal disease (LD) biomarkers can reveal valuable information about disease status. Lyso-globotriaosylceramide (lyso-Gb_3), glucosylsphingosine (lyso-Gb_1), galactosylsphingosine (psychosine), and glucose tetrasaccharide (Glca1-6Glca1-4Glca1-4Glc, Glc_4) are biomarkers associated with Fabry, Gaucher, Krabbe, and Pompe disease, respectively. Clinical biomarker testing is performed to guide patient management, including monitoring disease progression and initiating treatment, and in diagnostic evaluations of either symptomatic patients or asymptomatic individuals with a positive family history or abnormal newborn screen. Biomarker analysis can be performed through independent analysis of a single analyte or as a multiplex assay measuring analytes for more than one disorder utilizing liquid chromatographic separation and tandem mass spectrometric detection. These guidelines were developed to provide technical standards for biomarker analysis, results interpretation, and results reporting, highlighting Fabry, Gaucher, Krabbe, and Pompe diseases as examples.

The Board of Directors of the American College of Medical Genetics and Genomics approved this technical standard on 24 June 2024.

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Introduction

Biochemical and clinical features of lysosomal diseases

Lysosomal diseases (LDs) comprise a clinically heterogeneous group of inherited conditions caused by lysosomal enzyme deficiencies and subsequent intra-lysosomal accumulation of macromolecules (eg, glycogen, glycosaminoglycans, and glycosphingolipids).[1] Substrate accumulation leads to lysosomal dysfunction and dysregulation of downstream pathways in affected tissues, resulting in a multi-systemic disorder and a broad spectrum of clinical manifestations. LDs are progressive with variable age of onset and clinical severity, with early age of onset associated with a more severe clinical course.[2] A growing number of LDs are identified by newborn screening in some states or countries, including Pompe disease, mucopolysaccharidosis type I (MPS-I), mucopolysaccharidosis type II (MPS-II), Krabbe disease, Fabry disease, Gaucher disease, and acid sphingomyelinase deficiency (ASMD).[3,4] At the time of this report, those included on the Recommended Uniform Screening Panel (RUSP) are Pompe disease, MPS-I, MPS-II, and Krabbe disease (https://www.hrsa.gov/advisory-committees/heritable-disorders/rusp). Gaucher disease, metachromatic leukodystrophy (MLD), and ASMD have been recommended as candidates for pilot programs by the newborn screening translational research network.[5]

The diagnosis of a specific LD is confirmed by demonstration of decreased enzyme activity and pathogenic variants in the relevant gene. Biomarker analysis in the diagnostic setting may also help to clarify variants of uncertain clinical significance and functions to provide a baseline value. Longitudinal biomarker analysis in patients offers an additional tool to guide therapeutic intervention and/or response. Available treatment may include enzyme replacement therapy (ERT), substrate reduction therapy (SRT), chaperone therapy, or hematopoietic stem cell transplantation and is available for a minority of LDs. Earlier therapeutic intervention has been shown to be successful in reducing disease symptoms and slowing disease progression.[6-8]

Biomarkers in lysosomal diseases

A biomarker, or biological marker, is defined as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention."[9] Biomarkers for LDs arise directly or indirectly from defective degradation of macromolecular substrates and derivatives. The first LD biomarker described was glucosylceramides (Gb_1), isolated in 1974 from the spleen of a patient with Gaucher disease.[10] The following year, globotriaosylceramide (Gb_3) was found to be elevated in the plasma from a male and affected family members with Fabry disease.[11] Additional biomarker-disease associations have since been discovered, including galactosylsphingosine in Krabbe disease,[12,13] lyso-sphingomyelin in ASMD,[14,15] and glucose tetrasaccharide (Glca1-6Glca1-4Glca1-4Glc, Glc_4) in glycogen storage diseases (GSDs), including Pompe disease,[16] and disorders of autophagy.[17]

Early in the development of enzyme replacement therapies, biomarkers were measured in conjunction with enzyme activity to assess the drug's effect on circulating substrate levels.[18,19] More recently, they have been used as surrogate endpoints in drug development, clinical trials, and therapeutic assessment to evaluate efficacy and toxicity.[9] Additionally, biomarkers may be used to assess disease severity, as an indicator of disease progression, to monitor compliance in SRT-treated patients, to augment newborn screening, and to clarify ambiguous situations in asymptomatic individuals. As such, biomarker monitoring has been incorporated in recommendations and consensus guidelines for patients with Fabry disease, Gaucher disease, Krabbe disease, and Pompe disease.[6,20-25]

This technical standard focuses on methods for analyzing lyso-Gb_3, lyso-Gb_1, galactosylsphingosine, and Glc_4 in the diagnosis or monitoring of patients with Fabry disease, Gaucher disease, Krabbe disease, and Pompe disease, respectively. We have chosen these LDs because they are among the most common LDs, are included in the RUSP, and/or have available therapies. As more centers consider adding these LD biomarkers to their menus and as these tests become integrated into the follow-up of abnormal newborn screening results, this laboratory technical standard will provide a reference for the measurement, interpretation, and reporting of these analytes. As therapeutic options emerge for other LDs, including MLD and Niemann-Pick type C, future revisions may consider further discussion on methodologies for their respective biomarkers.

Clinical description of Fabry disease

Fabry disease is one of the most common LDs with global prevalence estimates of 1 in 40,000 to 1 in 170,000.[26] This X-linked disorder of globoside degradation is caused by pathogenic variants in *GLA* (HGNC: 4296; NM_000169) and a deficiency of lysosomal α-galactosidase A (α-Gal A; EC 3.2.1.22), resulting in impaired degradation of Gb_3 and its subsequent accumulation in cells of the vascular, renal, and autonomic nervous systems, which leads to disease symptoms.

Affected males with a classic phenotype present in early childhood with neuropathic pain, hypohidrosis, gastrointestinal involvement, angiokeratomas, and corneal whorls.[27] Disease progression in adulthood (when untreated) leads to end stage renal disease, hypertrophic cardiomyopathy, and strokes with death occurring in the late fifth to early sixth decade from kidney failure, cardiac involvement, and strokes.[28] Heterozygous females typically manifest symptoms of the disease, although generally at a later age than

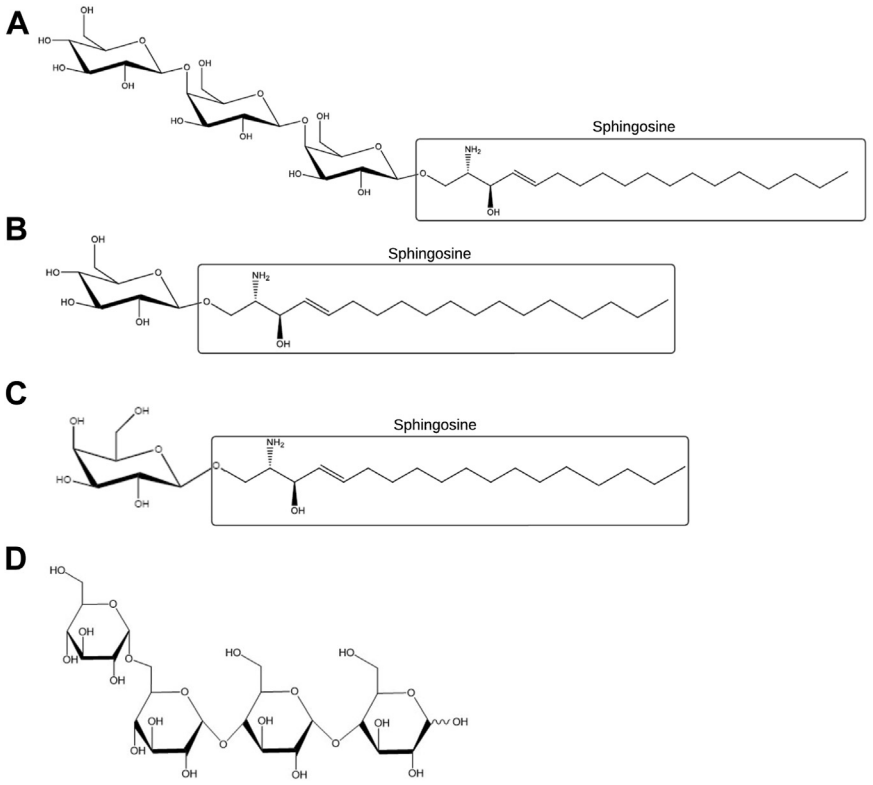

Figure 1 Biomarker structures. Structures of (A) lyso-Gb_3, (B) lyso-Gb_1, (C) galactosylsphingosine, and (D) Glc_4.

affected males, and present with varying signs, symptoms, and degrees of severity due at least in part to X-chromosome inactivation.[29-31] Attenuated phenotypes have been observed in patients with Fabry disease, such as those harboring the pathogenic variant c.644A>G p.(Asn215Ser), who present primarily with cardiac involvement and milder symptoms in other organs.[27] Food and Drug Administration (FDA)-approved therapies for Fabry disease include ERT (agalsidase beta) and chaperone therapy (migalastat).

The diagnosis of Fabry disease is established in males by enzyme testing demonstrating deficient α-Gal A activity.[32] In heterozygous females, enzyme testing is unreliable because of X-inactivation, and the diagnosis is based on gene sequencing.[33] Biomarker testing often supports the diagnosis and can be particularly useful in patients with an attenuated phenotype. Elevations of deacylated Gb_3, lyso-Gb_3 (Figure 1A), are highest in patients with classic Fabry disease when compared with atypical forms and are also higher in affected males than females.[34,35] Assessment of plasma lyso-Gb_3 has been shown to improve diagnostic outcomes in females with normal α-Gal A activity, although false-negative results may occur for attenuated phenotypes.[36,37] Normal lyso-Gb_3 levels have been observed in some patients with attenuated phenotypes harboring the *GLA* variants c.644A>G p.(Asn215Ser), c.335G>A p.(Arg112His), c.956T>C p.(Ile319Thr), c.640-801G>A, and c.431G>A p.Gly144Asp.[34,38,39] Finally, lyso-Gb_3 is not specific to Fabry disease because it has also been reported in patients with MPS-I, MPS-II, and MPS-III, suggesting that its accumulation origin may not be directly related to α-Gal A deficiency.[40]

Clinical description of Gaucher disease

Gaucher disease is the most common LD with global prevalence estimates of 1 in 26,000 to 1 in 63,000 for type 1 Gaucher disease and 1 in 2000 to 1 in 8000 for types 2 and 3 Gaucher disease.[41] Disease incidence estimates in individuals of Ashkenazi Jewish descent are as high as 1 in 450 births for type 1 Gaucher disease.[42] This autosomal recessive sphingolipidosis is caused by biallelic pathogenic variants in *GBA* (HGNC: 4177; NM_00157) and enzymatic deficiency of acid β-glucosidase (GBA1; EC 3.2. 1.45) with subsequent accumulation of lysosomal glucosylceramide and its sphingoid base, lyso-Gb_1 (Figure 1B).[43,44] Type 1 Gaucher disease, the most common form in the United States, can be distinguished from types 2 and 3 by its limited neurological involvement. Patients with type 2 (acute infantile neuronopathic) and type 3 (chronic neuropathic) Gaucher disease account for ~1% and 5% of patients in the United States, respectively.[45]

The primary phenotypic consequences of type 1 Gaucher disease include hepatosplenomegaly, thrombocytopenia, growth delay, bone involvement, and/or pulmonary involvement with age of onset ranging from childhood to late adulthood.[43,46] Patients with type 1 Gaucher disease have an increased risk for multiple myeloma and Parkinsonism.[47]

Type 2 Gaucher disease follows a rapidly progressive and often fatal course that presents with neonatal hydrops fetalis, hepatosplenomegaly, anemia, thrombocytopenia, interstitial lung disease, and neurological manifestations, including seizures, bulbar palsy, hypertonia, and supranuclear gaze palsy.[27,47] Type 3 Gaucher disease is characterized by infantile-childhood onset with a progressive disease course and clinical features of hepatosplenomegaly, interstitial lung disease, anemia, thrombocytopenia, and characteristic bone and neurological involvement.[27]

FDA-approved ERT (imiglucerase, velaglucerase alfa, and taliglucerase alfa) and SRT (miglustat and eliglustat) are currently available treatment options for symptomatic patients with types 1 and 3 Gaucher disease, although they are generally not effective in the amelioration of neurological manifestations for patients with type 3 Gaucher disease.[2]

The findings of low enzyme activity together with biallelic pathogenic variants and/or elevated biomarker can establish the diagnosis of Gaucher disease. Biomarker testing is also useful in evaluating therapeutic response and treatment compliance.[48-51] Established biomarkers for monitoring patients with Gaucher disease include tartrate resistant acid phosphatase, chitotriosidase activity, angiotensin converting enzyme, and/or C-C motif ligand 18/pulmonary-activated-related chemokine; however, their levels reflect macrophage activation and/or lipid storage and are not specific to Gaucher disease.[52] As a byproduct of the enzymatic defect, lyso-Gb_1 levels directly reflect sphingolipid turnover and correlate with levels of tartrate resistant acid phosphatase, chitotriosidase activity, angiotensin converting enzyme, and C-C motif ligand 18/pulmonary-activated-related chemokine.[53] The deacylated species accumulates in plasma and tissues of patients with non-neuronopathic and neuronopathic disease types,[50,51,54,55] as well as in patients with saposin C deficiency (because of pathogenic variants in *PSAP*), a rare, atypical form of Gaucher disease.[56] Current evidence supports lyso-Gb_1 as the most reliable biomarker for diagnosis, prognosis, and disease and therapeutic monitoring.[57]

Clinical description of Krabbe disease

Krabbe disease, also known as globoid cell leukodystrophy, is an autosomal recessive LD caused by a deficiency in galactocerebrosidase (GALC; EC 3.2.1.46) activity.[25,58] This disorder is caused by homozygous or compound heterozygous pathogenic variants in the galactocerebrosidase gene (HGNC: 4115) with an estimated frequency of 1 in 400,000. Krabbe disease can be divided into 4 subtypes based on the age of onset and clinical presentation, with a majority of patients developing the infantile-onset form characterized by rapid neurodegeneration and early death.[59] Patients with the late-onset type exhibit ataxia, weakness, vision loss, and psychomotor regression.[58,59]

Hematopoietic stem cell transplantation is the only treatment available for patients with Krabbe disease and is shown to have the potential to be effective when performed several weeks before the onset of symptoms, which, in the infantile form, translates to the need for intervention in the 1st 4 to 6 weeks of life.[60] Several states, corresponding to 1/3 of the US newborn population, have incorporated Krabbe disease into their newborn screening program, and it was added to the RUSP in July 2024. Galactosylsphingosine, one of the 4 substrates degraded by galactocerebrosidase enzyme (Figure 1C), is shown to be neurotoxic at elevated concentrations, is elevated in Krabbe disease and saposin A deficiency (due to pathogenic variants in *PSAP*) and may be useful to monitor disease progression before and after treatment.[61,62] Quantification of galactosylsphingosine can be used for confirmation of primary screening results that are based on lack of enzyme activity.

Clinical description of Pompe disease

Pompe disease (acid α-glucosidase (GAA) deficiency; EC 3.2.1.20) is an autosomal recessive GSD that results from enzymatic deficiency of lysosomal α-glucosidase leading to multisystemic glycogen accumulation. Glucose tetrasaccharide (Glca1-6Glca1-4Glca1-4Glc, Glc_4; Figure 1D), a marker of glycogen accumulation, is elevated in the urine of patients with Pompe disease, GSD-III, GSD-VI, and GSD-IX.[63-65]

Pompe disease has a variable age of onset and presents as infantile-onset Pompe disease (IOPD) or late-onset Pompe disease (LOPD). Prevalence estimates in the US for IOPD and LOPD is approximately 1 in 28,000.[66] IOPD is apparent shortly after birth and presents with hypotonia, muscle weakness, failure to thrive, and hypertrophic cardiomyopathy. When left untreated, death from respiratory failure often occurs in the first 2 years of life.[23,67] In contrast, patients with LOPD have measurable residual enzyme activity and present anytime between late infancy and adulthood with a myopathy that progresses to respiratory insufficiency if left untreated; however, cardiac involvement is rare.[68] FDA-approved ERT for patients with IOPD includes alglucosidase alfa and FDA-approved ERT for patients with LOPD includes alglucosidase alfa and avalglucosidase alfa-ngpt.

A strong clinical suspicion and absent enzyme activity supports a clinical diagnosis of Pompe disease. Biomarker analysis of urinary Glc_4 may assist in distinguishing patients with IOPD from LOPD. In neonates, Glc_4 excretion is typically elevated in patients with IOPD and within the normal range in patients with LOPD.[65,69,70] In contrast, urinary Glc_4 excretion is typically elevated in untreated juveniles and adults with LOPD[65] and can be used to monitor response to ERT.[71-74] ERT-treated patients may have an increase in Glc_4 excretion during intercurrent illness, such as infection, that is usually temporary.[73] Furthermore, Glc_4 excretion may be temporarily increased in otherwise unaffected individuals secondary to certain malignancies, acute pancreatitis, muscle trauma, during pregnancy, and after ingestion of starch and glycogen derived from meat.[64,75]

Materials and Methods

The laboratory technical standard and guideline was informed by a review of the literature, including any current guidelines, and expert opinion. Resources consulted included PubMed (search terms: biomarkers, lysosomal storage disorders, glycosphingolipids, Fabry disease, lyso-globotriaosylceramide, lyso-Gb_3, Pompe disease, glucose tetrasaccharide, Glc_4, hexose tetrasaccharide, Hex_4, Gaucher disease, glucosylsphingosine, lyso-Gb_1, glycopsychosine, Krabbe disease, galactosylsphingosine, psychosine, and tandem mass spectrometry), the American College of Medical Genetics and Genomics (ACMG) Standards and Guidelines for Clinical Genetics Laboratories, Clinical and Laboratory Standards Institute (CLSI) guidelines, Clinical Laboratory Improvement Amendments (CLIA) regulations, Online Mendelian Inheritance in Man, GeneReviews, the Centers for Disease Control and Prevention Morbidity and Mortality Weekly Report on Good Laboratory Practices for Biochemical Genetics Testing and Newborn Screening for Inherited Metabolic Disorders.[76] When the literature provided conflicting or insufficient evidence about a topic, the authors used expert opinion to inform the recommendations. Expert opinion included the coauthors of the document, members of the Biochemical Genetics Subcommittee of the Laboratory Quality Assurance Committee, and any experts consulted outside the workgroup and acknowledged in this document. Any conflicts of interests for workgroup members are listed at the end of the article. The ACMG Laboratory Quality Assurance Committee reviewed the document providing further input on the content, and a final draft was delivered to the ACMG Board of Directors for review and approval to send out for member comment. The final draft of the document was posted on the ACMG website, and an email link was sent to ACMG members inviting all to provide comment. All members' comments were assessed by the authors and our recommendations were amended as deemed appropriate. Member comments and author responses were reviewed by a representative of the Laboratory Quality Assurance Committee and the ACMG Board of Directors. The final document was approved by the ACMG Board of Directors.

Biomarker Testing

Clinical indications for testing

Biomarker analysis can be used to assess disease burden and monitor compliance with treatment in known patients. It has also been shown to aid in the diagnosis of patients presenting with symptoms or with a positive family history of one of these disorders. Biomarker analysis may be most useful for newborn screening to reduce false-positive results, to follow up ambiguous results of enzymatic analysis in symptomatic patients, and to aid in the interpretation of variants of uncertain clinical significance identified by molecular genetic testing.

Preanalytical requirements

The laboratory should provide clear instructions to clients regarding the type of specimen, volume, shipping, and storage conditions. Acceptable sample types and analyte stability should be established by the testing laboratory during validation and communicated to ordering health care professionals upon request. The appropriate sample type for each disorder discussed in this technical standard may vary.

Sample type, collection, handling, and storage

Clinical testing for LD biomarkers is performed in plasma or dried blood spots for lyso-Gb_3 and lyso-Gb_1 analysis; whole blood, dried blood spots, or cerebrospinal fluid for galactosylsphingosine analysis; and urine for Glc_4 analysis. Lyso-Gb_3, lyso-Gb_1, and Glc_4 analysis is also possible in cerebrospinal fluid and may be useful in clinical trial design or evaluating therapeutic response in patients.[77,78]

Analysis of lyso-Gb_3 and lyso-Gb_1 requires approximately 300 μL of plasma or serum obtained from approximately 1 mL of whole blood collected by venipuncture in a lavender top (EDTA), green top (sodium heparin and lithium heparin), yellow top (ACD B), or red top (SST) tube. Galactosylsphingosine testing can be done in whole blood or dried blood spots from 0.5 to 2.0 mL blood collected in EDTA (preferred) or heparin (alternative). Galactosylsphingosine analysis from cerebrospinal fluid requires 0.15 mL. For Glc_4 analysis, preprandial or fasting urine samples are preferred to avoid dietary artifacts, specifically from formula additives for infants.[75]

Ideally, plasma, serum, and cerebrospinal fluid should be shipped frozen on dry ice and stored frozen until analysis. Whole blood should be shipped with wet ice and stored at refrigerated temperature until analysis. Blood spots should be collected on filter paper that meets the physical characteristics and performance requirements specified by CLSI guidelines (eg, Whatman 903).[79] Blood spot samples should be dried completely before shipping, kept away from direct sunlight, and should avoid exposure to high humidity, heat, and corrosive liquids, such as bleach and alcohol. Urine should be collected in a clean container with no preservatives and frozen, shipped on dry ice, and stored frozen until ready for testing.

Method validation

Calibration and quantitation

At the time of this report, clinical laboratories in the United States utilize stable isotope dilution liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods to quantify lyso-Gb_3, lyso-Gb_1, galactosylphingosine, and Glc_4. Quantitation by these methods requires the use of a calibration curve with isotope labeled internal standards (deuterium or carbon-13) to control for matrix effects and variations in extraction efficiency that could otherwise affect method performance. Reagents and internal standards are commercially available. When an internal standard is not

available, laboratories may request an internal standard be synthesized by a manufacturer or synthesize an internal standard themselves. Performance of all internal standards and reagents must be validated by each laboratory before being put into use. As with any analytical assay, each laboratory must establish their analytical protocols by assessing various time points, analytical measurement range, and effects of interference. Performance characteristics should be verified on a regular basis as detailed by CLSI[80] and specified by CLIA.

Laboratories should prepare and evaluate performance characteristics of quality control (QC) samples that are analyzed alongside each batch of patient samples. The QC samples should be prepared in the appropriate sample matrix and include at least 2 levels that span the analytical measurement range. Target ranges should be established for each analyte by analyzing replicates over several runs, ideally by multiple operators. Plasma and serum from normal individuals can be pooled and used as a normal/low QC for plasma/serum-specific analytes. Similarly, urine from unaffected individuals can be pooled and used as the low QC for urine-specific analytes. Plasma, serum, and urine samples can also be spiked with standards and used as a high QC; however, the stock solution of standards used to spike controls should be different than the stock solution used to prepare calibrators. The prepared high QC sample should measure near the upper limit of quantitation and within the range of values observed in patients. The use of Westgard rules for clinical specimen analysis further controls the parameters for quality patient diagnosis and reporting.[81]

Proficiency testing

Clinical laboratories must participate in an ongoing proficiency testing (PT) program for biomarker testing adhering to the requirements of regulatory agencies. The European Research Network for Evaluation and Improvement of Screening, Diagnosis and Treatment of Inherited Disorders of Metabolism provides a commercially available, external PT service for the quantitative analysis of lyso-Gb_1 and lyso-Gb_3 in plasma/serum. Currently, no external PT programs for galactosylsphingosine and Glc_4 analysis are available. As an alternative PT approach, laboratories can perform interlaboratory comparison through split sample analysis, store aliquots of samples from affected individuals and rerun these samples periodically, or perform clinical correlation through the review of the electronic medical record.[82]

Reference ranges

Reference ranges for all reportable analytes should be established by the performing laboratory and periodically verified and updated per the recommended CLSI guidelines.[83] Before testing, mean biomarker levels should be determined in a cohort of unaffected individuals, if possible, and in disease-affected individuals to obtain both a normal reference range and an affected range. It is important to note that Glc_4 reference ranges are age dependent. Reference ranges should be established or verified for each specimen type used for clinical testing during validation because there may be significant differences between sample types.

Testing personnel

Initial training and ongoing competency assessment must be established and documented by the performing laboratory. Qualifications for personnel performing high complexity testing must meet CLIA requirements and, at a minimum, have an associate degree in laboratory science or certification in medical laboratory technology from an accredited program. Stricter requirements and regulations determined by individual states may apply.

Biomarker analysis

Several methods detailed below have been published for biomarker analysis in biological samples. These include approaches for sample preparation, chromatographic separation, derivatization, and analyte detection and quantification. All offer high sensitivity and sufficiently low detection limits for clinical applications but differ in the equipment required, workflow, and capital equipment cost. Chromatographic methods, immunoassays, and fluorescence or ultraviolet (UV) detection are simpler and available at a lower capital cost than LC-MS/MS or gas chromatography-mass spectrometry methods.

Sample preparation

A specimen cleanup step to remove interfering compounds is a general prerequisite for biomarker analysis via fluorescence detection, UV detection, gas chromatography-mass spectrometry, and/or LC-MS/MS detection. Stable isotope dilution is used in combination with LC-MS/MS to improve analytical performance. Urine samples generally require an initial centrifugation step to remove debris before the addition of the stable isotope internal standard. Liquid-liquid extraction,[84-88] solid-phase extraction,[87,89] or an in-line column cleanup method[90] are commonly used techniques to obtain extracts containing the analyte of interest with the latter offering a simpler and faster method of protein precipitation and phospholipid removal.

Analytical methods for lyso-Gb_3

Purified extracts are subjected to LC coupled with fluorescence detection or triple quadrupole MS/MS to detect and quantify lyso-Gb_3. Fluorescence-based methods require precolumn derivatization with o-phtaldialdehyde reagent, which can lead to interference from other o-phtaldialdehyde-reactive compounds.[34] Compounds are separated using high-performance liquid chromatography (HPLC), detected at excitation and emission wavelengths of 340 and 435 nm, respectively, and quantified relative to an external standard curve.

Chromatographic separation can be achieved using HPLC or ultra-performance liquid chromatography with a C18 reverse phase or hydrophilic interaction chromatography

column. Nano-LC-MS applications have also been described.[84] Although no structural isomers of lyso-Gb_3 have been reported, several isoforms with modifications to the sphingosine backbone have been identified.[85-88] MS/MS is performed using electrospray ionization in positive mode, and peaks are detected by selected reaction monitoring for the transitions *m/z* 786→282 (underivatized lyso-Gb_3 $[M+H]^+$ ion) and 793→289 (d_7-lyso-Gb_3).

Analytical methods for lyso-Gb_1 and galactosylsphingosine

Lyso-Gb_1 and galactosylsphingosine are structural isomers (isobars) and therefore are detected by the same mass transition with MS/MS. Chromatographic separation before mass spectrometry analysis is required to distinguish between the 2, using a C18 reverse phase, BEH amide, or hydrophilic interaction chromatography column.[91] For known patients with Gaucher disease, quantification of the total hexosylsphingosine (lyso-Gb_1 plus galactosylsphingosine) fraction offers an alternative approach for lyso-Gb_1 analysis to reduce run times.[61,92-95] Purified extracts are subjected to LC coupled with fluorescence detection or triple quadrupole MS/MS to detect and quantify lyso-Gb_1 and galactosylsphingosine. MS/MS is performed using electrospray ionization in positive mode, and peaks are detected by selected reaction monitoring for the transitions *m/z* 462→282 for underivatized lyso-Gb_1 and galactosylsphingosine $[M+H]^+$ ion, *m/z* 468→282 for $^{13}C_6$-lyso-Gb_1, and m/z 467→287 for d_5-galactosylsphingosine.[96]

Fluorescence-based methods offer an alternate method for lyso-Gb_1 analysis and require precolumn derivatization with 4-fluoro-7-nitrobenzofurazan (NBD-F) reagent.[89] Compounds are separated using HPLC, detected at excitation and emission wavelengths of 470 and 530 nm, respectively, and quantified relative to an external standard curve.

Analytical methods for Glc4

Purified extracts are subjected to GC or LC coupled with UV detection, MS, or triple quadrupole MS/MS to detect and quantify Glc_4. Precolumn derivatization with para-aminobenzoic acid (PABA)[71] reagent is required before UV detection at a wavelength of 304 nm and quantified relative to an external standard curve.[97] In addition to PABA, 1-phenyl-3-methyl-5-pyrazolone[98] derivatization may be used before LC-MS/MS detection, although derivatization is not required for analysis.[64]

HPLC separation with a C18 reverse phase is required to resolve Glc_4 from other glycans. MS/MS is typically performed using electrospray ionization in positive mode[64,71]; however, matrix-assisted laser desorption ionization offers an alternative. PABA-derivatized peaks are detected by selected reaction monitoring for the transitions *m/z* 866→509 for Glc_4 $[M+H]^+$ ion and *m/z* 872→509 for $^{13}C_6$-Glc_4. Underivatized Glc_4 identified and quantified by MS/MS in negative ion mode monitors the transition *m/z* 665→179 $[M-H]^-$.[64] Separation of Glc_4 from its isomer maltotetraose (M_4) can be achieved with chromatography by an ultra-performance liquid chromatographyamide stationary-phase column.[64]

Results Interpretation

Results of biomarker analysis should be reviewed and interpreted by an American Board of Medical Genetics and Genomics board-certified clinical biochemical geneticist or another qualified individual.[99] The interpretation should consider not just the numeric result but also the age of the patient, previous test results, and other supporting information, such as a reason for referral, family history, or known treatment. Although not always available, this information can be helpful to provide more targeted recommendations for future testing (either sequential analysis for monitoring purposes or more conclusive diagnostic analyses if biomarker testing may have been ordered inappropriately or inappropriate specimen type was collected).

Because results for Glc_4 are reported as excreted amount per unit of creatinine, spurious elevations of Glc_4 may be due to low creatinine values for the submitted specimen. Although this is most commonly observed in infants, it can also be seen in older individuals.

It is important to note that although results of biomarker analysis can help to support a particular diagnosis, a normal result does not rule out disease because patients with attenuated phenotypes may not show evidence of substrate accumulation at the time of testing. Interpretations may include the assessment of other laboratory findings (eg, enzyme analysis and gene sequencing results) and take into consideration the clinical and treatment history, when available.

Reporting

Patient reports must include patient and specimen information as specified by CLIA and in accordance with the ACMG Standards and Guidelines for Clinical Genetics Laboratories, sections 2.4, 2.41, and 2.42. In addition to the required patient and specimen information, written reports should specify the targeted analyte, include the reported concentration, units of measurement (eg, μmol/L or ng/mL), reference range, results interpretation (see "Interpretation"), the type of testing performed (eg, investigational, laboratory-developed, or FDA-cleared), and any limitations of testing that may be observed. Recommendations for follow-up testing (ie, confirmation with enzyme analysis and/or molecular testing) may also be included, when appropriate.

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Conflict of Interest

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