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# ACMG TECHNICAL STANDARD

# Biochemical testing for congenital disorders of glycosylation: A technical standard of the American College of Medical Genetics and Genomics (ACMG)

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ABSTRACT

Congenital disorders of glycosylation (CDG) are a large and continually expanding group of disorders that present with a variety of clinical findings and have been linked to over 170 genes. Individually, CDGs are rare; however, the true incidence may be underestimated because of the variability of the clinical findings, and the multiple testing strategies needed to diagnosis them across multiple pathways. Testing for CDGs has evolved over recent years with the availability of high-throughput molecular testing and improved gene discovery techniques. Biochemical testing to detect defects in glycosylated proteins or enzymatic deficiency still plays a critical role in the diagnosis of affected individuals, and both testing modalities are often required to finalize a diagnosis. Emerging therapeutic approaches targeting improvements in glycosylation require reliable and reproducible biochemical testing for therapeutic monitoring, dose adjustment, and avoidance of dose-related side effects. To maintain clinical sensitivity and specificity and to ensure reproducibility across laboratories performing complex biochemical testing, the American College of Medical Genetics and Genomics has developed the following technical standard.



The Board of Directors of the American College of Medical Genetics and Genomics approved this technical standard on 29 October 2024.

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## Introduction

Congenital disorders of glycosylation (CDGs) are a continuously expanding group of over 170 monogenic human diseases with defects in the synthesis and transfer of glycans. Synthetic pathways for glycans have many steps, each dependent on the prior step, which require careful regulation. The clinical spectrum of CDGs reflects the functional impact of disrupted glycosylation on human development and physiology with multisystem and multiorgan involvement being cardinal features of this group of disorders, although the clinical spectrum is constantly expanding both within the larger class of disorders and in many of the disorders individually. Large-scale genomic sequencing has resulted in an explosion of the number of human disorders linked to errors in glycosylation. A chapter on CDGs from a biochemical genetics textbook published in 2009 identified 17 unique disorders in this group.[1] A publication from 2017 places the number of CDGs at 104,[2] and a recent article in 2023 places the number of described CDGs at >170,[3] and the count in 2024 sits at >200.[4] It is believed that up to 2% of all human genes may have a role in glycosylation, which still leaves considerable room for further expansion of this disease group.[5]

The most common and best remembered description of a CDG is the historic picture of PMM2-CDG (OMIM 212065), previously known as CDG-Ia: a severely affected infant with abnormal fat pads over the buttocks, inverted nipples, hypotonia, and failure to thrive. The path to diagnosis for an infant with this clinical picture should include the analysis of transferrin isoforms, although specific methodology may vary. This clinical presentation of PMM2-CDG is accurate, and transferrin isoform analysis is a good first step. The optimal testing strategy for an individual will depend on the overall clinical picture, because of the increasing expansion of the clinical and genetic variability in this group of disorders.[3,6,7] Moving past the commonly known CDGs introduces even greater clinical and laboratory variability. Many of these disorders have each only been described in a small number of families, increasing the challenge of getting generalizable information.

Clinical testing for CDGs has traditionally centered around the analysis of transferrin, because of its abundance and availability in accessible sample matrices.[8] Targeted enzyme activity analysis, N-glycan and O-glycan profiles, apolipoprotein CIII (ApoCIII) analysis, flow cytometry, and urine oligosaccharide screening have expanded the spectrum of testing that could lead to the diagnosis of a CDG. This does not touch on the many laboratory abnormalities that may be observed in affected individuals that support a diagnosis but do not specifically point toward a CDG, such as liver function tests and coagulation studies. Expanded molecular techniques and the wider availability of untargeted sequencing via exome and genome analysis have resulted in an explosion of disorders linked to underlying defects in glycosylation, not all of which are detectable by biochemical testing. The timing and order of testing that can lead to a CDG diagnosis are in a state of flux, with molecular and biochemical methods being done as first- or second-tier testing depending on clinical findings, urgency, and insurance coverage.

## Glycosylation in human disease

The broad scope of disorders that have been linked to defects in glycosylation is easier to understand when paralleled with the extent of glycosylation observed in human systems. Historically, glycosylation is defined as the process of systematic alteration of proteins in the endoplasmic reticulum (ER) and/or Golgi apparatus by the addition of monosaccharides or oligosaccharides after synthesis, as a form of post-translational modification. A more recent symposium dedicated to CDGs defined glycosylation as "the synthesis of fully functional glycans, and their covalent enzymatic attachment to other molecules including proteins, lipids, and small RNA."[9] Because glycosylation is the most abundant post-translational protein modification, this explains the large number of CDGs and the breadth of clinical presentations. Although up to 2% of human genes may be linked to glycosylation,[5] it is unlikely that all will be involved in human disease, and there remains debate over when impaired glycosylation becomes a CDG. Some defects will be embryonic lethal, such as homozygosity for the common *PMM2* pathogenic variant[10] or recent reports of fetal demise in DPAGT1-CDG (formerly CDG-Ij) (OMIM 608093),[11] and some will be tolerated without a clinical phenotype, as seen in some individuals with asymptomatic phosphomannose isomerase deficiency (MPI-CDG) (OMIM 154550).[12]

CDG naming has evolved over time, mainly as a necessity because of the increase in the number of conditions and the expanding type of glycosylation disorders. Initially, the abbreviation CDG stood for "carbohydrate-deficient glycoprotein," and many early publications refer to that overarching disease grouping.[13-15] Early CDGs were named based on the pattern produced by transferrin isoform analysis, either type I or type II and then a letter based on the order of discovery. This naming convention has been effectively deprecated by the large number of identified CDGs and the ability to identify causative genes; however, it retains utility for the description of laboratory findings. Historically, a type I CDG profile represents a defect in the synthesis or transfer of oligosaccharides onto proteins in the ER, whereas a type II CDG profile shows evidence of a defect in the processing of N-linked glycans in the ER or the Golgi apparatus. The description of transferrin isoform profiles as showing type I or type II patterns is still commonly used in laboratories to classify and describe their findings; however, the scope of glycosylation disorders has broadened considerably.

CDGs exhibit multiorgan involvement with broad phenotypic variation, both within and between genetic

causes of CDGs.[16] CDGs can be divided into groups based on glycosylation type and modified biomolecule. The largest known groupings include protein N-linked glycosylation defects, protein O-linked glycosylation defects, glycosylphosphatidylinositol (GPI) anchor glycosylation defects, lipid glycosylation defects, and defects in multiple glycosylation pathways.[17] Glycans are grouped by their protein linkage; N-glycans are linked to asparagine via the amide group, and O-glycans are linked to serine or threonine via the hydroxyl group.[8,18]

N-glycan synthesis can be broken down into 3 stages: (1) formation of the nucleotide-linked sugars takes place in the cytosol, (2) stepwise addition of monosaccharides and transfer to protein in the ER, and (3) final glycan processing in the Golgi apparatus. O-glycan synthesis occurs mainly in the Golgi apparatus and is focused on assembly; no additional processing takes place.

## Clinical description of glycosylation disorders

Not all genes implicated in glycosylation have been linked to a CDG. A basic description, agreed upon at an expert symposium, defined CDGs as "inherited (recessive, dominant, or X-linked) or de novo disorders that cause 'substantial' hypoglycosylation in one or more cell types."[9] This definition is followed throughout this technical standard, although it must be recognized that this definition may continue to evolve. N-linked glycosylation defects comprise the largest group, including the most common single genetic cause of CDG, which are defects caused by pathogenic variants in *PMM2*. These disorders typically exhibit multisystem manifestations with significant neurologic involvement with the notable exception of MPI-CDG and phosphoglucomutase 1 deficiency (PGM1-CDG), which typically have normal development.[19] The most common perinatal findings include hypotonia, nonspecific dysmorphic features (inverted nipples or abnormal fat pads occasionally present), feeding problems, growth delay, hepatopathy with elevated transaminases, and abnormal coagulation profiles. Additional features include neonatal hemorrhages (including cerebral hemorrhage) and thrombotic events, pericardial effusion, strabismus, nystagmus, neonatal seizures, abnormal thyroid function screening results, and nonimmune hydrops.[20,21] The majority of N-linked CDGs can be readily identified with transferrin isoform analysis. As general advice, it has been recommended to consider ruling out a CDG with any unexplained clinical presentation, particularly with multisystem involvement.[1,2]

The clinical presentation of O-linked glycosylation defects varies significantly depending on which type of O-glycosylation is affected. For example, deficiency of mucin-type O-N-acetylgalactosamine linkage can lead to familial tumoral calcinosis with hyperphosphatemia and massive calcium deposits in the skin and subcutaneous tissues,[22] whereas a defect in O-fucosylation has been shown to cause Peters plus syndrome, characterized by anterior eye chamber defects, disproportionate short stature, developmental delay, and cleft lip and/or palate.[23] O-mannosylation defects lead to hypoglycosylation of α-dystroglycan,[24] an important glycoprotein needed to link the intracellular cytoskeleton of muscle to the extracellular matrix, causing α-dystroglycanopathies.[25,26] O-glycosylation types may be detected by ApoCIII glycosylation testing, whereas disorders such as α-dystroglycanopathies, often associated with elevated creatine kinase, may require a muscle biopsy to identify the specific defect. ApoCIII glycosylation abnormalities primarily detect disruption of Golgi homeostasis and trafficking, which also affects N-glycosylation; therefore, detecting combined N- and O-glycosylation disorders. GALNT2-CDG (OMIM 618885), a mucin-type O-glycosylation disorder with childhood epilepsy and developmental delay, can be specifically detected via ApoCIII glycosylation analysis given that ApoCIII is the direct glycosylation target of *GALNT2*.[27] The biochemical signature of ApoCIII glycosylation abnormalities in GALNT2-CDG is distinct from that of combined N- and O-glycosylation disorders given the lack of entire O-glycans in the former.[27]

Typically, individuals affected with GPI anchor disorders have epilepsy and intellectual disability, along with multiple congenital anomalies, including heart, skeletal (particularly distal fingertip anomalies), endocrine, ophthalmologic, and facial anomalies (dysmorphic features), with possible elevations in alkaline phosphatase levels depending on the specific genes involved.[28,29] Testing for cell-surface expression of GPI-anchored proteins using flow cytometry can confirm defective GPI anchor synthesis. This may need to be specifically requested.

## Incidence

The combined incidence and prevalence of CDGs as a group have not been established, and any published estimates are likely low.[30] The estimated prevalence in European and African American individuals is 1/10,000 based upon heterozygote frequencies of potentially pathogenic variants in 53 genes in which pathogenic variants are known to cause a CDG.[30] PMM2-CDG, the most frequently diagnosed CDG, has an estimated calculated disease frequency of 1/20,000 in the Dutch and Danish population[31] and 1/77,000 in Estonia.[32] Given the increasing availability of large genomic databases, further estimates of CDG prevalence have been made. A recent study found PMM2-CDG to be the only CDG with an estimated prevalence greater than 1 to 100,000. The reported prevalence in this article may be overestimated because it did not exclude those homozygous for the common p.Arg141His amino acid substitution, which is associated with embryonic lethality.[10,31] A 2022 study reviewing published CDG cases identified 3057 individuals with an identified CDG, and >1000 were reported to have PMM2-CDG. Only 3 other CDGs included in the analysis (ALG6-CDG [OMIM 614566], EXT1 [OMIM

133700]/EXT2-CDG [OMIM 133701], and FKTN-CDG [OMIM 253800]) have over 100 cases reported.[33]

## Mode of inheritance

CDGs have been identified with all inheritance patterns, except through mitochondrial DNA. The most common inheritance for CDGs is autosomal recessive. Table 1 lists the genes currently known to be associated with CDGs and their described inheritances. Lists of genes associated with CDGs are continually expanding.

## Treatment of glycosylation disorders

A small fraction of known CDGs have an available treatment. Treatment and management for most known CDGs is supportive and palliative. There is substantial mortality in the first years of life because of severe infection or vital organ failure.[34] Small molecule supplementation is currently the most used treatment, although extensive research, including drug repurposing and gene therapy, is underway.[35-37] The best known example of a treatable CDG is MPI-CDG, in which oral mannose[38] ameliorates protein losing enteropathy, coagulopathy, and hyperinsulinism but does not necessarily halt the progression of the liver disease, which may stem from developmental liver defects requiring liver transplantation in later life.[39] D-galactose supplementation improves hypoglycemia, coagulopathy, rhabdomyolysis, and endocrinopathy in PGM1-CDG (OMIM 614921),[40,41] TMEM165-CDG (OMIM 614727),[42] and SLC39A2-CDG (OMIM 612166).[43] Symptom improvement for specific disorders has also been observed with targeted supplementation. Manganese and galactose have been used to treat seizures in SLC39A8-CDG (OMIM 616721).[41] In individuals affected with SLC35C1-CDG (OMIM 266265), recurrent infections with leukocytosis may respond to oral fucose supplementation.[44] Seizures in PIGM-CDG (OMIM 610293) and PIGO-CDG (OMIM 610293) have responded to butyrate[35] and vitamin B6 supplementation, respectively.[37,45] Uridine and uridine triacetate have been reported to improve seizures and anemia in CAD-CDG (OMIM 616457).[46] Fresh frozen plasma and/or protein C concentrate has been used to prevent bleeding episodes in multiple CDGs, and improve capillary leakage and edema, particularly during times of infection.[47] Therapeutic approaches that target symptomatic improvement through improvements in glycosylation require reliable and reproducible biochemical testing for therapeutic monitoring, dose adjustment, and avoidance of dose-related side effects, underlying the need for this American College of Medical Genetics and Genomics (ACMG) technical standard.

## Materials and Methods

This laboratory technical standard was informed by a review of the literature, including any current guidelines, and expert opinion. Resources consulted included PubMed (search terms included: congenital disorder(s) of glycosylation, glycosylation defect, transferrin isoform analysis, CDG, glycan profiling, N-glycan, and O-glycan), Clinical and Laboratory Standards Institute guidelines, and Clinical Laboratory Improvement Amendments regulations. When the literature provided conflicting evidence about a topic or when there was insufficient evidence, the authors used expert opinion to inform the recommendations. Expert opinion included the coauthors of the document and members of the Biochemical Genetics Subcommittee of the Laboratory Quality Assurance (Lab QA) Committee. Any conflicts of interest for workgroup members or consultants are listed. The ACMG Lab QA 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 document was posted on the ACMG website, and an email link was sent to ACMG members inviting all to provide comments. All members' comments were assessed by the authors; additional evidence was also included, and our

**Table 1** Genes and inheritance linked to known glycosylation disorders, classified with at least "Moderate" evidence in GenCC database

| Inheritance Pattern | Genes Involved |
|---|---|
| Autosomal recessive (120) | *ALG1, ALG11, ALG12, ALG14, ALG2, ALG3, ALG6, ALG8, ALG9, ATP6V0A2, ATP6V1A, ATP6V1E1, B3GALNT2, B3GALT6, B3GAT3, B3GLCT, B4GALNT1, B4GALT1, B4GALT7, B4GAT1, CAD, CANT1, CCDC115, CHST14, CHST3, CHST6, CHSY1, COG1, COG2, COG5, COG6, COG7, COG8, COPB2, CRPPA, CSGALNACT1, DDOST, DOLK, DPAGT1, DPM1, DPM2, DPM3, DSE, EOGT, EXTL3, FKRP, FKTN, FUT8, G6PC3, GALNT3, GFPT1, GMPPA, GMPPB, GOSR2, LARGE1, LFNG, MAN1B1, MGAT2, MOGS, MPDU1, MPI, NANS, NDST1, PGAP1, PGAP2, PGAP3, PGM1, PGM3, PIGB, PIGC, PIGG, PIGH, PIGK, PIGL, PIGM, PIGN, PIGO, PIGP, PIGQ, PIGS, PIGT, PIGU, PIGV, PIGW, PIGY, PMM2, POMGNT1, POMGNT2, POMK, POMT1, POMT2, RFT1, RXYLT1, SAR1B, SEC24D, SLC10A7, SLC35A1, SLC35A3, SLC35C1, SLC35D1, SLC39A8, SRD5A3, ST3GAL3, ST3GAL5, STT3A, TGDS, TMEM165, TMEM199, TRAPPC11, TRAPPC12, TRAPPC2L, TRAPPC4, TRAPPC6B, TRAPPC9, TRIP11, TUSC3, UGDH, UGP2, XYLT1, XYLT2* |
| Autosomal dominant (14) | *ARCN1, COG4*[a], *COPA, DHDDS*[a], *EXT1, EXT2*[a], *GANAB, GNE*[a], *NUS1*[a], *POFUT1, POGLUT1*[a], *PRKCSH, SEC23B*[a], *SEC63* |
| X-linked (11) | *ALG13*[b], *ATP6AP1, ATP6AP2, MAGT1, OGT, PIGA, SLC35A2*[b], *SLC9A7, SSR4, TRAPPC2, VMA21* |

[a]Have demonstrated both recessive and dominant inheritance.
[b]Have been found de novo.

recommendations were amended as deemed appropriate. Member comments and author responses were reviewed by a representative of the Lab QA Committee and the ACMG Board of Directors. The final document was approved by the ACMG Board of Directors.

## Preanalytical requirements

### Specimen requirements

Most laboratory testing that is specific for CDGs is performed on serum or plasma samples. Serum and plasma provide readily available specimens that show abnormal glycans and other metabolites at levels detectable with available equipment. Whole blood for isolation of leukocytes for enzymology may be obtained for some disorders. Specimens for urine oligosaccharide analysis often require freezing before shipment, although this should be verified with the performing laboratory. Laboratories performing or initiating testing for CDGs should consider sample type requirements, and collection, processing, shipping, storage, and stability as part of the method validation process. Methods describing CDG screening from both dried blood spots (transferrin isoforms and ApoCIII)[48] and dried serum spots (transferrin isoforms only)[49] for the purposes of identifying CDGs have been published. Testing of these sample types is not currently available on a clinical basis in the United States.

### Sample handling, shipping, and storage

Specimen requirements for sample handling, shipping, and storage should be established or verified by the performing laboratory during method validation. Once established, these should be made available to all clients and health care professionals who may order testing. Serum, plasma, and urine samples should be frozen immediately after collection and shipped to the testing laboratory on dry ice. Specimens submitted for enzyme analysis should be sent as whole blood to arrive at the testing laboratory as quickly as possible for preparation. If timely shipping of whole blood is not possible, it may be necessary to extract leukocytes and ship prepared and frozen to the testing laboratory. The testing laboratory should make a protocol for the isolation of leukocytes available.

### Preanalytical variables

CDG testing does not require fasting; however, if other studies are being collected under these conditions, it will not negatively affect CDG testing. The abnormal glycans and intermediates detected during screening are not affected by short term dietary intake. Enzyme analysis requires no special patient preparation.

Dietary treatment, such as mannose for MPI-CDG, may normalize the abnormal profiles seen with both transferrin and N-glycan analyses. Transfusions, particularly fresh frozen plasma, may affect screening for glycosylation disorders by introducing normal glycan species or simply by dilution. Transferrin has a circulating half-life of 8-10 days. If possible, samples for diagnostic testing should be collected before a transfusion. If the patient is transfusion dependent, samples should be collected immediately before a scheduled transfusion to maximize the time since the most recent transfusion. In all cases, the testing laboratory should be notified of these potential confounding factors for testing.

Abnormal results by transferrin isoform and N-glycan analysis are not necessarily indicative of a glycosylation disorder. Liver dysfunction, including caused by chronic alcohol ingestion[50] or other inherited diseases, such as classic galactosemia, peroxisomal disorders,[51] and hereditary fructose intolerance,[52] can result in type I CDG transferrin profiles and increases in immature glycans that are commonly seen in PMM2-CDG and MPI-CDG. A summary of CDG testing performance and known confounders is shown in Table 2. It is important to recognize that negative biochemical testing for CDGs does not necessarily rule out a CDG. Because of the location of specific glycosylation defects and in some cases, tissue specific expression, clinically available laboratory tests are often negative, even in the presence of clearly disease-causing variants. Many newly described CDGs have only a small number of affected individuals, and their full scope of presentations may not be known. A small study in 2016 showed that only 7 of 15 individuals with a confirmed CDG had clearly abnormal results on clinically available testing and 5 of 15 had normal results by all available testing.[53] Precise sensitivity and specificity data for clinically available CDGs are not available and would be highly variable. Based on how disorders were classified in the past, transferrin isoform analysis would likely have high sensitivity for those disorders previously known as type I CDGs; however, false-positive and false-negative transferrin screening results have been reported when testing is performed shortly after birth.[54] Affected individuals with PMM2-CDG and normal transferrin profiles have been described[55]; however, this is uncommon and seems associated with a small number of causative variants.[56] As always, laboratory findings should be correlated with the clinical presentation and the results of complementary studies to identify the underlying cause in any individual patient.

Laboratories may find it beneficial to obtain a referral indication for such testing, or to create unique testing options for different indications. Depending on the volume of testing ordered, and the rarity of CDGs, it may be more helpful for testing laboratories to reach out for clinical information when needed. Common laboratory practice in the past relied on the assumption that all pediatric tests were for inherited disorders and all adult testing was for alcohol ingestion.[12] The expansion of the clinical spectrum of CDGs in recent years has made that paradigm less accurate; however, it may narrow the number of ordered tests that require additional inquiry.[12] If molecular results are available before testing, they should be provided to the testing

**Table 2** Summary of known performance limitations for clinically available testing for glycosylation disorders

| Assay/Analyte | False Positive | False Negative | Other Considerations |
|---|---|---|---|
| Transferrin isoforms | • Classic galactosemia<br>• Fructose intolerance (uncontrolled)<br>• Liver disease<br>• Alcohol use<br>• Hemolytic uremic syndrome | • Prematurity<br>• Transfusion<br>• O-linked or mixed disorders | Transferrin sequence variations may result in false-positive results if profiles are not properly interpreted |
| N-glycans | • Liver disease<br>• Peroxisomal disease<br>• Inflammation | • Neutropenia<br>• Transfusion<br>• GPI anchor disorders | |
| O-glycans (methodology varies) | | • Neutropenia<br>• Transfusion<br>• GPI anchor disorders | Defects may be tissue specific (skeletal or cardiac muscle) |
| Flow cytometry | | • Neutropenia<br>• Transfusion | May require specific request to ordering facility |
| Enzyme analysis | • Sample integrity<br>• Carrier status | • Bone marrow transplant | Only available for PMM2-CDG and MPI-CDG |

*CDG*, congenital disorder of glycosylation; *GPI*, glycosylphosphatidylinositol.

laboratory so that they can be reviewed in tandem with the biochemical testing results and an integrated interpretation can be provided.

## Method validation

Each laboratory must validate the performance characteristics of its specific analytical protocol and periodically verify its performance in accordance with local regulations. In the United States, this includes Clinical Laboratory Improvement Amendments and the College of American Pathologists requirements and potentially state regulations. All assays commonly performed to screen for CDGs are classified as laboratory developed tests and should be documented and validated as such, including compliance with all applicable local, state, and federal regulations. Examples of published method validation approaches are provided by the Clinical and Laboratory Standards Institute.[57,58] Laboratories should also devise procedures to address analytical values outside of their established criteria for performance and for clinical specimens outside established stability parameters.

Matrix-specific quality control (QC) specimens should be included with each batch and typically should include 2 levels of QC, a normal and abnormal specimen. Commercially available QC materials are not available for CDG screening. Laboratories should develop a system for using patient samples or contrived patient samples to ensure the assay's daily performance is acceptable before the release of results. Normal QC samples can be obtained by pooling laboratory specimens or from volunteer donors. Laboratories using residual patient samples for abnormal QC should verify stability and document any changes in QC performance over time. For enzyme assays, heat-inactivated samples may be suitable for abnormal QC materials, although patient samples that demonstrate isolated targeted enzyme deficiencies are preferable. For CDG assays, such as transferrin isoforms and enzyme analysis in which quantitative values are obtained and reported, QC ranges should be established before new QC specimens are put into use. For assays such as glycan analysis, in which there are no quantitative results obtained, any QC samples should be analyzed before use to determine performance and acceptance criteria for each day's run.

## Proficiency testing

Laboratories should participate in proficiency testing programs for all analytes as required by applicable local regulations and accreditations. There is an external proficiency testing program for qualitative analysis of carbohydrate-deficient transferrin through the European Research Network for evaluation and improvement of screening, diagnosis and treatment of inherited disorders of metabolism. Because specialized tests for CDGs are run in a limited number of locations, laboratories will need to develop and maintain internal proficiency testing programs through split samples, interlaboratory exchange, or clinical verification. Laboratories are responsible for ensuring that proficiency testing programs meet all relevant regulations for their respective licensure and accreditation requirements.

## Reference intervals

Laboratory-specific, age-appropriate reference intervals for all reported analytes should be established and periodically validated per applicable recommendations.[59] When literature-based intervals are used, they must be verified by the laboratory before implementation. For all applications, reference intervals should be established on the appropriate specimen type, including all appropriate collection variables (tube type, storage, and transport). For assays that rely largely on qualitative interpretation, such as glycan and oligosaccharide profiles, reference ranges are not provided. For the diagnosis of rare disorders, analysis of confirmed

cases is important to understand the disease range and profiles for interpretation. Given the rarity of most CDGs, interpretations should note any limitations with respect to the described biochemical abnormalities in known patients. Broader identification of affected individuals usually results in an expanded clinical and laboratory phenotype; therefore, generalizing from small numbers of cases should be done with caution. In the case of enzyme analyses, it is important to establish abnormal ranges for confirmed positives and heterozygotes.

## Testing for glycosylation disorders

### Carbohydrate-deficient transferrin analysis

The most common test considered when screening for CDGs is the analysis of transferrin to detect aberrant glycosylation. Transferrin is the second most abundant glycosylated protein in the human body, and it is readily accessible for analysis in plasma and serum. These qualities made it an ideal target molecule for glycosylation screening assays. Because the spectrum of glycosylation disorders has expanded, more and more confirmed disorders are identified as having normal transferrin screening results. Many genetic causes of CDG, such as GPI anchor synthesis disorders or disorders in the glycosylation of α-dystroglycan, do not affect N-glycosylation and are expected to have normal transferrin screening results.

Early methods for the analysis of transferrin isoforms used isoelectric focusing,[50,60,61] however, most laboratories now use tandem mass spectrometry (MS/MS) for transferrin isoform analysis. The 2 most common MS or MS/MS techniques for transferrin analysis are liquid chromatography (LC)-electrospray ionization[62] and matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS).[63] Because of differences in mass resolution, transferrin isoform analysis by MALDI-TOF MS is not able to detect the smaller mass differences observed in some disorders, commonly referred to as type II CDGs; however, it does have an extremely high sensitivity for type I CDG profiles.[64]

Independent of instrument setup differences, the principles of sample preparation are similar. Plasma or serum samples can be used for the analysis of transferrin isoforms. Laboratories should ensure that their validation is appropriate for all accepted sample types. Sample preparation can be done manually or using an automated online process.[62,65] The transferrin in the specimen is enriched using an anti-human (commonly rabbit or goat) transferrin antibody-coupling affinity column. The sample is cleaned up to remove anything (buffers, albumin, and other matrix components) that could interfere with either ionization or MS analysis. Cleanup can be accomplished via solid-phase extraction either offline before injection into the LC system or online via an automated extraction system integrated with the LC.

Depending on the ionization method used, MS/MS detection of transferrin isoforms can be based on multiply charged species (common with electrospray ionization and LC sample introduction systems), which need to be deconvoluted/reconstructed by instrument specific software before interpretation, or singly charged species (common with MALDI and non-LC front ends). In the interpretation of transferrin isoform analyses in their simplest form, a normal specimen should have large amounts of di-oligo (or tetrasialo) transferrin and small (or absent) amounts of the mono-oligo (disialo) species. The absolute amounts of transferrin detected can vary greatly; the determination of normal vs abnormal profiles is based on the ratios between the species rather than absolute abundance. Figure 1 shows examples of normal (A), and type I (B and C) transferrin isoform profiles. Common sequence variations (previously described as common polymorphisms) can change the appearance of transferrin profiles; however, they should be distinguished from true abnormals by experienced interpretation.[61] Abnormalities of galactosylation, such as TMEM165-CDG and PGM1-CDG, can also be identified from transferrin isoform analysis.[66,67]

### ApoCIII glycoform analysis

The relative quantification of apolipoprotein CIII glycoforms can be used to provide clarification of the subtype when transferrin isoform analysis shows a type II profile, and the identification of mixed CDG profiles. This analysis is designed to identify glycoforms with 0, 1, or 2 sialic acids, denoted ApoCIII-0, ApoCIII-1, and ApoCIII-2, respectively. Analysis of the ratio to ApoCIII-2 can be informative for type II and mixed type CDGs. ApoCIII analysis can be multiplexed with transferrin isoform analysis, when performed by LC-MS/MS, as described in the previous section.

### Glycan profiles

High-resolution MS involving TOF MS has allowed for the detection and interpretation of additional abnormal glycans beyond transferrin isoform analysis. Rather than investigating the glycan structure from a single protein, sample preparation for structural glycan analysis cleaves glycans from plasma or serum proteins and uses high-resolution MS to identify structural changes directly.[63] Analysis of both N- and O-linked glycans follows similar principles. Serum samples are incubated to cleave the glycan linkages using PNGaseF for N-linked glycans and β-elimination for O-linked glycans. An incubation step may be added to assist in denaturing the proteins before cleavage. Glycan cleavage typically requires an incubation time of 16 to 24 hours. After cleavage, the freed glycans need to be purified using graphite solid-phase extraction columns. Cleaved and purified glycans must undergo permethylation to improve reproducibility by protecting the terminal glycosidic linkage. After permethylation, additional extraction steps via

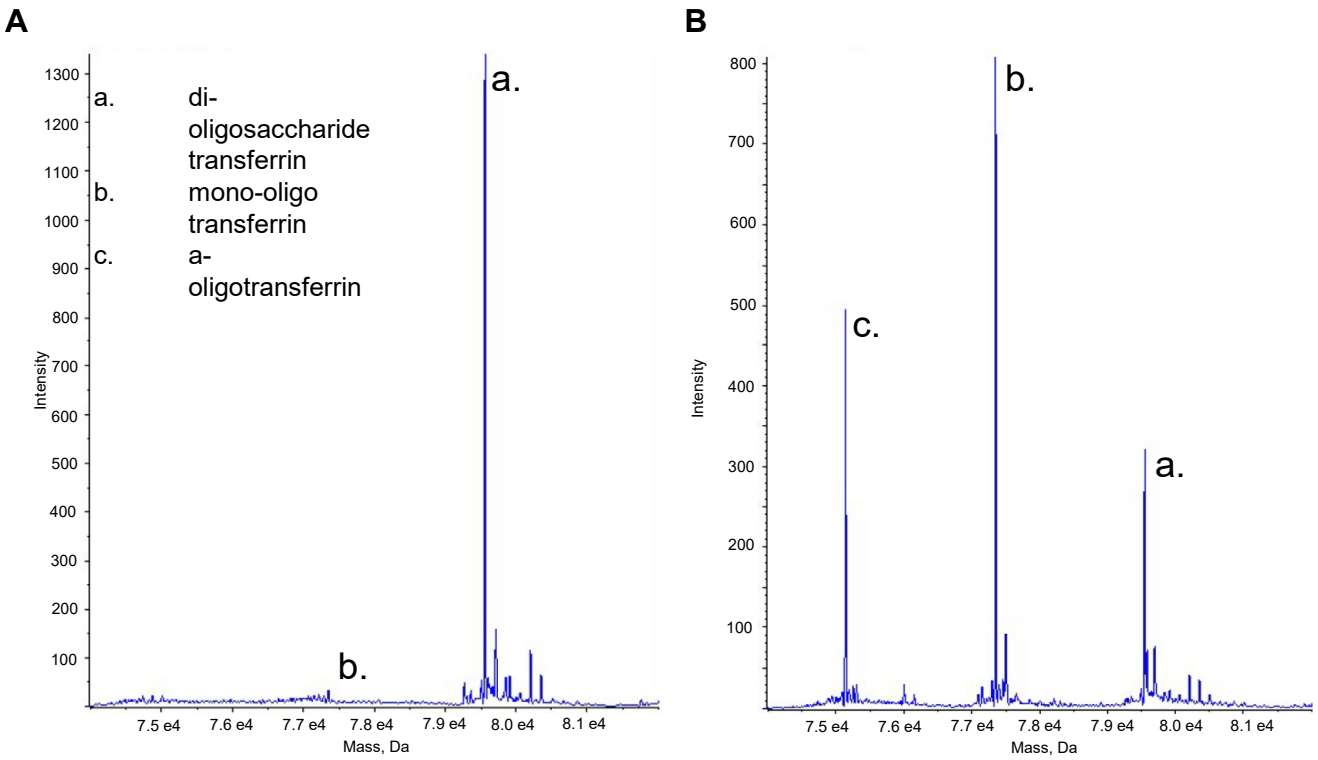


**Figure 1 Transferrin isoform profiles for (A) normal result and (B) PMM2-CDG.** CDG, congenital disorder of glycosylation.

liquid extraction and solid-phase extraction are needed. After the final extraction, the eluate should be dried completely under ultrahigh purity nitrogen.[63]

Analysis using MALDI-TOF MS is most effective for higher-mass glycan species (m/z > 2000); however, it can provide reliable results down to a m/z of ~600. For analysis of N-glycans, this mass range is sufficient. Assessment of O-glycans requires analyses using LC-MS/MS to quantify these species (T-antigen and ApoCIII have been used clinically), although MALDI-TOF MS methods have been published.[68] Dried samples must be reconstituted in an appropriate solvent mixture before spotting on an appropriate MALDI plate for analysis. Sample volumes used for MALDI analysis are very small, typically only 1 to 2 μL. Capturing the profiles is performed using specialized instrument specific software. A common issue with MALDI profiles is low overall intensity, which may cause low-abundance glycans to be missed. Acceptance criteria for profile acceptability should be developed and documented. Resampling from the existing spot or creating a new spot on the MALDI plate from the reconstituted sample are quick troubleshooting steps that should be tried if the intensity is not acceptable. Multiple profiles (replicates) may be helpful for the identification of normal variation compared with true abnormalities. Profiles may be analyzed using instrument software or transferred to image viewing software for easier viewing and interpretation.

Glycan profiles should be reviewed by a qualified and trained laboratory director. Structural predictions and mass variations may be used for the identification of normal and abnormal glycans. The overall profile of abnormal glycans may suggest a specific glycosylation disorder or pathway. N-glycan analysis can provide qualitative identification of a tetrasaccharide that is seen in PMM2-CDG, MPI-CDG, and ALG1-CDG (OMIM 608540), which may be helpful for suggesting a specific diagnosis of these more common disorders.[69]

### Enzyme analysis

Confirmatory enzyme analysis is only clinically available for 2 CDGs, PMM2-CDG and MPI-CDG. Both assays are performed using colorimetric detection on leukocytes or fibroblasts. Isolated leukocytes are incubated with enzyme-specific substrates and buffers to generate a colored product, which is proportional to the amount of nicotinamide adenine dinucleotide phosphate in the reaction mixture. Leukocytes are isolated from whole blood samples according to appropriate protocols. An aliquot, typically standardized for a specific protein concentration, is incubated with enzyme-specific substrates. Testing for PMM2-CDG is the analysis of phosphomannose mutase activity using mannose-1-phosphate as a substrate, and testing for MPI-CDG is the analysis of phosphomannose isomerase activity using mannose-6-phosphate as a substrate. Substrates should be prepared fresh on the day the analysis is being performed. A known quantity of the respective substrate is added to the leukocyte aliquot, and the reaction proceeds. The reaction is performed on a plate reader, allowing the

measurement of absorbance at multiple time points for calculation of enzyme activity. Enzyme activity is calculated using the reaction time, sample volume, protein concentration, well size, and extinction coefficient for nicotinamide adenine dinucleotide phosphate.[70]

These enzyme analyses cannot be multiplexed because the reaction product being measured is the same for both. For both enzymes, there is an overlap in enzyme activity between heterozygotes and affected individuals; therefore, correlation with clinical findings and other laboratory testing is required. During validation, in addition to the reference range from unaffected individuals, heterozygotes and affected individuals should also be analyzed to determine the appropriate ranges. Although the clinical overlap between PMM2-CDG and MPI-CDG is not extensive, performing both enzyme analyses simultaneously is effective at ruling out decreased activity because of sample handling issues, similar to the recommended inclusion of a reference enzyme for lysosomal enzyme analysis.[71] Ideally, leukocyte samples from affected patients should be used as controls, although specimens from affected patients are difficult to find and maintain in sufficient quantity for routine analysis. The use of cultured and prepared fibroblasts as affected controls could be considered. Heat-inactivated controls are an option, although they cannot mimic single-enzyme defects.

### Other laboratory investigations

The scope of this technical standard was limited to targeted assays for the diagnosis of CDGs that are presently clinically available. There are many publications describing methods for the detection of CDGs in both targeted and untargeted approaches; however, these are limited to specific laboratories or performed on a research basis. As clinical utility is proven and knowledge advances, these may become clinically available to patients in the future and should be addressed at that time.

Although not specifically developed or intended to identify CDGs, abnormalities may be present on other assays, including basic laboratory studies and more complex profiles. Urine oligosaccharide screening was developed to identify lysosomal storage disorders based on characteristic excretion patterns in the urine of suspected patients. MS methods have resulted in a significant expansion of the disorders detected by these assays, compared with their original thin-layer chromatography iterations.[72,73] The excretion of abnormal glycan intermediates in urine had not been widely reported; however, after the improved assay had been in use clinically, reports of unusual metabolites that were characteristic of particular CDGs were published.[69] Based on these findings and the relative ease of obtaining a urine sample for screening, high-resolution oligosaccharide screening should be considered as an early screen for CDGs. It can further define a diagnosis when molecular testing results are equivocal for a disorder known to have a characteristic oligosaccharide profile. NGLY1-CDDG (OMIM 615273) was a disorder not thought to have a distinctive biochemical profile until the discovery of an abnormal oligosaccharide in the urine of affected individuals.[74] Additionally, sorbitol, an analyte commonly included in the analysis of polyols for the identification of transaldolase deficiency, has been proposed as a severity biomarker for PMM2-CDG and a potential biomarker for treatments currently being trialed.[75] Flow cytometry can be used for the identification of several glycosylation disorders, specifically those involving defects of the GPI anchor, which can be detected by specialized methods to isolate GPI deficient neutrophils (National Institutes of Health, unpublished data). Individuals with CDGs may have abnormalities on other laboratory tests, including coagulation factors and liver function tests, although these findings are not diagnostic, they can provide important information about the overall clinical picture and supporting evidence when a diagnosis is suspected. Information about liver function and coagulation status can be critical for clinical management.

### Molecular testing

Molecular testing (exome/genome analysis, targeted gene sequencing, gene panels, or copy-number analysis) can be used to confirm a diagnosis suggested by biochemical testing or to identify a glycosylation disorder for which no known clinically available biochemical testing is available. As testing paradigms shift, enzyme and metabolite test results are increasingly being used to confirm uncertain or equivocal results identified by high-throughput sequence analysis. Full technical details about molecular testing and interpretation for CDGs are beyond the scope of this document. Further details regarding next-generation sequencing,[76] variant interpretation,[77] and curation of gene panels[78] have been covered extensively by previous ACMG technical standards and should be referred to for guidance regarding the setup of molecular analysis for CDGs.

## Test interpretation and reporting

### Interpretation

Results should be reviewed and interpreted by an American Board of Medical Genetics and Genomics-certified biochemical geneticist or other similarly qualified individual. Quantitative results should be compared with established reference ranges, and the significance of any deviation should be noted. Comparison with established disease and carrier ranges may be helpful for enzyme assays. When reports are interpreted on a strictly qualitative basis, laboratory directors should ensure that all individuals who are performing reporting understand the scope of the test and are able to accurately identify normal and abnormal profiles, and specific qualitative abnormalities that may point to more specific disorders. Much of this training can be accomplished during method validation by analyzing a wide variety of samples from individuals with normal and

abnormal profiles; however, if that is not possible, stored samples and collaborative review with an experienced laboratory director should be undertaken. QC samples should be selected to represent an array of nonclinically significant conditions (age, gender, and feeding status) to reflect the diversity of normal profiles as accurately as possible.

### Reporting

Important elements to include in patient reports are appropriate patient and specimen information, analyte values reported against reference limits, and interpretive comments. Each report should provide clear information about the results of the assay performed, integrating clinical information and the results of other laboratory testing as available and appropriate. Many aspects of abnormal testing for glycosylation disorders are based on qualitative profile review by experienced laboratorians, rather than deviations from established reference ranges. These observations should be described accurately and concisely, and attempts should be made to standardize the language used to minimize confusion. Disorder nomenclature for CDGs has been standardized in recent years to focus on the gene involved in the defect,[17] and this should be reflected in laboratory reports. Common name reporting may still be used, particularly for more common CDGs, or when referencing older literature. Laboratory reports should include appropriate next steps, such as further testing (molecular analysis, familial testing, or further biochemical studies) or appropriate consultations, and known limitations of the assay.

Laboratory testing for CDGs is complex and requires a high degree of technical and interpretive expertise. The performance of each assay, including limitations, is important for all parties to understand, and using the complementary aspects of biochemical and molecular testing to confirm or rule out diagnoses in this expanding group of disorder.

## Conflict of Interest

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## References

1. Korner C, Thiel C, Hoffmann G. Congenital disorders of glycosylation. In: Sarafoglou K, Hoffmann G, Roth K, eds. *Pediatric Endocrinology and Inborn Errors of Metabolism*. McGraw-Hill; 2009:339-352.
2. Jaeken J, Péanne R. What is new in CDG? *J Inherit Metab Dis*. 2017;40(4):569-586. http://doi.org/10.1007/s10545-017-0050-6
3. Himmelreich N, Kikul F, Zdrazilova L, et al. Complex metabolic disharmony in PMM2-CDG paves the way to new therapeutic approaches. *Mol Genet Metab*. 2023;139(3):107610. http://doi.org/10.1016/j.ymgme.2023.107610
4. Ng BG, Freeze HH, Himmelreich N, Blau N, Ferreira CR. Clinical and biochemical footprints of congenital disorders of glycosylation: proposed nosology. *Mol Genet Metab*. 2024;142(1):108476. http://doi.org/10.1016/j.ymgme.2024.108476
5. Bruneel A, Cholet S, Tran NT, Mai TD, Fenaille F. CDG biochemical screening: where do we stand? *Biochim Biophys Acta Gen Subj*. 2020;1864(10):129652. http://doi.org/10.1016/j.bbagen.2020.129652
6. De Graef D, Ligezka AN, Rezents J, et al. Coagulation abnormalities in a prospective cohort of 50 patients with PMM2-congenital disorder of glycosylation. *Mol Genet Metab*. 2023;139(2):107606. http://doi.org/10.1016/j.ymgme.2023.107606
7. Tiwary H, Hecht LE, Brucker WJ, Berry GT, Rodig NM. The development of end stage renal disease in two patients with PMM2-CDG. *JIMD Rep*. 2022;63(2):131-136. http://doi.org/10.1002/jmd2.12269
8. Lefeber DJ, Morava E, Jaeken J. How to find and diagnose a CDG due to defective N-glycosylation. *J Inherit Metab Dis*. 2011;34(4):849-852. http://doi.org/10.1007/s10545-011-9370-0
9. Freeze HH, Jaeken J, Matthijs G. CDG or not CDG. *J Inherit Metab Dis*. 2022;45(3):383-385. http://doi.org/10.1002/jimd.12498
10. Pajusalu S, Vals MA, Mihkla L, Šamarina U, Kahre T, Õunap K. The estimated prevalence of N-linked congenital disorders of glycosylation across various populations based on allele frequencies in general population databases. *Front Genet*. 2021;12:719437. http://doi.org/10.3389/fgene.2021.719437
11. Tao H, Sun Y, Zhai J, Wu J. DPAGT1-CDG: recurrent fetal death. *Birth Defects Res*. 2023;115(13):1185-1191. http://doi.org/10.1002/bdr2.2219
12. Helander A, Jaeken J, Matthijs G, Eggertsen G. Asymptomatic phosphomannose isomerase deficiency (MPI-CDG) initially mistaken for excessive alcohol consumption. *Clin Chim Acta*. 2014;431:15-18. http://doi.org/10.1016/j.cca.2014.01.018
13. Carchon H, Van Schaftingen E, Matthijs G, Jaeken J. Carbohydrate-deficient glycoprotein syndrome type IA (phosphomannomutase-deficiency). *Biochim Biophys Acta*. 1999;1455(2-3):155-165. http://doi.org/10.1016/s0925-4439(99)00073-3
14. Schachter H, Jaeken J. Carbohydrate-deficient glycoprotein syndrome type II. *Biochim Biophys Acta*. 1999;1455(2-3):179-192. http://doi.org/10.1016/s0925-4439(99)00054-x
15. Gordon N. Carbohydrate-deficient glycoprotein syndromes. *Postgrad Med J*. 2000;76(893):145-149. http://doi.org/10.1136/pmj.76.893.145
16. Freeze HH, Schachter H, Kinoshita T. Genetic disorders of glycosylation. In: Varki A, Cummings RD, Esko JD, et al., eds. *Essentials of Glycobiology*. 3rd Ed.: Cold Spring Harbor Laboratory Press; 2015:569-582.
17. Jaeken J, Hennet T, Matthijs G, Freeze HH. CDG nomenclature: time for a change. *Biochim Biophys Acta*. 2009;1792(9):825-826. http://doi.org/10.1016/j.bbadis.2009.08.005

18. Jaeken J, Matthijs G. Congenital disorders of glycosylation: a rapidly expanding disease family. *Annu Rev Genomics Hum Genet*. 2007;8:261-278. http://doi.org/10.1146/annurev.genom.8.080706.092327
19. Sparks SE, Krasnewich DM. Congenital disorders of N-linked glycosylation and multiple pathway overview. In: GeneReviews. Seattle: University of Washington; 2005. In: Adam MP, Mirzaa GM, Pago RA, et al., eds. Last update January 12, 2017. Accessed January 27, 2023. https://www.ncbi.nlm.nih.gov/sites/books/NBK1332/
20. Funke S, Gardeitchik T, Kouwenberg D, et al. Perinatal and early infantile symptoms in congenital disorders of glycosylation. *Am J Med Genet A*. 2013;161A(3):578-584. http://doi.org/10.1002/ajmg.a.35702
21. Makhamreh MM, Cottingham N, Ferreira CR, Berger S, Al-Kouatly HB. Nonimmune hydrops fetalis and congenital disorders of glycosylation: a systematic literature review. *J Inherit Metab Dis*. 2020;43(2):223-233. http://doi.org/10.1002/jimd.12162
22. Freeze HH, Schachter H. Genetic disorders of glycosylation. In: Varki A, Cummings RD, Esko JD, et al., eds. *Essentials of Glycobiology*. 2nd ed. Cold Spring Harbor Laboratory Press; 2009:585-600.
23. Lesnik Oberstein SAJ, Kriek M, White SJ, et al. Peters Plus syndrome is caused by mutations in B3GALTL, a putative glycosyltransferase. *Am J Hum Genet*. 2006;79(3):562-566. http://doi.org/10.1086/507567
24. Endo T. Glycobiology of α-dystroglycan and muscular dystrophy. *J Biochem*. 2015;157(1):1-12. http://doi.org/10.1093/jb/mvu066
25. Mercuri E, Messina S, Bruno C, et al. Congenital muscular dystrophies with defective glycosylation of dystroglycan: a population study. *Neurology*. 2009;72(21):1802-1809. http://doi.org/10.1212/01.wnl.0000346518.68110.60
26. Topaloglu H. Abnormal glycosylation of the alpha-dystroglycan: deficient sugars are no good. *Neurology*. 2009;72(21):1798-1799. http://doi.org/10.1212/WNL.0b013e3181a4e451
27. Zilmer M, Edmondson AC, Khetarpal SA, et al. Novel congenital disorder of O-linked glycosylation caused by GALNT2 loss of function. *Brain*. 2020;143(4):1114-1126. http://doi.org/10.1093/brain/awaa063
28. Jezela-Stanek A, Ciara E, Piekutowska-Abramczuk D, et al. Congenital disorder of glycosylphosphatidylinositol (GPI)-anchor biosynthesis—the phenotype of two patients with novel mutations in the PIGN and PGAP2 genes. *Eur J Paediatr Neurol*. 2016;20(3):462-473. http://doi.org/10.1016/j.ejpn.2016.01.007
29. Wu T, Yin F, Guang S, He F, Yang L, Peng J. The glycosylphosphatidylinositol biosynthesis pathway in human diseases. *Orphanet J Rare Dis*. 2020;15(1):129. http://doi.org/10.1186/s13023-020-01401-z
30. Freeze HH, Chong JX, Bamshad MJ, Ng BG. Solving glycosylation disorders: fundamental approaches reveal complicated pathways. *Am J Hum Genet*. 2014;94(2):161-175. http://doi.org/10.1016/j.ajhg.2013.10.024
31. Schollen E, Kjaergaard S, Legius E, Schwartz M, Matthijs G. Lack of Hardy-Weinberg equilibrium for the most prevalent PMM2 mutation in CDG-Ia (congenital disorders of glycosylation type Ia). *Eur J Hum Genet*. 2000;8(5):367-371. http://doi.org/10.1038/sj.ejhg.5200470
32. Vals MA, Pajusalu S, Kals M, Mägi R, Õunap K. The prevalence of PMM2-CDG in Estonia based on population carrier frequencies and diagnosed patients. *JIMD Rep*. 2018;39:13-17. http://doi.org/10.1007/8904_2017_41
33. Piedade A, Francisco R, Jaeken J, et al. Epidemiology of congenital disorders of glycosylation (CDG)—overview and perspectives. *J Rare Dis*. 2022;1(1):3. http://doi.org/10.1007/s44162-022-00003-6
34. Grünewald S. Congenital disorders of glycosylation: rapidly enlarging group of (neuro)metabolic disorders. *Early Hum Dev*. 2007;83(12):825-830. http://doi.org/10.1016/j.earlhumdev.2007.09.016
35. Almeida AM, Murakami Y, Baker A, et al. Targeted therapy for inherited GPI deficiency. *N Engl J Med*. 2007;356(16):1641-1647. http://doi.org/10.1056/NEJMoa063369
36. Brasil S, Allocca M, Magrinho SCM, et al. Systematic review: drug repositioning for congenital disorders of glycosylation (CDG). *Int J Mol Sci*. 2022;23(15):8725. http://doi.org/10.3390/ijms23158725
37. Brasil S, Pascoal C, Francisco R, et al. CDG therapies: from bench to bedside. *Int J Mol Sci*. 2018;19(5):1304. http://doi.org/10.3390/ijms19051304
38. Thiel C, Körner C. Therapies and therapeutic approaches in congenital disorders of glycosylation. *Glycoconj J*. 2013;30(1):77-84. http://doi.org/10.1007/s10719-012-9447-5
39. Janssen MCH, de Kleine RH, van den Berg AP, et al. Successful liver transplantation and long-term follow-up in a patient with MPI-CDG. *Pediatrics*. 2014;134(1):e279-e283. http://doi.org/10.1542/peds.2013-2732
40. Morava E. Galactose supplementation in phosphoglucomutase-1 deficiency; review and outlook for a novel treatable CDG. *Mol Genet Metab*. 2014;112(4):275-279. http://doi.org/10.1016/j.ymgme.2014.06.002
41. Verheijen J, Tahata S, Kozicz T, Witters P, Morava E. Therapeutic approaches in congenital disorders of glycosylation (CDG) involving N-linked glycosylation: an update. *Genet Med*. 2020;22(2):268-279. http://doi.org/10.1038/s41436-019-0647-2
42. Morelle W, Potelle S, Witters P, et al. Galactose supplementation in patients with TMEM165-CDG rescues the glycosylation defects. *J Clin Endocrinol Metab*. 2017;102(4):1375-1386. http://doi.org/10.1210/jc.2016-3443
43. Witters P, Tahata S, Barone R, et al. Clinical and biochemical improvement with galactose supplementation in SLC35A2-CDG. *Genet Med*. 2020;22(6):1102-1107. http://doi.org/10.1038/s41436-020-0767-8
44. Marquardt T, Lühn K, Srikrishna G, Freeze HH, Harms E, Vestweber D. Correction of leukocyte adhesion deficiency type II with oral fucose. *Blood*. 1999;94(12):3976-3985.
45. Kuki I, Takahashi Y, Okazaki S, et al. Vitamin $B_6$-responsive epilepsy due to inherited GPI deficiency. *Neurology*. 2013;81(16):1467-1469. http://doi.org/10.1212/WNL.0b013e3182a8411a
46. Rymen D, Lindhout M, Spanou M, et al. Expanding the clinical and genetic spectrum of CAD deficiency: an epileptic encephalopathy treatable with uridine supplementation. *Genet Med*. 2020;22(10):1589-1597. http://doi.org/10.1038/s41436-020-0933-z
47. Brucker WJ, Croteau SE, Prensner JR, et al. An emerging role for endothelial barrier support therapy for congenital disorders of glycosylation. *J Inherit Metab Dis*. 2020;43(4):880-890. http://doi.org/10.1002/jimd.12225
48. Wada Y, Kadoya M, Okamoto N. Mass spectrometry of transferrin and apolipoprotein CIII from dried blood spots for congenital disorders of glycosylation. *Mass Spectrom (Tokyo)*. 2022;11(1):A0113. http://doi.org/10.5702/massspectrometry.A0113
49. Pérez-Cerdá C, Quelhas D, Vega AI, Ecay J, Vilarinho L, Ugarte M. Screening using serum percentage of carbohydrate-deficient transferrin for congenital disorders of glycosylation in children with suspected metabolic disease. *Clin Chem*. 2008;54(1):93-100. http://doi.org/10.1373/clinchem.2007.093450
50. Xin Y, Lasker JM, Rosman AS, Lieber CS. Isoelectric focusing/western blotting: a novel and practical method for quantitation of carbohydrate-deficient transferrin in alcoholics. *Alcohol Clin Exp Res*. 1991;15(5):814-821. http://doi.org/10.1111/j.1530-0277.1991.tb00607.x
51. Magalhães APPS, Burin MG, Souza CFM, et al. Transferrin isoelectric focusing for the investigation of congenital disorders of glycosylation: analysis of a ten-year experience in a Brazilian center. *J Pediatr (Rio J)*. 2020;96(6):710-716. http://doi.org/10.1016/j.jped.2019.05.008
52. Li H, Byers HM, Diaz-Kuan A, et al. Acute liver failure in neonates with undiagnosed hereditary fructose intolerance due to exposure from widely available infant formulas. *Mol Genet Metab*. 2018;123(4):428-432. http://doi.org/10.1016/j.ymgme.2018.02.016
53. Lam C, Levy D, Ciccone C, Krasnewich D, Gahl W, Wolfe L. The yield of biochemical screens for congenital disorders of glycosylation. *Mol Genet Metab*. 2016;117:267.
54. Thiel C, Meßner-Schmitt D, Hoffmann GF, Körner C. Screening for congenital disorders of glycosylation in the first weeks of life. *J Inherit*

*Metab Dis*. 2013;36(5):887-892. http://doi.org/10.1007/s10545-012-9531-9

55. Vermeer S, Kremer HPH, Leijten QH, et al. Cerebellar ataxia and congenital disorder of glycosylation Ia (CDG-Ia) with normal routine CDG screening. *J Neurol*. 2007;254(10):1356-1358. http://doi.org/10.1007/s00415-007-0546-3
56. Hall PL, Liedke K, Turgeon C, et al. Sensitivity of transferrin isoform analysis for PMM2-CDG. *Mol Genet Metab*. 2024;143(1-2):108564. http://doi.org/10.1016/j.ymgme.2024.108564
57. Clinical and Laboratory Standards Institute. *Statistical Quality Control for Quantitative Measurement Procedures: Principles and Definitions:* C24-A3. 3rd ed. Clinical and Laboratory Standards Institute; 2006. Accessed January 27, 2023. https://clsi.org/standards/products/clinical-chemistry-and-toxicology/documents/c24/
58. Clinical and Laboratory Standards Institute. *Liquid Chromatography-Mass Spectrometry Methods:* C62. 2nd ed. Clinical and Laboratory Standards Institute; 2022. Accessed January 27, 2023. https://clsi.org/standards/products/clinical-chemistry-and-toxicology/documents/c62/
59. Clinical and Laboratory Standards Institute. *Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory*: EP28-A3C. 3rd ed. Clinical and Laboratory Standards Institute; 2010. Accessed January 27, 2023. https://clsi.org/standards/products/method-evaluation/documents/ep28/
60. Kilár F, Hjertén S. Fast and high resolution analysis of human serum transferrin by high performance isoelectric focusing in capillaries. *Electrophoresis*. 1989;10(1):23-29. http://doi.org/10.1002/elps.1150100107
61. Sebetan IM, Oshida S, Akaishi S. Transferrin (Tf) polymorphism: an analysis by isoelectric focusing. *Forensic Sci Int*. 1982;19(3):281-286. http://doi.org/10.1016/0379-0738(82)90091-3
62. Lacey JM, Bergen HR, Magera MJ, Naylor S, O'Brien JF. Rapid determination of transferrin isoforms by immunoaffinity liquid chromatography and electrospray mass spectrometry. *Clin Chem*. 2001;47(3):513-518. http://doi.org/10.1093/clinchem/47.3.513
63. Xia B, Zhang W, Li X, et al. Serum N-glycan and O-glycan analysis by mass spectrometry for diagnosis of congenital disorders of glycosylation. *Anal Biochem*. 2013;442(2):178-185. http://doi.org/10.1016/j.ab.2013.07.037
64. Li X, Raihan MA, Reynoso FJ, He M. Glycosylation analysis for congenital disorders of glycosylation. *Curr Protoc Hum Genet*. 2015;86:17.18.1-17.18.22. http://doi.org/10.1002/0471142905.hg1718s86
65. O'Brien JF, Lacey JM, Bergen HR. III Detection of hypo-N-glycosylation using mass spectrometry of transferrin. *Curr Protoc Hum Genet*. 2007; Chapter 17:Unit 17.4 https://doi.org/10.1002/0471142905.hg1704s54.
66. Foulquier F, Amyere M, Jaeken J, et al. TMEM165 deficiency causes a congenital disorder of glycosylation. *Am J Hum Genet*. 2012;91(1):15-26. http://doi.org/10.1016/j.ajhg.2012.05.002
67. Altassan R, Radenkovic S, Edmondson AC, et al. International consensus guidelines for phosphoglucomutase 1 deficiency (PGM1-CDG): diagnosis, follow-up, and management. *J Inherit Metab Dis*. 2021;44(1):148-163. http://doi.org/10.1002/jimd.12286
68. Wada Y, Okamoto N. Apolipoprotein C-III O-glycoform profiling of 500 serum samples by matrix-assisted laser desorption/ionization mass spectrometry for diagnosis of congenital disorders of glycosylation. *J Mass Spectrom*. 2021;56(4):e4597. http://doi.org/10.1002/jms.4597
69. Zhang W, James PM, Ng BG, et al. A novel N-tetrasaccharide in patients with congenital disorders of glycosylation, including asparagine-linked glycosylation protein 1, phosphomannomutase 2, and mannose phosphate isomerase deficiencies. *Clin Chem*. 2016;62(1):208-217. http://doi.org/10.1373/clinchem.2015.243279
70. Dave MB, Dherai AJ, Udani VP, Ashavaid TF. Leukocyte phosphomannomutase and phosphomannose isomerase activity in an Indian cohort. *Indian J Clin Biochem*. 2022;37(2):238-241. http://doi.org/10.1007/s12291-020-00930-5
71. Strovel ET, Cusmano-Ozog K, Wood T, Yu C. Measurement of lysosomal enzyme activities: a technical standard of the American College of Medical Genetics and Genomics (ACMG). *Genet Med*. 2022;24(4):769-783. http://doi.org/10.1016/j.gim.2021.12.013
72. Bonesso L, Piraud M, Caruba C, Van Obberghen E, Mengual R, Hinault C. Fast urinary screening of oligosaccharidoses by MALDI-TOF/TOF mass spectrometry. *Orphanet J Rare Dis*. 2014;9:19. http://doi.org/10.1186/1750-1172-9-19
73. Xia B, Asif G, Arthur L, et al. Oligosaccharide analysis in urine by MALDI-TOF mass spectrometry for the diagnosis of lysosomal storage diseases. *Clin Chem*. 2013;59(9):1357-1368. http://doi.org/10.1373/clinchem.2012.201053
74. Hall PL, Lam C, Alexander JJ, et al. Urine oligosaccharide screening by MALDI-TOF for the identification of NGLY1 deficiency. *Mol Genet Metab*. 2018;124(1):82-86. http://doi.org/10.1016/j.ymgme.2018.03.002
75. Ligezka AN, Radenkovic S, Saraswat M, et al. Sorbitol is a severity biomarker for PMM2-CDG with therapeutic implications. *Ann Neurol*. 2021;90(6):887-900. http://doi.org/10.1002/ana.26245
76. Rehm HL, Bale SJ, Bayrak-Toydemir P, et al. ACMG clinical laboratory standards for next-generation sequencing. *Genet Med*. 2013;15(9):733-747. http://doi.org/10.1038/gim.2013.92
77. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015;17(5):405-424. http://doi.org/10.1038/gim.2015.30
78. Bean LJH, Funke B, Carlston CM, et al. Diagnostic gene sequencing panels: from design to report-a technical standard of the American College of Medical Genetics and Genomics (ACMG). *Genet Med*. 2020;22(3):453-461. http://doi.org/10.1038/s41436-019-0666-z