CAT
Critically Appraised Topic

Titel: Laboratory diagnosis of galactosemia

Author: Ben Masure
Supervisor: Prof. Dr. Pieter Vermeersch
Search/methodology verified by: Prof. Dr. Pieter Vermeersch
Date: 17/8/2026

Clinical bottom line

Galactosemia is a disease caused by deficient activity of one of four enzymes of the Leloir-pathway which results in elevated blood galactose concentration. Clinical presentations can be categorized into two main clinical scenarios: neonatal liver dysfunction and cataracts. Galactose-1-phosphate uridylyltransferase (GALT) deficiency, the most frequent form of galactosemia, typically presents with neonatal liver dysfunction and can be lethal if undetected due to E. coli sepsis. Galactokinase (GALK1) deficiency causes cataract and uridine diphosphate galactose-4-epimerase (GALE) deficiency only presents with liver failure in its rare generalized form, while the far more common peripheral and intermediate forms are clinically benign. The clinical significance of galactose mutarotase (GALM) deficiency is unclear.
Currently, in contrast to surrounding regions such as the Netherlands, Germany and Wallonia-Brussels, GALT deficiency is not part of the newborn screening in the Flemish community. Of note, while newborn screening is performed to identify GALT deficiency, other forms of galactosemia are detected as "by-catch". The diagnosis of GALT deficiency in Flanders therefore depends on clinical suspicion and selected laboratory tests.
Measurement of GALT activity in red blood cells is the preferred test as the test does not require patients to be on a diet containing galactose. The only contra-indication is blood transfusion. Measurement of galactose and galactose-1-phosphate (Gal-1-P) in red blood cells and galactitol (urine) can be false-negative in patients on a galactose-free diet. These two tests do, however, have the advantage that they can also help identify patients with the other 3 forms of galactosemia. The measurement of reducing sugars, a simple biochemical test which was previously used for screening for GALT deficiency, has become obsolete due to its limited sensitivity, in particular in patients with a low lactose intake.

Clinical/Diagnostic scenario

Clinical scenario
For galactosemia two important clinical scenarios exist.

The most severe with jaundice, hepatomegaly, vomiting, hypoglycemia, sepsis (E. coli) and failure to thrive in a neonate in the first days after the start of milk feeding. This is the typical clinical presentation of classic galactosemia (type I galactosemia or GALT deficiency) or more rarely of the generalized form of GALE deficiency (type III galactosemia). A key difference between generalized epimerase deficiency galactosemia and intermediate or peripheral epimerase deficiency galactosemia is that individuals with generalized epimerase deficiency galactosemia develop clinical findings on a normal milk diet, while infants with peripheral or intermediate epimerase deficiency galactosemia remain clinically well, at least in the neonatal period (Openo & Schulz, 2009).

The other possible presentation is cataracts during infancy. This presentation is typical for galactokinase deficiency (type II galactosemia, GALK1) and the recently discovered galactose mutarotase deficiency (type IV galactosemia, GALM).

Duarte variant galactosemia (D/G) and peripheral or intermediate form of GALE are benign forms but can have abnormal screening tests.

Currently, in contrast to surrounding regions (Loeber et al., 2021), classic galactosemia is not part of the newborn screening in the Flemish community.

Diagnostic scenario
Historically, screening of galactosemia was performed with Benedict's test, a screening test for reducing sugars, followed by thin layer chromatography (TLC). The appropriateness of those tests has been questioned due to a

lack of sensitivity since the rise of GC-MS for the measurement of galactose and/or galactitol and the availability of enzyme testing in red blood cells. Both the Benedict's test and measurement of galactose/galactitol can be false-negative in patient on a galactose-free (or lactose-free) diet.
The turn-around time (TAT) of GC-MS measurement of galactose and/or galactitol, the sugar alcohol of galactose, is typically around 1 to 2 weeks.

The gold standard method for the detection of GALT deficiency is the measurement of GALT enzyme activity in red blood cells. This quantitative test is very sensitive and can differentiate between classic GALT deficiency (no significant residual GALT activity), Duarte (significant albeit low residual enzyme activity), carriers and unaffected individuals. The test can be performed in patients on a galactose-free diet. Of note, the test is unreliable if the patient has received a recent blood transfusion. Measurement of GALT activity in dried blood spots as used on some newborn screening programs is only semiquantitative. Measurement of Gal-1-P in red blood cells or measurement of total galactose (sum of galactose and Gal-1-P) can also be used but can be false negative if the patient is on a galactose-free (or lactose-free diet) diet. Lactose is the major source of galactose in children. Measurement of total galactose is used in some newborn screening programs. As galactose is converted to Gal-1-P in red blood cells except in patients who lack galactokinase, measurement of total galactose can detect all forms of galactosemia.

Genetic testing is also possible, but has a longer TAT and might be inconclusive in case of variants of unknown significance.

In the Flemish community of Belgium, classic galactosemia is not part of the newborn screening (NBS). This is in contrast to the Wallonia-Brussels community and neighboring countries.

Question(s)

1) Question 1

Which types of galactosemia exist, and what is the clinical scenario?

2) Question 2

Which tests are needed for diagnosis?

3) Question 3

What tests are needed for follow-up of galactosemia?

4) Question 4

Which tests can be used for NBS?

Search terms

1) MeSH Database (PubMed): MeSH term: " (galactosemia[MeSH Terms]) AND (diagnosis[MeSH Terms])"
2) American College of Medical Genetics and Genomics (ACMG)
3) *UpToDate Online*

Relevant evidence/References

Alonso-Fernández, J. R., & Patel, V. (2012). Dietary sugars: TLC screening of sugars in urine and blood samples. Royal Society of Chemistry. https://doi.org/10.1039/9781849734929-00186

Badiu Tișa, I., Achim, A. C., & Cozma-Petruț, A. (2022). The importance of neonatal screening for galactosemia. Nutrients, 15(1), 10. https://doi.org/10.3390/nu15010010

Benedict, S. R. (1909). A reagent for the detection of reducing sugars. Journal of Biological Chemistry, 5, 485–487.

Fridovich-Keil, J. L., Gambello, M. J., Singh, R. H., & Sharer, J. D. (2020). Duarte variant galactosemia. In M. P. Adam, J. Feldman, G. M. Mirzaa, R. A. Pagon, S. E. Wallace, & A. Amemiya (Eds.), GeneReviews. University of Washington, Seattle.

Hennermann, J. B., Schadewaldt, P., Vetter, B., Shin, Y. S., Mönch, E., & Klein, J. (2011). Features and outcome of galactokinase deficiency in children diagnosed by newborn screening. Journal of Inherited Metabolic Disease, 34(2), 399–407. https://doi.org/10.1007/s10545-010-9270-8

Ko, D. H., Jun, S. H., Park, H. D., Song, S. H., Park, K. U., Kim, J. Q., Song, Y. H., & Song, J. (2010). Multiplex enzyme assay for galactosemia using ultraperformance liquid chromatography–tandem mass spectrometry. Clinical Chemistry, 56(5), 764–771. https://doi.org/10.1373/clinchem.2009.139618

Loeber, J. G., Platis, D., Zetterström, R. H., Almashanu, S., Boemer, F., Bonham, J. R., Borde, P., Brincat, I., Cheillan, D., Dekkers, E., Dimitrov, D., Fingerhut, R., Franzson, L., Groselj, U., Hougaard, D., Knapkova, M., Kocova, M., Kotori, V., Kozich, V., … Schielen, P. C. J. I. (2021). Neonatal screening in Europe revisited: An ISNS perspective on the current state and developments since 2010. International Journal of Neonatal Screening, 7(1), 15. https://doi.org/10.3390/ijns7010015

Morell-Garcia, D., Bauça, J. M., Barceló, A., Perez-Esteban, G., & Vila, M. (2014). Usefulness of Benedict's test for the screening of galactosemia. Clinical Biochemistry, 47(9), 857–859. https://doi.org/10.1016/j.clinbiochem.2014.02.005

Openo, K. P., & Schulz, J. M. (2009). Epimerase deficiency galactosemia. In M. P. Adam, G. M. Mirzaa, R. A. Pagon, S. E. Wallace, L. J. H. Bean, K. W. Gripp, & A. Amemiya (Eds.), GeneReviews. University of Washington, Seattle. https://www.ncbi.nlm.nih.gov/books/NBK51671/

Pasquali, M., Yu, C., & Coffee, B. (2018). Laboratory diagnosis of galactosemia: A technical standard and guideline of the American College of Medical Genetics and Genomics (ACMG). Genetics in Medicine, 20(1), 3–11. https://doi.org/10.1038/gim.2017.172

Satekge, T. M., Kiabilua, O., Krause, A., & Pillay, T. S. (2020). Five years of screening for galactosaemia in South Africa: Pitfalls of using Benedict's test and thin layer chromatography to screen for galactosaemia in a developing country. Clinica Chimica Acta, 505, 73–77. https://doi.org/10.1016/j.cca.2020.02.018

Succoio, M., Sacchettini, R., Rossi, A., Parenti, G., & Ruoppolo, M. (2022). Galactosemia: Biochemistry, molecular genetics, newborn screening, and treatment. Biomolecules, 12(7), 968. https://doi.org/10.3390/biom12070968

Teixeira, L. F., Prauchner, G. R. K., Gusso, D., & Wyse, A. T. S. (2024). Classical hereditary galactosemia: Findings in patients and animal models. Metabolic Brain Disease, 39(1), 239–248. https://doi.org/10.1007/s11011-023-01281-9

Wada, Y., Kikuchi, A., Arai-Ichinoi, N., Sakamoto, O., Takezawa, Y., Iwasawa, S., Niihori, T., Nyuzuki, H., Nakajima, Y., Ogawa, E., Ishige, M., Hirai, H., Sasai, H., Fujiki, R., Shirota, M., Funayama, R., Yamamoto, M., Ito, T., Ohara, O., … Kure, S. (2019). Biallelic GALM pathogenic variants cause a novel type of galactosemia. Genetics in Medicine, 21(6), 1286–1294. https://doi.org/10.1038/s41436-018-0340-x

Welling, L., Bernstein, L. E., Berry, G. T., Burlina, A. B., Eyskens, F., Gautschi, M., Grünewald, S., Gubbels, C. S., Knerr, I., Labrune, P., van der Lee, J. H., MacDonald, A., Murphy, E., Portnoi, P. A., Õunap, K., Potter, N. L., Rubio-Gozalbo, M. E., Spencer, J. B., Timmers, I., … Bosch, A. M. (2017). International clinical guideline for the management of classical galactosemia: Diagnosis, treatment, and follow-up. Journal of Inherited Metabolic Disease, 40(2), 171–176. https://doi.org/10.1007/s10545-016-9990-5

Yuzyuk, T., Viau, K., Andrews, A., Pasquali, M., & Longo, N. (2018). Biochemical changes and clinical outcomes in 34 patients with classic galactosemia. Journal of Inherited Metabolic Disease, 41(2), 197–208. https://doi.org/10.1007/s10545-018-0136-9

Appraisal

Which types of galactosemia exist, and what is the clinical scenario?

Galactosemia is a disease caused by deficient activity of one of four enzymes of the Leloir-pathway which results in elevated blood galactose concentration. The four enzymes are galactose-1-phosphate uridylyltransferase (GALT), galactokinase (GALK1), uridine diphosphate galactose-4-epimerase (GALE) (Pasquali et al., 2018) and galactose mutarotase (GALM) (Wada et al., 2019). (See attachment 1,2,3)

GALT deficiency (classic galactosemia; type I galactosemia) is an enzyme deficiency in the third enzyme of the Leloir pathway (galactose-1-phosphate uridylyltransferase). Patients often present with life-threatening jaundice, vomiting and hepatomegaly during the first days of life, which resolves after dairy restriction, and with long-term complications including cognitive impairment, memory impairment, executive dysfunction, language disability and cataract later in life (Teixeira et al., 2024).

Duarte variant galactosemia is also an enzyme deficiency in GALT, however with still up to 25% of residual enzyme activity compared to controls. Patients are typically asymptomatic and show no difference in prevalence of acute issues seen in the general newborn population and no detectable differences in developmental outcome (Fridovich-Keil et al., 2020).

GALK1 deficiency (type II galactosemia) is an enzyme deficiency in the second enzyme of the Leloir pathway. GALK1 catalyses the phosphorylation of D-galactose to form Gal-1-P. Phenotypes differ between patients, the most severe is associated with insoluble enzyme, while milder phenotypes are characterized by soluble enzyme with impaired catalytic function (Pasquali et al., 2018).

GALE deficiency (type III galactosemia) is a deficiency in the enzyme that catalyzes the (reversible) conversion of UDP-galactose to UDP-glucose and of UDP-N-acetylgalactosamine to UDP-N-acetylglucosamine. The clinical features of GALE deficiency are variable. Three clinical forms exist:

1. Generalized form, with profound deficiency in all tissues. Clinical symptoms are like classic galactosemia.
2. Intermediate form, with profound deficiency in red blood cells (RBCs) and white blood cells (WBCs) and partial deficiency in other tissues. Patients with intermediate form are typically asymptomatic.
3. Peripheral form, with deficiency limited to RBCs and WBCs. Patients are typically asymptomatic (Pasquali et al., 2018).

GALM encodes galactose mutarotase, which catalyzes epimerization between β- and α-D-galactose, the first step of the Leloir pathway. Type IV galactosemia is just recently discovered in 2018 through positive results in newborn screening in Japan, without deficiencies in the other enzymes of the Leloir pathway, Fanconi-Bickel syndrome, portosystemic shunts, cholestasis or other metabolic disorders. Of the 8 discovered patients, 2 developed cataracts. Other clinical features typical for galactosemia were not yet discovered in patients with GALM deficiency (Wada et al., 2019).

Which tests are needed for diagnosis?

1. Biochemical enzyme assays

GALT activity in red blood cells (type I, classic galactosemia)

This enzyme assay is specific for classic galactosemia. Two important types of assays exist, one based on UDP-glucose consumption and the second on UDP-galactose production.

The consumption assays are based on patient hemolysates incubated with UDP-glucose and Gal-1-P. The residual, unconsumed UDP-glucose is subsequently quantified by adding NAD^+ and uridine 5′-diphosphoglucose dehydrogenase, which converts UDP-glucose to UDP-glucuronic acid with concomitant formation of NADH. Since NADH exhibits absorbance at 340 nm, the increase in absorbance is directly proportional to the residual UDP-glucose concentration and inversely proportional to GALT enzyme activity. For each measurement, a blank control is prepared in which Gal-1-P is omitted. The presence of endogenous NAD^+ may cause falsely low apparent GALT activity (false-positive results), particularly in neonates and infants, as erythrocytic NADase activity is not yet fully developed. Preincubation of samples with NADase can prevent this interference (Pasquali et al., 2018). However, at UZ Leuven, we have a completely home-made assay, that is not sensitive to this interference.

In-house GALT assay (UZ Leuven)

At UZ Leuven, GALT activity is measured with a fully home-made assay that quantifies the formation of the second reaction product, glucose-1-phosphate. To specifically assess the activity of GALT present in the red blood cells, the sample is centrifuged, the plasma and buffy coat are aspirated and the remaining RBCs are lysed. In a first step, GALT converts Gal-1-P and UDP-glucose into UDP-galactose and glucose-1-phosphate. To allow the glucose-1-phosphate formed to be measured, phosphoglucomutase (PGM) is added, which converts glucose-1-phosphate to glucose-6-phosphate. Glucose-6-phosphate is subsequently oxidized to 6-phosphogluconate by glucose-6-phosphate dehydrogenase (G6PDH). The coupled reduction of $NADP^+$ to NADPH is measured spectrophotometrically at 340 nm and is directly proportional to GALT activity. (See attachment 4).

The UDP-galactose production method can be by radioenzymatic assays and ultraperformance liquid chromatography coupled with tandem mass spectrometry (UPLC-MS/MS) methods.
Radioenzymatic methods directly quantify the formation of UDP-galactose from a radiolabeled substrate. Patient hemolysates are incubated with UDP-glucose and ^{14}C-labeled galactose-1-phosphate (^{14}C-Gal-1-P) at 37 °C for 10–30 minutes. Following incubation, unreacted ^{14}C-Gal-1-P is separated from the reaction product (^{14}C-UDP-galactose) by ion-exchange chromatography. The radioactivity of each fraction is then measured by liquid scintillation counting or an equivalent radioisotope detection technique. The measured radioactivity corresponding to ^{14}C-UDP-galactose is directly proportional to GALT enzyme activity.
Although specific, this method is not practical for high-throughput diagnostics due to the use of radioactive material and the labor-intensive separation step (Pasquali et al., 2018).
UPLC-MS/MS has been developed as a non-radioactive alternative. These methods allow accurate and reliable quantification of UDP-galactose formation, providing a robust measurement of GALT activity with higher throughput and improved safety (Ko et al., 2010).

RBC GALE enzyme activity (type III) and GALK1 enzyme activity (type II)

Traditionally, GALK1 and GALE activities are assessed using radioenzymatic assays. In this approach, GALK1 activity is measured by monitoring the formation of ^{14}C-galactose-1-phosphate from ^{14}C-galactose, whereas GALE activity is determined by detecting ^{14}C-UDP-glucose generated from ^{14}C-UDP-galactose. Separation of radiolabeled substrates and products is achieved using weak cation-exchange matrices, such as diethylaminoethyl (DEAE) cellulose (Pasquali et al., 2018).
More recently, LC-MS/MS-based methods have been developed for the measurement of GALK1 and GALE activity in RBCs and/or dried blood spots (DBS). These assays employ ^{13}C-labeled substrates as safer, non-radioactive alternatives, offering improved analytical performance and clinical applicability (Pasquali et al., 2018).

2. Metabolite assays

Urine reducing substances (Benedict's test)

In the presence of a reducing sugar, the Cu^{2+} in Benedict's reagent is reduced to Cu^{+} (red-brown copper(I) oxide precipitate) while the sugar is oxidized. Whether a sugar is reducing is pH-dependent: in the alkaline Benedict's reagent, ketoses such as fructose tautomerize to aldoses and thus also reduce Cu^{2+}, while at neutral or acidic pH fructose is not reducing (Benedict, 1909). Benedict's test is associated with a relatively low sensitivity and a high false-positive rate, limiting its reliability as a screening tool for galactosemia. Awareness of these limitations is essential. In regions lacking a newborn screening program that includes galactosemia, assessment of erythrocyte GALT activity and Gal-1-P concentration remains crucial for the differential diagnosis of neonatal hypoglycemia with hepatic involvement (Morell-Garcia et al., 2014). Although Benedict's test has historically been used as a rapid, low-cost screening tool for galactosemia, its diagnostic accuracy is limited by both false positives and false negatives. False negatives may occur when urinary galactose excretion is low at the time of testing, for example in neonates who have not yet been exposed to significant amounts of dietary lactose or after a period of fasting. In such cases, the concentration of reducing substances in urine may fall below the detection threshold of the assay, giving a misleadingly normal result despite significant enzyme deficiency. While total galactose (DBS) Gal-1-P (RBC) and galactitol (urine) can also be false-negative in patients on a galactose-free diet, the sensitivity of these tests in babies with a limited lactose intake is significantly higher (Satekge et al., 2020).

Thin layer chromatography

TLC is an analytical technique in which the stationary phase, typically silica gel for carbohydrate separation, is applied as a thin layer on an inert supporting plate. The mobile phase, contained in a reservoir, migrates through the stationary phase by capillary action, enabling separation of analytes (Alonso-Fernández & Patel, 2012). It allows the rapid identification of reducing sugars in patients with a positive result for the Benedict test, but also lacks sensitivity.

Galactose-1-phosphate (Gal-1-P)

Analogous to the GALT assay, Gal-1-P in red blood cells can be quantified using a two-step enzymatic reaction coupled to spectrophotometric detection of reduced pyridine nucleotides. In this approach, alkaline phosphatase first hydrolyzes Gal-1-P to free galactose. The released galactose is subsequently oxidized by galactose dehydrogenase in the presence of NAD^{+} or $NADP^{+}$, generating NADH or NADPH, respectively, which are measured spectrophotometrically.
Alternatively, Gal-1-P can be directly quantified by stable isotope dilution gas chromatography–mass spectrometry (GC/MS). Compared with enzymatic assays, GC/MS provides superior sensitivity while maintaining a comparable linear range (Pasquali et al., 2018). While more sensitive than Benedict's test, this assay will be false-negative in patients on a lactose-free diet.

3. Molecular genetic testing

Targeted analysis for common pathogenic variants in GALT is frequently used in clinical practice after metabolite and enzyme assays. However, when results are discordant with biochemical findings, further evaluation with full

GALT gene sequencing or gene-targeted deletion/duplication analysis is recommended, as these approaches aid in clarifying equivocal enzyme activity results.
This is particularly relevant because different genotypes can produce similar enzyme activity levels. For instance, carriers of classic galactosemia (N/G), Duarte variant homozygotes (D2/D2), and D1 (Los Angeles) variant compound heterozygotes (D1/G) all typically show ~50% of normal GALT activity. Molecular testing allows these combinations to be distinguished. Importantly, the D1 allele is not associated with reduced GALT activity and should be reported as benign when identified.
Molecular testing of the proband, together with parental studies, enables precise determination of the familial GALT alleles. This provides a more accurate assessment of reproductive risk for classic galactosemia than biochemical testing alone.
For GALE and GALK1, diagnostic evaluation generally involves full-gene sequencing followed by deletion/duplication testing, rather than targeted mutation analysis (Pasquali et al., 2018).

What tests are needed for follow-up of galactosemia?

RBC Gal-1-P is the most widely used marker for follow-up of GALT-deficiency. It falls rapidly after lactose restriction but rarely normalizes completely. It can be used to track metabolic control, and persistent elevation can expose hidden lactose intake (Welling et al., 2017).

Galactitol in urine or RBCs can be measured using the same GC/MS methodology as for Gal-1-P (Pasquali et al., 2018). Urine galactitol is especially useful in the monitoring of GALK1-deficiency (Hennermann et al., 2011).

Which tests can be used for NBS?

Most newborn screening programs in the United States employ fluorometric Beutler-based assays to semiquantitatively measure GALT activity in dried blood spots (DBS). Approximately one third of programs measure total galactose (galactose plus Gal-1-P) in DBS, either as a primary screening tool or in combination with GALT activity assays. Elevated galactose levels in the presence of normal GALT activity are suggestive of GALK1, GALE or GALM deficiency. Measurement of total galactose alone, however, may yield false-negative results in infants already receiving lactose-free formula or total parenteral nutrition. Conversely, false-positive results have been reported in newborns with citrin deficiency, Fanconi–Bickel disease, hepatic dysfunction, or under the influence of certain medications (Pasquali et al., 2018).

Neonatal screening is crucial for the early detection of classic galactosemia. Without prompt diagnosis and immediate dietary restriction of galactose sources, affected infants may sustain severe, irreversible, and sometimes fatal damage to vital organs such as the liver, kidneys, and brain. Early recognition and treatment significantly improve the likelihood of a favourable prognosis. In contrast, untreated infants and children face profound health complications, including intellectual disability, behavioural disturbances, impaired growth and development, nutritional deficiencies, and long-term sequelae necessitating intensive medical care (Badiu Țișa et al., 2022; Yuzyuk et al., 2018).

Attachments

Attachment 1

Type	Onset	Typical presentation	Key complications	Clinical severity
Classic galactosemia (Type I, GALT deficiency)	Neonatal (first days after milk feeding)	Jaundice, hepatomegaly, vomiting, hypoglycemia, sepsis (E. coli), failure to thrive	Liver failure, sepsis, developmental delay, premature ovarian insufficiency	Severe, life-threatening if untreated
Duarte variant (D/G)	Newborn (screen-detected)	Usually asymptomatic; occasionally mild feeding problems	Generally, none; long-term development usually normal	Mild/benign
Galactokinase deficiency (Type II, GALK1)	Infancy to early childhood	Isolated cataracts, otherwise well	Visual impairment from cataracts if not treated early	Mild, non-life-threatening
Galactose epimerase deficiency (Type III, GALE)	Varies (newborn to childhood)	Peripheral form: asymptomatic. Generalized form resembles classic galactosemia (liver disease, FTT)	Generalized form: developmental delay, growth issues; peripheral form benign	Variable (benign → severe)
Galactose mutarotase deficiency (Type IV, GALM)	Neonatal	Neonatal hypergalactosemia; some infants develop cataracts, others remain asymptomatic	Mild phenotype; often improves on lactose restriction	Usually mild, variably symptomatic

Pasquali M, Yu C, Coffee B. Laboratory diagnosis of galactosemia: a technical standard and guideline of the American College of Medical Genetics and Genomics (ACMG). Genet Med. 2018 Jan;20(1):3-11. doi: 10.1038/gim.2017.172. Epub 2017 Oct 26. PMID: 29261178.

Wada Y, Kikuchi A, Arai-Ichinoi N, Sakamoto O, Takezawa Y, Iwasawa S, Niihori T, Nyuzuki H, Nakajima Y, Ogawa E, Ishige M, Hirai H, Sasai H, Fujiki R, Shirota M, Funayama R, Yamamoto M, Ito T, Ohara O, Nakayama K, Aoki Y, Koshiba S, Fukao T, Kure S. Biallelic GALM pathogenic variants cause a novel type of galactosemia. Genet Med. 2019 Jun;21(6):1286-1294. doi: 10.1038/s41436-018-0340-x. Epub 2018 Oct 19. Erratum in: Genet Med. 2020 Jul;22(7):1281. doi: 10.1038/s41436-020-0836-z. PMID: 30451973.

Attachment 2

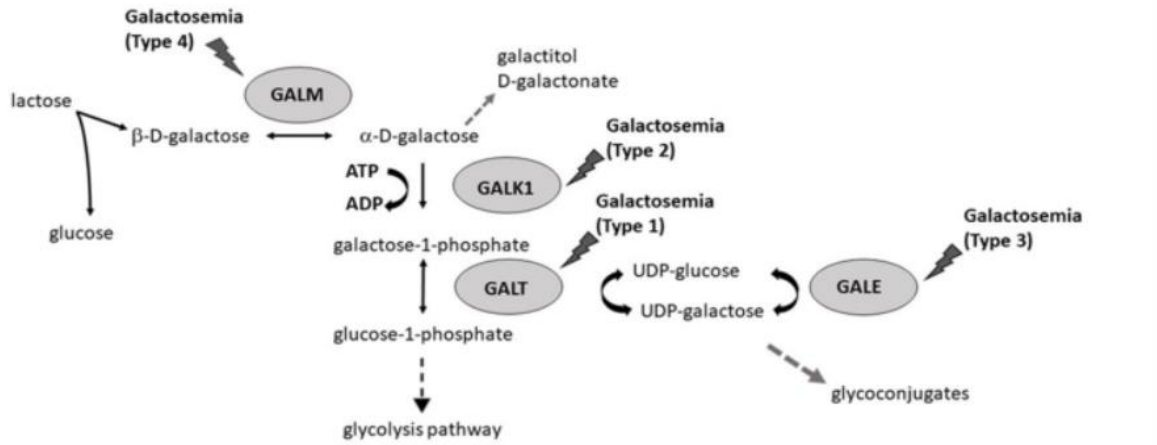


Figure 1. Schematic representation of the Leloir pathway and associated reactions. Several forms of galactosemia can be caused by deficiency of GALM, GALT, GALK1, or GALE.

Succoio M, Sacchettini R, Rossi A, Parenti G, Ruoppolo M. Galactosemia: Biochemistry, Molecular Genetics, Newborn Screening, and Treatment. Biomolecules. 2022 Jul 11;12(7):968. doi: 10.3390/biom12070968. PMID: 35883524; PMCID: PMC9313126.

Attachment 3

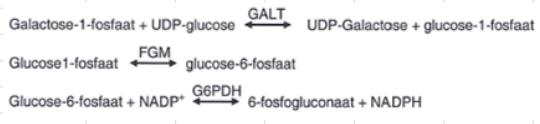
Forms of Galactosemia	Gene	Location	Gene Size	Exons	Variants
Type 1 galactosemia (OMIM 230400)	*GALT*	9p13.3	4.3 kb	11	Ser135Leu Lys285Asn Gln188Arg Asn314Asp del 5.5 kb del 5 kb
Type 2 galactosemia (OMIM 230200)	*GALK1*	17q25.1	7.3 kb	8	Pro28Thr Ala198Val
Type 3 galactosemia (OMIM 230350)	*GALE*	1p36.11	5 kb	13	Val94Met Lys257Arg Gly319Glu
Type 4 galactosemia (OMIM 618881)	*GALM*	2p22.1	68 kb	7	Arg82* Ile99Leufs*46Trp311* Gly142Arg Arg267Gly

Succoio M, Sacchettini R, Rossi A, Parenti G, Ruoppolo M. Galactosemia: Biochemistry, Molecular Genetics, Newborn Screening, and Treatment. Biomolecules. 2022 Jul 11;12(7):968. doi: 10.3390/biom12070968. PMID: 35883524; PMCID: PMC9313126.

Attachment 4

Testprincipe

Om specifiek de activiteit van het in de rode bloedcellen aanwezige GALT te onderzoeken, wordt het staal gecentrifugeerd om het plasma en de buffy coat af te zuigen en worden vervolgens de RBC gelyseerd.
In een eerste stap zet het GALT galactose-1-fosfaat en UDP-glucose om in UDP-galactose en glucose-1-fosfaat. Om de vorming van glucose-1-fosfaat te kunnen meten, wordt fosfoglucomutase (FGM) toegevoegd dat glucose-1-fosfaat kan omvormen tot glucose-6-fosfaat. Glucose-6-fosfaat wordt vervolgens geoxideerd tot 6-fosfogluconaat door glucose-6-fosfaat-dehydrogenase (G6PDH). De hieraan gekoppelde reductie van $NADP^+$ tot NAPDH kan spectrofotmetrisch worden gemeten.



Testprinciple of the home-made UZ Leuven GALT –assay.