DNA-TECHNOLOGY

GENETICS

LACTOSE INTOLERANCE

REAL-TIME PCR GENOTYPING KIT FOR DETECTION OF THE -13910 T>C POLYMORPHISM IN THE *MCM6* GENE ASSOCIATED WITH ADULT TYPE HYPOLACTASIA

CLINICAL SIGNIFICANCE

About 60% of the world population has difficulties digesting lactose, a disaccharide found in dairy products. This ratio varies from 28% in European population to 70% in Middle East [1]. Lactose intolerance is clinically manifesting congenital or acquired inability to digest a lactose due to the decreased lactase activity (lactase deficiency). The undigested lactose has an osmotic activity, which increases the osmotic pressure in the small intestine and accelerates the intestinal peristalsis. The fermentation of lactose by the bacterial flora in small intestine leads to a high production of short-chain fatty acids and gases. This is followed by the onset of abdominal pain, diarrhea, and flatulence [2].

In some cases, the inability to digest lactose is asymptomatic, and only manifested cases with secondary metabolic disorders, delayed development and/or affecting social activity are considered to be a disease.

The clinical classification of lactase defiency [3]:

Congenital lactase deficiency	A relatively rare disorder caused by a mutation in the lactase gene (*LCT*) that manifests itself from the first days of life of the child. It may be accompanied by a metabolic acidosis due to diarrhea and hypercalcemia
Transient lactase deficiency	Observed in premature infants and characterized by a low lactase activity — immaturity of enzyme systems at the birth. In the first weeks of life, the lactase activity is normalized
Primary lactase deficiency	A widespread condition caused by genetic polymorphisms in the lactase gene enhancer. The lactase activity is high during the first months of the child's life. It decreases after the end of breastfeeding, however enterocytes stay morphologically intact. It is inherited by autosomal recessive type
Secondary lactase deficiency	Reduced lactase activity associated with enterocyte damage is possible in infectious (intestinal infection), immune (intolerance of cow's milk proteins), inflammatory processes in the intestine, as well as atrophic changes (in celiac disease, after a long period of complete parenteral nutrition, etc.) and lack of trophic factors.

Diagnosis methods of lactase deficiency [4]:

- **Diagnosis by dietary change** is based on relevant symptoms when lactose-containing foods are consumed and a reduction in symptoms on a lactose-free diet or when lactase enzyme is used.
- **Fecal laboratory tests** can include determination of the total carbohydrate content in the feces and fecal pH. It does not allow differentiating the different types of disaccharidase deficiencies (which is possible with stool carbohydrate chromatography), but in combination with clinical history it is sufficient for screening and monitoring the correct selection of a diet. Stool pH determination (normally 5.5 or higher) reflects the fermentation processes in the intestine: lactase deficiency leads to decrease of pH.
- **Detection of hydrogen, methane, or ^{14}C-labeled CO_2 in exhaled air**. The methods allow indirect estimation of microflora activity by lactose fermentation. It is reasonable to determine the concentration of gases after a dosed load of normal or labeled lactose.
- **Glycemic load test with lactose**. Glycemic levels recorded before and after lactose loading reflect the cumulative result of lactose digestion and absorption in the small intestine.
- **Determination of lactase activity in biopsy specimens of small intestine mucosa**. Invasiveness, complexity and high cost of the method limit its application in everyday practice. Besides, there is no clear correlation between the degree of lactase reduction in the intestine and clinical picture; the results obtained may be influenced by the biopsy specimen location.
- **Genetic testing** can be used to diagnose genetic forms of lactose intolerance — congenital and primary lactase deficiency.

The primary lactase deficiency is associated with single nucleotide variants in a regulatory region of *LCT* gene which is located in *MCM6* gene. In European populations two polymorphisms are the most common: -13910 T>C and -22108 G>A:

	Normal lactase activity	Lactase deficiency
-13910 T>C	TT, CT	CC
-22108 G>A	AA, GA	GG

There is a correlation between the -13910 T>C and -22018 G>A genotypes. According to studies conducted in Poland, 42.4% of people with the -13910CC genotype have the -22018GG variant. At the same time, -13910CC genotype was detected in all people with -22018GG, but not vice versa [5]. These genotypes are widespread in Europe and in the Russian Federation [6].

Genetic testing for lactose intolerance based only on the-13910 T>C polymorphism is more informative and reduces cost of the assay.

The presence of the -13910CC genotype is not always associated with lactose intolerance. Such factors as the amount of lactose consumed, *Lactobacillus* and *Bifidobacterium* bacteria in the gut microbiota capable of fermenting lactose, the timing of food movement through the GI tract, may influence the clinical picture [7].

Lactose Intolerance Genotyping kit is designed to determine the -13910 T>C polymorphism in the *MCM6* gene associated with lactose intolerance by Real-time PCR.

THE ASSAY IS RECOMMENDED

to diagnose or predict the occurrence of lactase deficiency associated with *MCM6* gene polymorphisms.

BIOMATERIAL

- whole peripheral blood;
- dried blood spots;
- buccal epithelium.

SPECIAL FEATURES OF THE KIT

- **Multiplex format** — simultaneous detection of several DNA targets in one tube.
- **Internal control** — assessment of the quality of polymerase chain reaction.
- **Automatic generation** of the results form when using the recommended Real-time PCR instruments of the DT series and RealTime_PCR software.
- **Availability of preset templates with test parameters**, which automatically set the necessary settings and calculate the results.

KIT SPECIFICATIONS

Composition of the kit:

- PCR-mix: *MCM6*: -13910 T>C;
- TechnoTaq MAX polymerase;
- PCR buffer;
- mineral oil.

The kit is designed for 48 tests, including positive and negative controls.

Hands-on time
(without sample preparation): from 1.5 hour.

DETECTION CHANNELS OF AMPLIFICATION PRODUCTS:

PCR-mix	Detection channels of allele variants and internal control				
	Fam	Hex	Rox	Cy5	Cy5.5
MCM6: -13910 T>C	C	T	–	IC*	–

* Internal control (IC) system is a human genomic DNA amplification system which allows to determine sufficiency of the extracted DNA for analysis.

Analytical sensitivity	at least 1.0 ng of human DNA per amplification tube (300 DNA copies).

RECOMMENDED MATERIALS AND EQUIPMENT

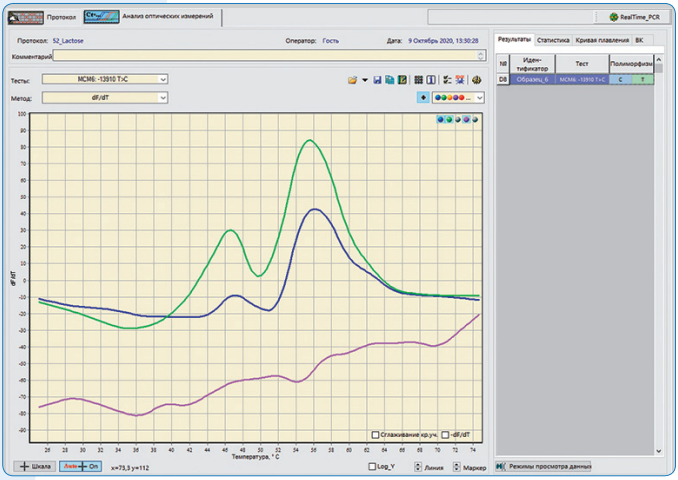
DNA extraction kits	Real-time PCR instruments
▪ PREP-GS Genetics ▪ PREP-RAPID Genetics ▪ PREP-CITO DBS ▪ PREP-MB MAX ▪ PREP-OPTIMA ▪ PREP-OPTIMA MAX	▪ DTprime ▪ DTlite

produced by DNA-Technology

SOFTWARE

- RealTime_PCR Software

Registration and interpretation of the reaction results are carried out automatically using the Real-Time PCR software for Real-time PCR instruments of the «DT» series manufactured by «DNA-Technology».



An example of the result of a PCR-assay using the «DT» series Real-time PCR instrument and related software: analysis of optical measurements.

AN EXAMPLE OF THE RESULTS FORM

Detection of the -13910 T>C polymorphism in *MCM6* gene associated with lactose intolerance by real-time PCR (Lactose Intolerance)

Date:
Number of tube:
Patient name:
Sex:
Age:
Organization:
Clinician name:
Comments:

Logo

Information about laboratory

Sample ID:

No.	Name of research	Results	
		Genotype	
1	MCM6: -13910 T>C	C	T

Study was carried out

Date:
Signature:

Results form was obtained using a real-time PCR instrument of the «DT» series and related software.

TRANSPORT AND STORAGE CONDITIONS

Transportation of the kit is carried out in thermocontainers with ice pack at temperature inside the container from 2 °C to 25 °C for no longer than 5 days.

All components of the kit, except for TechnoTaq MAX polymerase, must be stored at temperature from 2 °C to 8 °C over the storage period.

TechnoTaq MAX polymerase must be stored in a freezer at temperature from -18 °C to -22 °C throughout the shelf life of the kit.

LIST OF REFERENCES

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