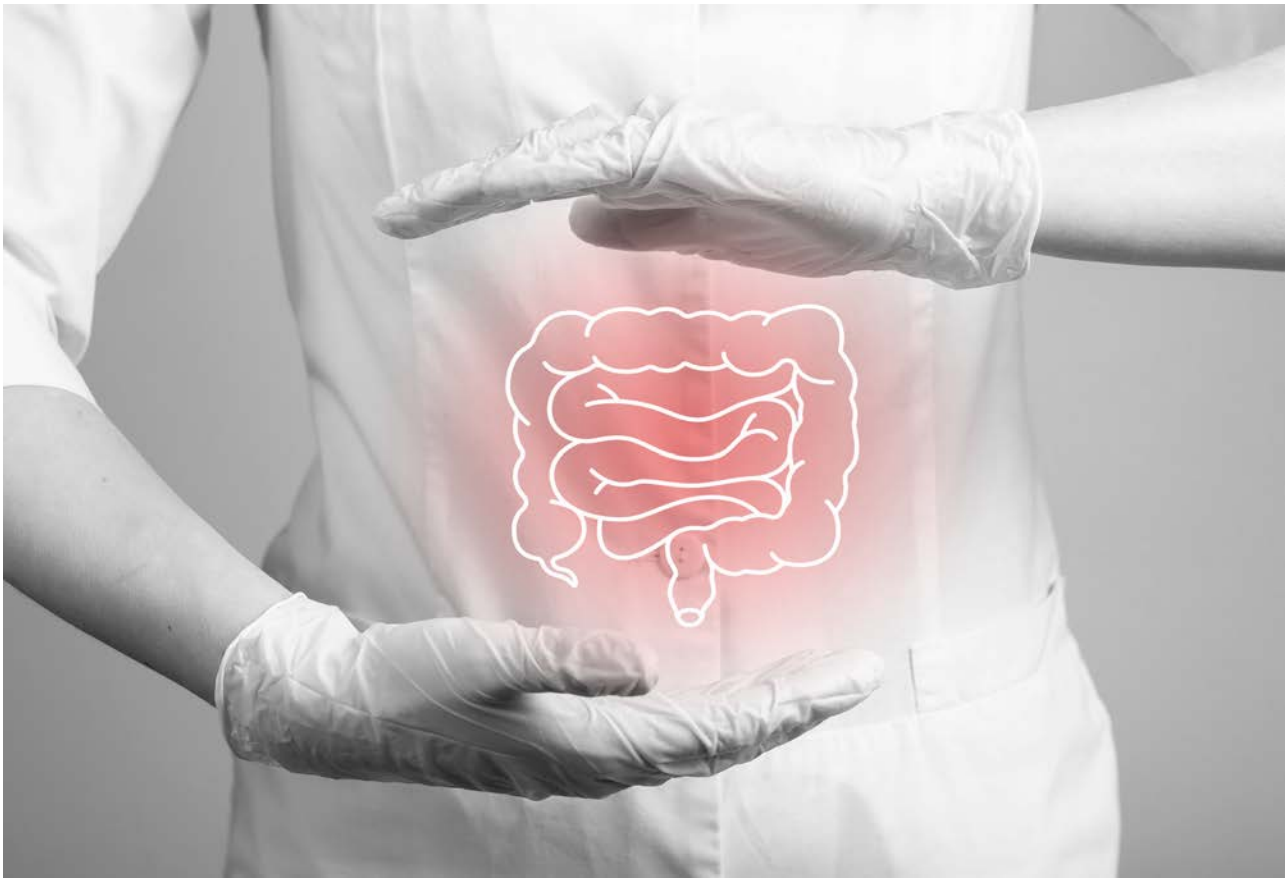
DNK s.r.o.
diagnostické centrum

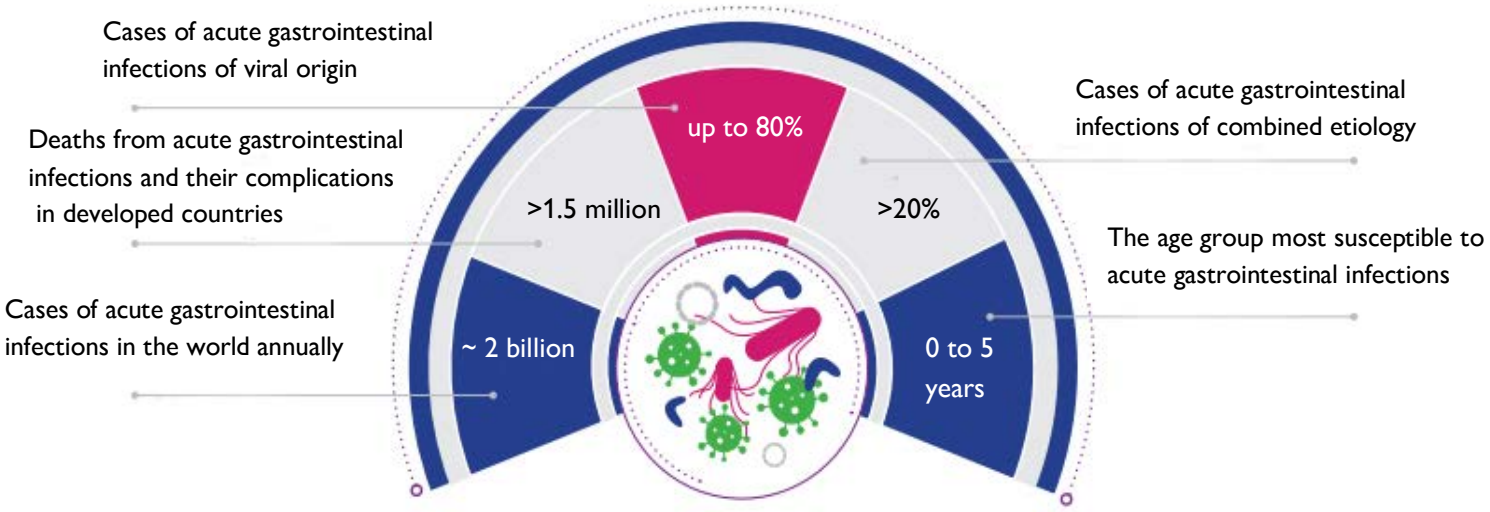
Laboratory diagnosis of acute gastrointestinal infections

by Real-Time PCR

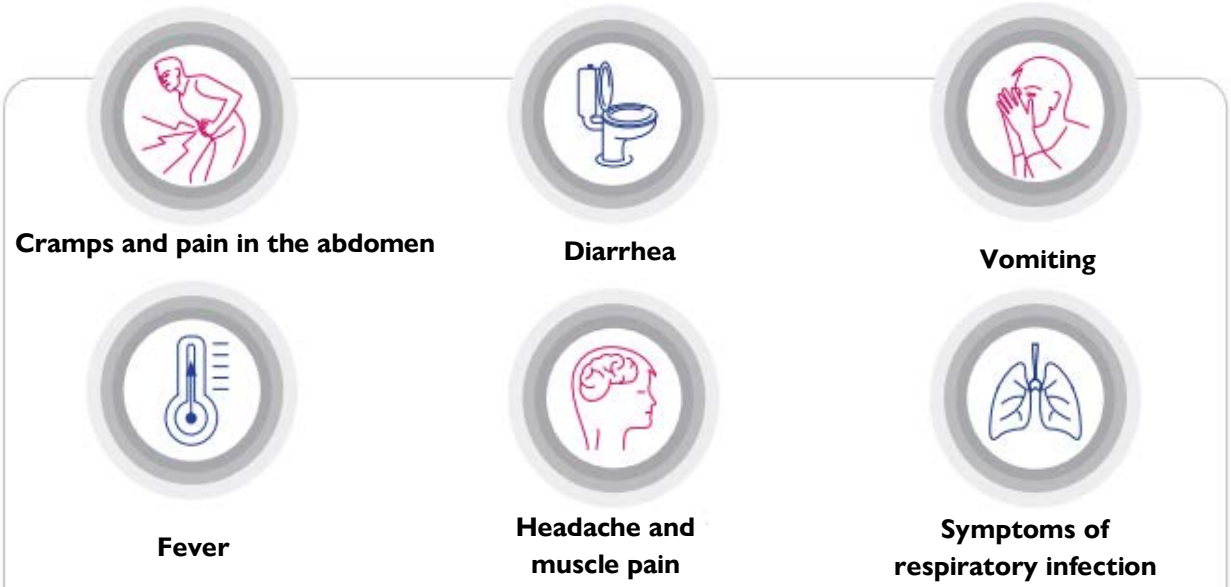


Acute gastrointestinal infections

Acute gastrointestinal infections belong to a group of acute infectious human diseases with a common route of transmission and a characteristic clinical picture. The causative agents of these infections are many viruses, bacteria and their toxins, as well as fungi and protozoa. They are some of the most common diseases in the world and rank second to acute respiratory viral infections among infectious diseases.

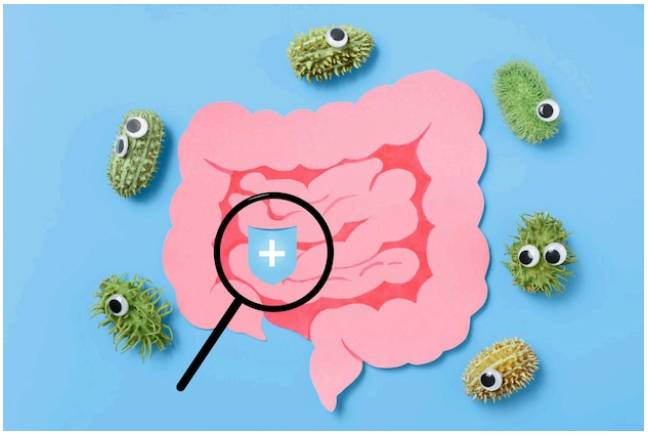


Clinical manifestations



In the absence of effective timely treatment, complications may develop: dehydration, acute renal failure, toxic shock syndrome, neurotoxicosis, circulatory diseases, hypovolemic shock.
Severe dehydration can lead to death.

- **Route of transmission of infections:** fecal-oral, through food, water or contact-home routes; with viral gastroenteritis, airborne transmission is also possible



- **Clinical signs:** acute onset of the disease, asymptomatic transmission, prolonged release of the pathogen during the recovery period
- **Epidemic process:** sporadic, group and nosocomial foci; rapid spread in the focus of disease
- **Stability in the outdoor environment:** high stability in the external environment; some pathogens are resistant to disinfectants

	Risk group	Incubation period	Seasonality of disease
Adenovirus serotypes 40 and 41	Newborns, children	8-10 days	All year round
Norovirus	Children and adults	12-48 hours	Autumn-winter
Rotavirus	Children aged 3 to 15 months	1-3 days	Winter
Astrovirus	Children under 2 y.	3-4 days	Cold season
Campylobacter	Children from 1 to 5 years	2-5 days	Summer
Salmonella spp.	Children and adults	12-24 hours	Summer-autumn
Shigella spp. a *EIEC*	Children from 1 to 5 years	1-7 days	Summer and autumn

Laboratory diagnosis of acute gastrointestinal infections by Real-Time PCR

For the timely implementation of preventive measures and effective therapy, it is necessary to establish the etiology of the disease in time. Differential diagnosis of acute gastrointestinal infections makes it possible to identify asymptomatic and chronic forms of the course of the disease and to control treatment.

Detection of DNA and RNA of acute gastrointestinal infectious agents by Real-Time PCR

- Molecular-genetic methods are considered a mandatory adjunct in the diagnosis of acute intestinal infections.

- Testing is performed within 24 hours from the moment the biological material arrives at the laboratory. In the case of a large number of samples, a representative sample shall be formed.

- Biological material should be obtained within the first 72 hours of disease onset and no later than 24 hours after hospitalization.

- In PCR testing, it is important to take into account properties such as high viral load (up to 10 virions/g (ml) of faeces) and a large number of PCR/RT-PCR inhibitors in the biological material studied. To obtain the correct results during the analysis, it is necessary to follow the recommendations of the manufacturer of the diagnostic kit.

RealBest® Technology: solution for PCR diagnostics of acute gastrointestinal infections

Preparation of faecal suspension

Remove the material from a clean, disposable surface or from a sterile plastic container. Immerse the working part of the swab completely in the sample (if you collect excess sample, leave the excess on the wall of the container).

Preparation of rectal swabs

The material is taken with a sterile swab. Introduce the working part of the tampon into the rectum to a depth of about 1 cm and make several circular movements.

Place the working part of the specimen swab in a test tube with **Transport solution (M), cat. No. C-8867** and carefully resuspend the specimen in solution, remove the probe.

The sample is ready for transport, storage and NA extraction

Temperature and storage time:

- (18–26) °C no more than 6 hours;
- (2–8) °C no more than 5 days;
- minus (18–40) °C no more than 1 month;
- under mínus 40 °C – long-term

Repeated freezing and thawing of samples is not permitted.

Isolation of DNA and RNA from 100 µl of faecal suspension/rectal swabs/aqueous concentrates

RealBest Extraction 100 for manual sample preparation
RealBest UniMag for RbMag/King Fisher Flex/RealFly automatic sample preparation stations

Before isolation, leave the sample at room temperature for 5 minutes until large suspension and particles settle; if necessary, centrifuge at 3000 rpm for 1 minute.

Identification of DNA and RNA of the main causative agents of acute gastrointestinal infections

- *Norovirus GI/GII*
- *Rotavirus A*
- *Astrovirus*
- *Adenovirus F*
- *Salmonella spp.*
- *Campylobacter* (thermophilic)
- *Shigella spp.*
- EIEC (enteroinvasive *E.coli*)

Reagent kits for the diagnosis of acute gastrointestinal infections by real-time PCR

Cat. №.	Kit name	Number of tests
	Kits for nucleic acids extraction	
8896	**RealBest extraction 100**	48 (6x8)
8883	**RealBest UniMag**	96 (4x24)
	Validation of biological material sampling	
8888 CE	**RealBest Sample Validation**	96
	Kits for acute gastrointestinal infections detection	
1696	**RealBest RNA Norovirus GI/GII**	96
1697	**RealBest RNA Rotavirus A/Astrovirus**	96
1698	**RealBest DNA Salmonella spp./Adenovirus F**	96
1699	**RealBest DNA Campylobacter (thermophilic)/Shigella spp. , EIEC**	96

Samples

Faecal suspension
Rectal swabs
Aqueous concentrates

Ready Master Mix for PCR/RT-PCR
96 tests
Complex positive control sample
Universal amplification protocol

Full automation of PCR analysis at RbMag station

Filling the tubes with biomaterial (transport solution M), extracting the nucleic acids and inserting the eluate into the finished reaction mixture

Storage at temperature

(2-8) °C for 18 months
Transport at 26 °C max. 10 days

Compatible devices

CFX96 (BioRad Laboratories Inc., USA), DTprime (DNA-Technology, Russia), Gentier 96E/R (Xi'an TianLong, Science and Technology Co., Ltd., China) and analogues

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