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INSTRUCTIONS FOR USE

Reagent kit for *Helicobacter pylori* DNA detection in human clinical material by PCR-RT "HP-test"

TS 21.20.23-059-97638376-2022

Version 2 dated June 2, 2023

Content

List of abbreviations

The following abbreviations and designations are used in this instruction:

PCR	polymerase chain reaction
DNA	deoxyribonucleic acid
NC	negative control sample
PC	positive control sample
ICS	internal control sample
SC	specificity control sample
SenC	sensitivity control sample

Introduction

Target analyte: A specific region of Helicobacter pylori genomic DNA.

The scientific validity of the target analyte lies in the specificity (DNA sequence uniqueness) in relation to the genome of the bacterium *Helicobacter pylori*.

H. pylori is transmitted from person to person, most often in childhood, and causes chronic active gastritis in all infected. [1] The main transmission way is within the family. The pathogen is transmitted mainly by the fecal-oral route.

H. pylori infection is one of the leading etiological factors in upper gastrointestinal tract diseases, such as chronic gastritis, gastric and duodenal ulcers, MALT lymphoma and gastric adenocarcinoma. [2] There is evidence linking *H. pylori* with the development of unexplained iron-deficiency anemia, idiopathic thrombocytopenic purpura, and vitamin B12 deficiency. [3]

H. pylori infection is associated with an increased risk of gastropathy in patients taking nonsteroidal anti-inflammatory drugs (NSAIDs) and low doses of acetylsalicylic acid (ASA). *H. pylori* diagnosis is mandatory before the scheduled prescription of NSAIDs and ASA. [4]

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[1] Bordin D. S., Shengelia M. I., Ivanova V. A. and others. *Helicobacter pylori*, clinical significance and principles of diagnosis // Infectious diseases: News. Opinions. Training – 2022, No. 1(40).

[2] Mayev I.V., Mkrtumyan A.M., Bektemirova L.G. and others. Efficacy of the *Helicobacter pylori* infection 1st line eradication therapy in patients with type 2 diabetes mellitus // Therapeutic Archive – 2022, No. 94(2), pp. 209-215.

[3] Sheptulin A.A. Key statements of the Maastricht-VI consensus (2022) on the diagnosis and treatment of *Helicobacter pylori infection* // Russian Journal of Gastroenterology, Hepatology, Coloproctology – 2022, No. 32(5), pp.70-74.

[4] Lazebnik L.B., Bordin D.S., Dehnich N.N. and others. VII National Recommendations for the diagnosis and treatment of diseases associated with *Helicobacter pylori* (VII Moscow Agreements) – 2021.

The scope of the reagent kit: clinical laboratory diagnostics of infectious diseases.

Indications for use: a reagent kit is recommended for use in clinical laboratory diagnostics for clinical material (gastric mucosal and duodenal biopsies, feces, saliva) testing in patients with suspected helicobacteriosis, regardless of the disease form and stage in all population groups.

Contraindications for use: no contraindications were identified if used by specially trained personnel and taking into account the intended use.

Population, demographic aspects of the medical device use: no population, demographic aspects of the reagent kit use were identified.

Sterility: the product is not sterile.

1. Intended use

Intended use: HP-test reagent kit is designed for *Helicobacter pylori* DNA qualitative detection by polymerase chain reaction with real-time hybridization-fluorescence detection in a DNA sample isolated from clinical material (gastric mucosal and duodenal biopsies, feces, saliva) in patients with suspected helicobacteriosis and associated gastrointestinal tract diseases.

Functional purpose: the obtained results can be used for early *Helicobacter pylori* infection diagnosis in patients with suspected helicobacteriosis and associated gastrointestinal tract diseases, regardless of the disease form and stage in all population groups. The results are taken into account in the comprehensive disease diagnosis.

Potential consumers of a medical device:

The kit is intended for professional use in medical centers and clinical diagnostic laboratories. The professional level of potential users is a clinical laboratory diagnostics doctor, a medical laboratory technician, and a laboratory technologist.

2. Method principle

Method

Qualitative PCR with real-time hybridization-fluorescence detection.

Test sample type

Material for PCR is DNA samples isolated from biopsies of the gastric mucosa and duodenum, feces and saliva.

Detection principle

DNA amplification occurs in a reaction buffer with primers specific to the corresponding DNA regions and *Taq* polymerase enzyme and is a series of repeated cycles of DNA temperature denaturation and primer annealing with their subsequent elongation.

The PCR mixture contains fluorescently labeled oligonucleotide probes that hybridize with a complementary region of the amplified DNA target and are hydrolyzed (destroyed) by *Taq* polymerase, as a result the fluorescence dye and quencher are separated and the fluorescence intensity increases. This allows to register the amplification specific product accumulation by measuring the fluorescent signal intensity in real time. The mixture also contains the uracil-DNA glycosylase enzyme, which prevents the reaction mixture contamination with amplicons.

The kit contains reagents for the detection of *Helicobacter pylori* DNA highly specific regions, as well as ICS (Table 1).

Table 1 – Test targets

Channel corresponding to the fluorophore	
FAM/Green	HEX/Yellow
Helicobacter pylori	ICS

ICS allows to evaluate DNA isolation effectiveness and the possible presence of inhibitors in the sample, which can lead to false negative results.

Method limitations

Saliva sample testing

It is recommended to be perform test before taking antibacterial agents and local antiseptics, since taking antibiotics shortly before the testing may skew the results.

For treatment monitoring it is recommended to conduct a saliva test no earlier than 10-14 days after the end of the corresponding local drugs use and no earlier than one month after systemic therapy.

When preparing a patient for the procedure, keep in mind:

- 6 hours before saliva sampling it is not recommended to use medications for oropharyngeal irrigation and lozenges;
- do not brush your teeth or use chew a gum/fresh breath lozenges before saliva sampling;
- before saliva sampling, rinse the mouth with saline solution or room temperature water.

Fecal sample testing

Fecal samples with a mass (volume) of approximately 1-3 g (1-3 ml) are used. Smear test is uninformative due to the low pathogens content in them. Transfer 1 g (approximately) sample into a special sterile bottle using a separate filter tip, or disposable spatulas, or a sterile cotton swab.

Sample pre-processing is required (see section 8.2 of the Instructions).

When monitoring treatment, biomaterial (feces) sampling for testing is carried out no earlier than 2-4 weeks after eradication therapy completion.

Biopsies of the gastric mucosa and duodenum testing

The material is collected by a doctor during gastroduodenoscopy. Preparation for testing:

- the last meal the night before no later than 18-20 hours.
- in the morning before the procedure do not eat, drink, take medications, do not smoke or brush your teeth.

A possible reason for obtaining a false positive result is contamination at DNA isolation or PCR reaction stages. A false positive result can be detected with a negative control sample.

A reagent kit cannot be used after the expiration date.

Do not use the reagent kit if the inner packaging is damaged, or the

reagent appearance does not match the description.

A reagent kit transported or stored in temperature regime violation cannot be used.

The clinical diagnosis conclusion cannot be based on the assay results with this medical device only. For diagnostic purposes, the results should be used in combination with other data: symptoms, the common clinical picture, the assay results from other test systems, the therapy used.

Total reaction time is 65 minutes (excluding sample preparation).

3. Reagent kit components

Configuration form

A reagent kit is designed in one configuration form.

Number of test samples

Each reagent kit contains reagents designed to carry out 96 reactions, it equates to:

detection of 94 test samples, negative and positive control samples in mass testing or 32 single test sample detections with negative and positive control samples in each test.

Reagent kit components

Table 2 – Reagent kit components

No.	Reagent name	Description	Quantity, volume
1	PCR Buffer	Transparent colorless liquid	1 tube, 480 µl
2	Primer Mix	Transparent colorless liquid, may have a shade of lilac	1 tube, 480 µl
3	PC	Transparent colorless liquid	1 tube, 480 µl
4	ICS	Transparent colorless liquid	1 tube, 950 µl
5	NC	Transparent colorless liquid	2 tubes, 1800 µl each

PCR Buffer is ready for use and contains all the basic reagents, including a thermostable hot start DNA polymerase, deoxynucleotide

triphosphates (dNTP), uracil-DNA glycosylase and a buffer optimized for PCR.

Primer Mix is ready for use and contains a multiplex mix of primers and probes:

1. primers and a probe for a specific region of *Helicobacter pylori* bacterium genomic DNA (FAM/Green);

2. primers and a probe for ICS (HEX/Yellow).

Positive control sample (PC) is ready for use and is a mixture of plasmid DNA with synthetic insertions of amplified DNA fragments: specific fragments of the *Helicobacter pylori* bacterium genome and ICS. PC does not undergo the NA isolation stage.

Internal control sample (ICS) is ready for use and is a plasmid DNA with a synthetic insertion of a specific amplified *Helicobacter pylori* bacterium DNA fragment. Add 10 µl of ICS into each test sample and NC after the sample preparation stage and before NA isolation.

Negative control sample (NC) is ready for use and is deionized DNase-free water. NC undergo NA isolation stage with the ICS addition.

The kit contains no products for medical use, materials of human or animal origin

4. Reagent kit characteristics

4.1. Technical and functional characteristics

Table 3 – HP-test reagent kit

Indicator	Characteristics and standards
1. Technical characteristics	
1.1. Appearance	
PCR Buffer	Transparent colorless liquid
Primer Mix	Transparent, colorless liquid, may have a shade of lilac
ICS	Transparent colorless liquid
PC	Transparent colorless liquid
NC	Transparent colorless liquid
1.2. Completeness	According to clause 1.4 TS 21.20.23-059-97638376-2022
1.3. Labelling	According to clause 4 TS 21.20.23-059-97638376-2022
1.4. Packaging	According to clause 5 TS 21.20.23-059-97638376-2022
2. Functional characteristics	
Positive result with PC	Fluorescence signal growth registered in tubes with PC in the FAM and HEX channels Ct ≤ 32

Reaction in NC+ICS sample	In the NC+ICS sample Ct is not indicated in the FAM channel (that is, there is no fluorescence accumulation curve), in the HEX channel Ct≤32.
Reaction in tubes with SC	Ct is not indicated in tubes with SC in the FAM and HEX channels (that is, there is no fluorescence accumulation curve) or Ct > 35
Reaction in SenC+ICS sample	In SenC+ICS sample, in the FAM channel in all repetitions (at least 4) Ct ≤ 35, and in the HEX channel Ct ≤ 32.

In case of a medical device malfunction, deviations in its functioning that may affect safety, or changes in the kit analytical characteristics, immediately stop using the medical device and inform the manufacturer (see Section 14 of the Instructions).

4.2. Analytical efficiency characteristics

4.2.1. Analytical specificity

It is specific to *Helicobacter pylori* DNA.

based on the cross-reactivity evaluation results carried out in the NA strains study of the following microorganisms:

Collection of Federal State Budgetary Institution "The Russian State Center for Animal Feed and Drug Standardization and Quality" (FGBU "VGNKI"): *Salmonella enteritidis*, *Salmonella choleraesuis*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Salmonella dublin*, *Salmonella typhi*, *Staphilococcus aureus*, *Salmonella abortusovis*, *Salmonella gallinarum-pullorum*, *Shigella flexneri*, *Campylobacter jejuni subsp. jejuni*, *Campylobacter fetus subsp. fetus*, *Listeria monocytogenes*, *Listeria monocytogenes*, *Proteus vulgaris*, *Morganella Morganii*

non-specific reactions were not detected.

4.2.2. Potentially interfering substances effect evaluation

The effect of potentially interfering substances on a reagent kit performance was studied regarding potentially interfering substances that may occur during a reagent kit normal use and presumably affect the reagent kit ability to produce valid results.

Interfering substances can originate from the following external and internal sources:

1) substances used for a patient's treatment (e.g., medicines);

2) substances found in specific sample types (e.g., blood hemoglobin);

The studied concentrations of interfering substances are shown in Table 4.

Table 4

Interfering substances	**Maximum concentration**
Endogenous interfering substances	
Hemoglobin	260 µg/ml
Exogenous interfering substances	
Drugs prescribed for anti-Helicobacter therapy	
Pantoprazole	0.008 mg
Esomeprazole	0.008 mg
Omeprazole	0.004 mg
Clarithromycin	0.1 mg
Amoxicillin	0.1 mg
Metronidazole	10 mg
De-Nol	0.06 mg

Based on the results of PCR reaction series with control samples without interfering substance, and control samples with added potentially interfering substances in concentrations that are expected to occur during normal HP-test reagent kit use, studied potentially interfering substances do not have an interfering effect on the kit operation and do not lead to PCR inhibition at concentrations not exceeding the permissible ones.

Limitations on the test material use:

- test material cannot be used in case of storage and transportation conditions violation (temperature, duration, repeated freezing and thawing);
- it is not allowed to use samples contaminated with extraneous biological material.
- **for saliva samples**: it is recommended to perform the study before taking antibacterial agents and local antiseptics, as taking antibiotics shortly before the testing may skew the result.

For treatment monitoring it is recommended to conduct a saliva test no earlier than 10-14 days after the end of the corresponding local drugs use and no earlier than one month after systemic therapy.

When preparing a patient for the procedure, keep in mind:

- 6 hours before saliva sampling it is not recommended to use medications for oropharyngeal irrigation and lozenges;
- do not brush your teeth or use chew a gum/fresh breath lozenges before saliva sampling;
- before saliva sampling rinse the mouth with saline solution or room temperature water.

- **for fecal samples:** 1-3 g (1-3 ml) fecal samples are used. Smear test is uninformative due to the low pathogens content in them. Transfer 1 g (approximately) sample into a special sterile bottle using a separate filter tip, or disposable spatulas, or a sterile cotton swab.

Sample pre-processing is required (see section 8.2 of the Instructions).

For treatment monitoring, biomaterial (feces) sampling for testing is carried out no earlier than 2-4 weeks after eradication therapy completion.

- **for biopsy samples of the gastric mucosa and duodenum:** the material is taken by a doctor during a gastroduodenoscopy. Preparation for testing:

- the last meal the night before no later than 18-20 hours.
- in the morning before the procedure do not eat, drink, take medications, do not smoke or brush your teeth.

4.2.3 Limit of detection

In accordance with GOST R 51352-2013 and taking into account international recommendations **CLSI EP-17A2**, the limit of detection (LOD) was determined by dilution analysis method of a standard enterprise sample ESS-*Helicobacter pylori*, which is a plasmid with a synthetic insertion of *Helicobacter pylori* genomic DNA fragment with 1000 copies concentration in 1 ml of 10% TE buffer (10 mM Tris, 1 mm EDTA), in deionized water in the range of the estimated detection limit – 200, 250, 300, 350, 400, 450, 500 copies/ml.

According to the assay results, the *Helicobacter pylori* DNA detection limit in 100 µl samples with 95% detection rate when using DNA-sorb-B isolation kits (RC No. FSR 2009/05220 dated March 5, 2019) and PREP-NA (RC No. FSR 2010/08867 dated October 13, 2016) for each cycler is:

Used cycler	Concentration, copies/ml (LOD) with 95% confidence probability	Confidence interval with 95% confidence probability
DNA-sorb-B		
DTprime	448	394.32-501.68
CFX 96	453	399.32-506.68
Rotor-Gene Q	456	402.32-509.68
Quant Studio 5	449	395.32-502.68
FLUORITE	467	413.32-520.68
PREP-NA		
DTprime	463	409.32-516.68
CFX 96	454	400.32-507.68
Rotor-Gene Q	464	410.32-517.68
Quant Studio 5	439	385.32-492.68
FLUORITE	454	400.32-507.68

4.2.4 Metrological traceability

Metrological traceability of control samples – positive control sample (PC), negative control sample (NC+ICS), control sample for sensitivity determination (SenC+ICS), specificity control sample (SC), standard enterprise sample (ESS-*Helicobacter pylori*+ICS) was confirmed by a spectrophotometric method, by checking the concentration of U-937 stock solution (produced by Thermo Fisher Scientific, USA), included in SC, and plasmid pl. *Escherichia phage* MS2 and pl. *Helicobacter pylori*, followed by multiplex allele-specific PCR-RT.

The obtained results confirmed that the positive control sample (PC) ensures HP-test reagent kit stable operation and is a mixture of plasmid DNA with amplified DNA fragments synthetic insertions: a specific fragment of *Helicobacter pylori* DNA and *Escherichia phage* MS2. PC is in a 10% TE buffer (1 mM Tris, 0.1 mM EDTA) with 0.05% sodium azide at 3900000 copies/ml and 2700000 copies/ml concentrations, respectively.

The assigned concentrations uncertainty of the control samples can be considered as the error of the measurement results obtained spectrophotometrically using the NanoDrop 2000c spectrophotometer (reference method), which is ± 1%.

The obtained results confirmed that the negative control sample (NC) ensures HP-test reagent kit stable operation, allows to control of the absence of false positive results, starting from the sample preparation stage, and does not contain DNA.

The obtained results of the PCR assay confirmed the correct operation of the control samples: a control sample for sensitivity determination (SenC + ICS), a specificity control sample (SC), a standard enterprise sample (ESS-*Helicobacter pylori* +ICS) and the possibility of their use to control the functional characteristics of finished products during acceptance tests.

4.2.5 Precision under repeatability conditions

To evaluate precision under repeatability conditions, positive control sample, sensitivity control sample (SenC) and specificity control sample (SC), ESS-*Helicobacter pylori,* and NC+ICS sample were tested in 10 repetitions each.

Repeatability data is obtained inside a laboratory for specific equipment and within a specific reagent kit batch.

To evaluate precision under repeatability conditions, the arithmetic mean of the sample, variance, standard deviation, and coefficient of variation are calculated based on the obtained values in control samples repetitions.

The assay results showed that the coefficient of variation under the kit repeatability conditions does not exceed 3%

4.2.6 Precision under reproducibility conditions

The test system reproducibility is evaluated similarly to the calculation of precision under repeatability conditions (Section 4.2.5), however, different batches of reagent kits are used for testing, reactions are performed in different laboratories, by different operators, on different days, on different PCR cyclers (Reproducibility unit 1, Reproducibility Unit 2, Reproducibility unit 3, Reproducibility unit 4).

When testing precision under reproducibility conditions, complete intra-assay, inter-assay and inter-series reproducibility was observed, the coefficient of variation does not exceed 5%.

4.3. Clinical efficiency characteristics

For clinical studies 178 samples of clinical material samples (41 - gastric mucosal biopsies, 40 - duodenal biopsies, 43 – fecal samples, 54 – saliva samples) were collected from 84 patients aged 4 to 83 years with suspected Helicobacter pylori infection and associated gastrointestinal tract diseases.

In accordance with the International Guideline CLSI EP09-A3 recommendations, it is recommended to perform clinical studies on 40 clinical samples at least. In order to conduct testing using clinical material on a larger sample size, in accordance with CLSI EP09-A3 recommendations, each sample was tested in 2 repetitions starting from the DNA isolation procedure.

Each tested clinical sample was tested in two series using HP-test reagent kit and the obtained data were compared with the obtained results:

- **for gastric mucosal biopsy samples, duodenal biopsy samples and fecal samples using a registered medical device** "Reagent kit for Helicobacter pylori DNA detection by polymerase chain reaction (Helicobacter pylori) according to TS 21.20.23-042-46482062-2019", manufactured by DNA-Technology TS LLC, Russia, registration certificate No. RZN 2020/12859 dated December 9, 2020;
- **for saliva samples by sequencing** using two oligonucleotide primers specific to conserved 16S rRNA regions on an Applied Biosystems 3500 Dx sequenator (Life Technologies Corporation, USA, RC No. FSZ 2011/09862 dated March 24, 2022) using a 318v2 chip. Bioinformatic processing was performed using the QIIME package.

The results matched, indicating that the medical device was functioning correctly.

To conduct a PCR assay using HP-test kit, the cyclers recommended by the reagent kit manufacturer were used:

- CFX96 (BioRad, USA, RC No. FSZ 2008/03399 dated June 21, 2016),
- DTprime (NPO DNA Technology LLC, Russia, RC No. FSR 2011/10229 dated March 3, 2011),
- Rotor-Gene Q (Qiagen, Germany, RC No. FSZ 2010/07595 dated August 10, 2010),
- QuantStudio 5 (Thermo Fisher Scientific, USA, RC No. RZN 2019/8446 dated June 6, 2019),
- FLUORITE (Xian TianLong Science and Technology Co, China,

RC No. RZN 2022/16415 dated January 24, 2022).

The result reproducibility for all used cyclers - 100%.

When testing **84 patients** aged 4 to 83 years with suspected Helicobacter pylori infection and associated diseases of the gastrointestinal tract, from the pool of samples submitted for testing in Federal State Budgetary Educational Institution of Higher Education «Samara State Medical University» of the Ministry of Health of the Russian Federation, as well as from the bank of residual aliquots of clinical material formed by FSBEI HE SamSMU MOH Russia (41 – gastric mucosal biopsy samples, 40 – duodenal biopsy samples, 43 – saliva samples, 54 – fecal samples), with the tested HP-test reagent kit in two series was determined, that:

- 20 gastric mucosal biopsy samples – true positive, 21 samples – true negative,
- 20 samples of duodenal biopsies – true positive, 20 samples – true negative,
- 26 fecal samples – true positive, 26 samples – true negative, 2 samples – false negative,
- 18 saliva samples – true positive, 21 samples – true negative, and 4 samples – false negative.

Confidence intervals (CI) of diagnostic characteristics will be calculated using the Clopper and Pearson Confidence Interval (Clopper, C., & Pearson, E. (1934). The Use of Confidence or Fiducial Limits Illustrated in the Case of the Binomial. Biometrika,26(4), 404-413. doi:10.2307/2331986). The diagnostic characteristics of the test kit were calculated with 95% confidence probability.

Test material type	Number of observations with positive samples	Number of observations with negative samples	Diagnostic sensitivity with 95% confidence probability	Diagnostic specificity with 95% confidence probability
Gastric mucosal biopsies	40	42	100% (95% CI:91.19%-100%)	100% (95% CI:91.59%-100%)
Duodenal biopsies	40	40	100% (95% CI:91.19%-100%)	100% (95% CI:91.19%-100%)
Feces	56	52	92,86% (95% CI:82.71%-98,02%)	100% (95% CI:93.15%-100%)
Saliva	44	42	81,82% (95% CI:67.29%-91,81%)	100% (95% CI:91.59%-100%)

5. Risks associated with the reagent kit use

The border risk zone includes the following hazards:

1. Loss of functional properties of the reagents included in the kit due to transportation, storage or operation under inappropriate conditions;
2. Clinical material contamination with inhibitory substances in concentrations exceeding permissible levels;
3. Reaction mixtures and DNA test samples contamination with contents from a PCR tube or amplification products;
4. Testing with a poor-quality DNA sample (low concentration and/or poor purification);
5. Failure to comply with sample preparation, analysis and disposal requirements due to unqualified personnel work;
6. Use of an unusable kit (use after the expiration date or in case of damaged packaging).

No risks were identified in the unacceptable risk zone.

The cumulative residual risk of using the reagent kit is acceptable, and the benefits of its use exceed the risk.

6. Safety precautions

The class, depending on the potential risk of use – 2b – in accordance with the medical devices nomenclature classification approved by the Order of the Ministry of Health of the Russian Federation No. 4n dated 06.06.2012.

All components and reagents included in the reagent kit belong to hazard class 4 (low-hazard substances) in accordance with GOST 12.1.007-76 "Occupational safety standards requirements. Harmful substances. Classification and general safety requirements".

Reagents included in the kit have low vapor elasticity and exclude the possibility of inhalation poisoning.

Reagents included in the kit are non-toxic, as they are prepared by mixing individual non-toxic components.

Work with material infected or suspected of infection is carried out in accordance with the requirements of SanPiN 3.3686-21 "Sanitary and epidemiological requirements for the prevention of infectious diseases".

It is necessary to simultaneously ensure and comply with the biological safety rules and requirements for the organization and conduct of these works by personnel in order to prevent premises and equipment contamination with nucleic acids and (or) amplicons of the tested samples.

The work should be carried out in a laboratory performing molecular biological (PCR) assays of clinical material in compliance with sanitary and epidemiological rules of SanPiN 2.1.3684-21 "Sanitary and epidemiological requirements for the maintenance of urban and rural settlements, water bodies, drinking water and drinking water supply, atmospheric air, soils, residential premises, operation of industrial, public premises, organization and implementation of sanitary and anti-epidemic (preventive) measures". Follow methodological recommendations "Guidelines for disinfection, pre-sterilization cleaning and sterilization of medical devices" (MU 287-113), MU "Organization of work of laboratories using nucleic acid amplification methods when working with material containing microorganisms of pathogenicity groups I–IV" (MU 1.3.2569-09).

The following requirements should always be met when working:

– remove unused reagents in accordance with SanPiN 2.1.3684-21 dated January 28, 2021 "Sanitary and epidemiological requirements for the maintenance of urban and rural settlements, water bodies, drinking

water and drinking water supply, atmospheric air, soils, residential premises, operation of industrial and public premises, organization and implementation of sanitary and anti-epidemic (preventive) measures";

ATTENTION! When removing waste after amplification (tubes containing PCR products), it is unacceptable to open the tubes and slash the contents, as this may lead to contamination of the laboratory area, equipment and reagents with PCR products;

- use the kit strictly for its intended purpose, according to these instructions;
- allow only specially trained personnel to work with the kit (a specialist with higher medical education who has been trained in licensed specialization courses for working with pathogenic biological agents of pathogenicity groups III-IV and PCR diagnostics, as well as a laboratory assistant with secondary specialized medical education);
- do not use the kit after the expiration date;
- avoid contact with skin, eyes and mucosa; in case of contact, rinse immediately the affected area with water and seek medical assistance.

The necessary precautions regarding the influence of magnetic fields, external electrical influences, electrostatic discharges, pressure or pressure changes, overload, sources of thermal inflammation are not provided.

The kit contains no substances of human or animal origin with a potential infectious nature, therefore, precautions against any special, unusual risks during the product use or sale are not provided.

7. Required equipment and materials

Work with a reagent kit is carried out in the working area 3 (for reaction mixtures preparation) (MU 1.3.2569-09).

PCR equipment:

1. Class II and III biosafety cabinet (e.g., microbiological safety boxes BMB-II-Laminar-C according to TS 32.50.50-010-51495026-2020, manufactured by Lamsystems CC, RC No. FSR 2012/13259 dated July 29, 2021 or Cabinet for clean operations DNA/RNA UV-Cleaner UVC/T-M-AR, Biosan, Latvia, RC No. RZN 2023/19369 dated January 18, 2023);

2. Vortex (e.g., Microspin 12 high-speed mini-centrifuge, BIOSAN SIA, Latvia, RC No. FSZ 2011/10116 dated July 11, 2011 or CM-70M centrifuge-mixer, manufactured by SIA ELMI, Latvia, RC No. RZN 2016/4616 dated May 31, 2023);

3. Variable volume dispensers that allow to select liquid volumes of 0.5–10 µl, 10-100 µl or 20-200 µl, 100-1000 µl (e.g., Eppendorf Research Plus, Germany, RC No. FSZ 2011/11028 dated November 15, 2011 or Biohit, Finland, RC No. FSZ 2012/12201 dated May 18, 2012);

4. Refrigerator from +2°C to +8°C with a freezer below -16°C (e.g., combined laboratory refrigerator XL-250 POZIS, XL-250-1 POZIS according to TS 9452-203-07503307-2012, manufactured by POZIS JSC, RC No. RZN 2016/4043 dated May 8, 2019);

5. Cycler [5] with real-time fluorescence detection in channels corresponding to FAM/Green, HEX/Yellow fluorophores:

- CFX96 (BioRad, USA, RC No. FSZ 2008/03399 dated June 21, 2016),
- DTprime (NPO DNA Technology LLC, Russia, RC No. FSR 2011/10229 dated March 3, 2011),
- Rotor-Gene Q (Qiagen, Germany, RC No. FSZ 2010/07595 dated August 10, 2010),
- QuantStudio 5 (Thermo Fisher Scientific, USA, RC No. RZN 2019/8446 dated June 6, 2019),

[5] Cyclers must be maintained, calibrated and used in accordance with the manufacturer's recommendations. Use of this kit in an uncalibrated device may affect the reagent kit performance.

- FLUORITE (Xian TianLong Science and Technology Co, China, RC No. RZN 2022/16415 dated January 24, 2022).

Materials and reagents not included in the kit:

ATTENTION! It is required to use only disposable sterile plastic consumables with "DNase-free" label when working with DNA.

1. Disposable tips with an aerosol barrier up to 1000 µl, 200 µl, 20 µl and 10 µl (Axygen, USA, RC No. FSZ 2012/12077 dated February 27, 2014);
2. Disposable 1.5–2.0 ml Eppendorf type tubes (Axygen, USA, RC No. FSZ 2012/11892 dated August 26, 2014);
3. Thin-walled disposable PCR tubes with an optically transparent lid (Axygen, USA, RC No. FSZ 2012/11892 dated August 26, 2014):

- 0.2 ml PCR tubes,
- 0.1–0.2 ml PCR tubes in strips,
- PCR plates with optically transparent film.

4. Separate lab coat and disposable talc-free gloves;
5. Container with disinfectant solution;
6. Test tube racks for 0.2 ml tubes or 0.2 ml tubes in strips (Axygen, USA, RC No. FSZ 2012/11892 dated August 26, 2014);
7. When testing feces, it is required to use a phosphate buffer (or sterile isotonic sodium chloride solution) to prepare a fecal suspension.
8. If long-term storage of the fecal suspension is required when testing feces it is necessary to use glycerin at 10-15% final concentration.
9. Place biopsies obtained during endoscopy (~0.1–1.0 g tissue pieces) in sterile 1.5–2.0 ml tubes with a transport medium (TE buffer, isotonic sodium chloride solution, phosphate buffer).
10. A kit for DNA isolation from clinical material (see Section 8.4 of the Instructions).

8. Test samples

Test sample type

Material for PCR is DNA samples isolated from gastric mucosal or duodenal biopsies, feces and saliva.

Before adding ICS into test samples during DNA isolation, mix thoroughly the contents by turning the tube 10 times or mixing on a vortex at low speed for 3-5 seconds, and then remove drops from the tube lids by short centrifugation.

Material sampling

ATTENTION! Before starting work, study the methodological recommendations "Taking, transporting, storing clinical material for PCR diagnostics" developed by the FBIS Central Research Institute of Epidemiology of Rospotrebnadzor, Moscow, 2012.

Clinical material sampling and its packaging is carried out by an employee of a medical organization trained in the requirements and rules of biological safety when working and collecting material suspected of infection with microorganisms of the pathogenicity group III.

8.1. Biopsy samples collection and preparation

The material is collected by a doctor during a gastroduodenoscopy. Preparation for testing:

- the last meal the night before no later than 18-20 hours.
- in the morning before the procedure do not eat, drink, take medications, do not smoke or brush your teeth.

Place biopsy samples obtained during endoscopy (~0.1–1.0 g tissue pieces) in sterile 1.5–2.0 ml tubes with a transport medium (TE buffer, isotonic sodium chloride solution, phosphate buffer).

ATTENTION! Sample pre-processing is not required.

Material storage conditions:

Transport and store samples at temperatures from 2°C to 8°C for up to 24 hours. If it is impossible to deliver the material to the laboratory within 24 hours, it is allowed to freeze the material once. It is allowed to store frozen material at -18°C… -22°C up to 1 month.

8.2. Fecal sample collection and preparation

Use fecal samples with approximate weight (volume) 1-3 g (1-3 ml). Smear test is uninformative due to the low pathogens content in them. Transfer 1 g (approximately) sample into a special sterile bottle using a separate filter tip, or disposable spatulas, or a sterile cotton swab.

When monitoring treatment, biomaterial (feces) sampling for testing is carried out no earlier than 2-4 weeks after eradication therapy completion.

Sample pre-processing is required! When testing native feces without prior freezing, a fecal suspension is prepared (if the feces have a watery consistency, a suspension is not prepared).

Fecal suspension preparation

Add 0.8 ml phosphate buffer (or sterile isotonic sodium chloride solution) into microcentrifuge 1.5–2.0 ml tubes (number of tubes corresponds to sample number). Add 0.1 g (0.1 ml) of feces into each tube with a separate filter tip (or disposable spatulas, or a sterile cotton swab) and resuspend carefully on a vortex until a homogeneous suspension is formed.

If it is impossible to test the material within 24 hours and / or long-term storage is required, add glycerin to a 10-20% fecal suspension in a phosphate buffer (or sterile isotonic sodium chloride solution) at 10-15% final concentration. Froze samples prepared in this way only after thorough homogenization and exposure to glycerin for 30-40 minutes.

Material and pre-processed sample storage and transportation conditions

Native fecal samples:

- at room temperature – up to 6 hours;
- at temperatures from 2°C to 8°C – up to 3 days.

Fecal suspension with glycerin, bacterial fraction and decolorized fecal extract:

- at -20°C – up to 1 week;
- at -70°C – for a long time.

8.3. Saliva sampling and sample preparation

It is recommended to be perform test before taking antibacterial agents and local antiseptics, since taking antibiotics shortly before the sampling may skew the results.

For treatment monitoring it is recommended to conduct a saliva test no earlier than 10-14 days after the end of the corresponding local drugs use and no earlier than one month after systemic therapy.

When preparing a patient for the procedure, keep in mind:

- it is not recommended to use medications for oropharyngeal irrigation and lozenges 6 hours before saliva sampling;
- do not brush your teeth or use chew a gum/fresh breath lozenges before saliva sampling;
- before saliva sampling rinse the mouth with saline solution or room temperature water.

Collect at least 1.0 ml saliva in disposable sterile plastic 2 ml tubes. Close the tube tightly with a lid.

ATTENTION! Sample pre-processing is not required.

Material storage conditions:

- at room temperature – up to 6 hours;
- at temperatures from 2°C to 8°C – up to 1 day;
- at -20°C – up to 1 week;
- at -70°C – for a long time.

It is allowed to freeze and thaw the material only once.

8.4. DNA isolation procedure from biological material

To isolate a human genomic DNA sample from biological material, it is recommended to use the following reagent kits:

- when using gastric mucosal or duodenal biopsies as clinical material:

- Reagent kit for DNA isolation from clinical material DNA-sorb-B according to TS 9398-003-01897593-2009 (FBIS Central Research Institute of Epidemiology of Rospotrebnadzor, Russia, RC No. FSR 2009/05220 dated March 5, 2019);

- when using saliva or feces as clinical material:

- Reagent kit for DNA isolation from clinical material DNA-sorb-B according to TS 9398-003-01897593-2009 (FBIS Central Research Institute of Epidemiology of Rospotrebnadzor, Russia, RC No. FSR 2009/05220 dated March 5, 2019);

- Reagent kit for RNA/DNA isolation from the clinical material "PREP-NA" according to TS 9398-035-46482062-2009 manufactured by DNA Technology LLC (registration certificate no. FSR 2010/08867 dated October 13, 2016).

ATTENTION! Simultaneously with DNA isolation from the tested clinical samples, it is required to carry out all sample preparation stages for 100 µl of negative control sample (NC) with 10 µl ICS addition, included in the reagent kit. Before DNA isolation from the test samples and NC, mix thoroughly the tube contents with ICS, turning the tube 10 times or mixing on a vortex at low speed for 3-5 seconds, and then remove drops from the tube lid by short centrifugation.

DNA test samples storage conditions:

- at temperatures from 2°C to 8°C – up to 1 day (24 hours);
- at temperatures from -18 °C to -22°C – up to 1 month;
- at -80°C – for a long time.

9. Kit components preparation for testing

The medical device does not require installation, assembling, adjustment, calibration for commissioning.

ATTENTION! It is required to use only disposable sterile plastic consumables with "DNase-free" label when working with DNA. It is mandatory to use a separate tip with an aerosol barrier for each reaction component.

ATTENTION! Mix reaction mixture components according to Table 5 in PCR tubes before analysis.

Preparation for testing

1. Mix thoroughly the tube contents with DNA isolated for analysis, NC that passed the DNA isolation stage, Primer Mix, PCR Buffer, PC, turning each tube 10 times or mixing on a vortex at low speed for 3-5 seconds, and then remove drops from the tube lids by short centrifugation;
2. Take the required number of strips or tubes for amplification of test and control DNA samples.

Before performing PCR, it is necessary to wet clean the PCR box, as well as the equipment and materials contained in it, with disinfectants suitable for use in PCR laboratories, turn on the UV lamp for 20-30 minutes.

10. Testing procedure

The PCR test consists of the following stages:

1. PCR preparation;
2. DNA amplification with real-time hybridization-fluorescent detection;
3. Result interpretation (described in detail in Section 11).

A) PCR preparation

(carried out in pre-PCR area – a room for reagent dispensing and preparation for PCR amplification)

Total reaction volume – 25 µl.

ATTENTION! It is forbidden to change the reaction volume. When the volume changes, the method sensitivity decreases dramatically!

To carry out 1 reaction, you need:

1. PCR Buffer – 5 µl;
2. Primer Mix – 5 µl;
3. Sample (test sample and NC that passed the DNA isolation stage) – 15 µl.

Total reaction volume – 25 µl.

Prepare reaction tubes according to Table 5 in the following order:

1. Label 0.1-0.2 ml PCR tubes;
2. In a separate disposable sterile Eppendorf type 1.5-2.0 ml tube, prepare the reaction mixture: (N+3) x5 µl of PCR Buffer + (N+3) x5 µl of Primer Mix, where N – the number of test samples. Mix on a vortex by short centrifugation;
3. Add 10 µl of the prepared reaction mixture into each PCR tube;
4. Add 15 µl of isolated DNA into the appropriate tubes for test samples. Do not add DNA preparation into tubes with PC and NC;
5. Add PC and NC into the appropriate tubes;
6. To remove drops from the walls, centrifuge the tubes for 1-3 seconds on a vortex microcentrifuge.

Table 5 - Tubes layout for analysis

	Sample 1	Sample N	PC	NC
Primer Mix	○	○	○	○

DNA PCR amplification with hybridization-fluorescence detection of amplification products in real time

(carried out in PCR area – a room for PCR amplification)

1. Install the tubes in a reaction module of a real-time PCR device. It is recommended to install the tubes in a thermal block center to evenly press the tubes with a heating lid.

2. Program the device to perform the corresponding amplification program and fluorescence signal detection, following the Instructions for the used device. Specify the analysis type: qualitative or quantitative with standards. PCR protocol is listed in Table 6.

Table 6 – PCR protocol

Stage	**Temperature, °C**	**Time, min:sec**	**Detection channels**	**Total cycles**
1	95	02:00	-	1
2	95	00:15	-	5
	64	00:20		
3	95	00:15	-	40
	64	00:20	FAM, HEX	

3. Specify the samples number and identifiers, mark the tubes location on the thermal block matrix in accordance with their layout.

4. Make sure that the FAM/Green and HEX/Yellow detection channels are included in optical measurement parameters of the amplification program.

5. Start PCR with fluorescent signal detection.

6. At the end of the program, start analyzing the results.

11. Result registration and interpretation

Results registration is carried out automatically during amplification with the used device software.

Recommendations on the threshold line setting

For cyclers of any models, a threshold line is set individually for each detection channel at a level corresponding to 10-20% of the maximum fluorescence level obtained for a positive control sample in the last amplification cycle.

The result interpretation is performed using Ct values of channels shown in Table 1. Only Ct values obtained at the PCR stage with fluorescence detection are taken into account (that is, corresponding to stage 3 – see Table 6).

First, the reaction outcome and Ct values in the control samples are evaluated. Result interpretation in test samples begins only after the correct PC and NC outcome.

In case of using Rotor-Gene Q cyclers, activate “Dynamic Tube”, “Noise slope correction” functions, set 10% value in “Outlier Removal” section.

Result interpretation in control samples

The following results should be obtained for PC and NC with ICS addition (Table 7).

Table 7 – Assay results for PC and NC

Control sample	Channel corresponding to the fluorophore	
	FAM/Green	HEX/Yellow
NC+ICS	Ct not indicated or > 35	Ct ≤ 32
PC	Ct ≤ 32	

When obtaining values for NC with added ICS that differ from those indicated in Table 8, the results of the entire series are considered unreliable. In this case, it is required to take special measures to eliminate possible contamination.

When obtaining values for PC that differ from those indicated in Table 8, repeat amplification of the entire sample batch.

When reobtaining values for PC that differ from those indicated in Table 8, it is required to replace the reagents.

Result interpretation in DNA test samples

Result analysis is shown in table 8.

Table 8 – Result interpretation principle during testing

Ct values		Result
FAM/Green (*Helicobacter pylori*)	HEX/Yellow (ICS)	
Ct ≤ 35	not considered	*Helicobacter pylori* DNA **detected**
Ct absent or Ct > 35	Ct ≤ 32	*Helicobacter pylori* DNA **not detected or below the detection limit**
Ct absent or Ct > 35	Ct absent or Ct > 32	**Invalid** result

The reason for obtaining an invalid result may be the presence of inhibitors in the DNA preparation obtained from clinical material, incorrect testing protocol implementation, non-compliance with the PCR temperature regime, etc.

The reason for obtaining a doubtful result may be an insufficient DNA concentration in the clinical sample.

In case of an invalid result, a conclusion is not issued, it is required to repeat biomaterial sampling from a patient and retest it.

If an invalid result repeats, it is required to repeat the test with a reagent kit from another manufacturer or by another method.

The diagnostic value of the obtained test result:

The obtained positive or negative test result can be used by a qualified specialist (doctor), taking into account the clinical picture and other test types data in combination, to diagnose helicobacteriosis in patients regardless of the disease form and stage of all population groups and therapy control.

12. Storage, transportation and operation conditions

Storage

Store a reagent kit in the manufacturer's packaging at -16°C… -24°C during the entire kit shelf life, it is allowed to store at 2°C…8°C up to 14 days.

It is not allowed to freeze/thaw a reagent kit more than 10 times.

A reagent kit stored in violation of the regulated regime cannot be used.

Transportation

Transport the reagent kit by all types of covered vehicles in accordance with the transportation rules applicable to this transport type.

Transport at temperatures from -16°C to -24°C during the entire kit shelf life. Transportation is allowed at temperatures from 2°C to 8°C up to 14 days. Atmospheric pressure is not subject to control, as it does not affect the product quality.

To ensure compliance with transportation conditions throughout the entire transportation period, a reagent kit is placed in a reusable polyurethane foam thermal container for temporary storage and transportation with prepared ice packs. The type, volume and quantity of ice packs placed in a thermal container with transported reagent kits, as well as the thermal container volume are selected depending on the transportation duration and conditions.

Reagent kits transported in violation of the temperature regime cannot be used.

Shelf life

A reagent kit shelf life is 12 months from the acceptance date of the manufacturer's QCD (Quality Control Dept.), if all transportation, storage and operation conditions are met. A reagent kit with an expired shelf life cannot be used.

Shelf life of opened kit components

12 months from the acceptance date of the manufacturer's QCD, if stored at -16°C…-24°C.

Shelf life of the kit components prepared for work

One hour under conditions that prevent the components from drying out, as well as contamination with extraneous biological material.

13. Disposal

Reagent kits that have become unusable, including due to expiration dates, must be disposed of in accordance with the requirements of SanPiN 2.1.3684-21 "Sanitary and epidemiological requirements for the maintenance of urban and rural settlements, water bodies, drinking water and drinking water supply, atmospheric air, soils, residential premises, operation of industrial, public premises, organization and implementation of sanitary and anti-epidemic (preventive) measures".

According to the medical waste classification, the kits belong to class A (epidemiologically safe waste, similar in composition to solid household waste). Unused reagents in accordance with paragraph 170 of SanPiN 2.1.3684-21 "Sanitary and epidemiological requirements for the maintenance of urban and rural settlements, water bodies, drinking water and drinking water supply, atmospheric air, soils, residential premises, operation of industrial, public premises, organization and conduct of sanitary and anti-epidemic (preventive) measures" are collected in reusable containers or disposable bags of any color (except yellow and red).

The remaining tubes and materials after the work are disposed of in accordance with the methodological recommendations "Guidelines for disinfection, pre-sterilization cleaning and sterilization of medical devices" (MU 287-113).

Liquid components (reagents) are destroyed by draining into the sewer with preliminary reagent dilution with tap water 1:100 and removal of the remaining packaging as industrial or household waste.

HP-test reagent kit packaging and tubes are subject to mechanical destruction with the removal of residues as industrial or household waste.

Personnel destroying a reagent kit must comply with the safety rules of a particular destruction method.

14. Warranty, contacts

The manufacturer guarantees the reagent kit quality and safety during shelf life if compliant with transportation and storage requirements as well as rules of operation.

If you have any complaints about the kit quality, undesirable events or incidents, please contact:

Limited Liability Company TestGene (TestGene LLC)
9, 44th Inzhenerny Proezd, office 13, Ulyanovsk, 432072, Russia
Phone number: +7 499 705 03 75
www.testgene.com

Technical Support Service:
Phone number: +7 927 981 58 81
E-mail: help@testgen.ru

Annex A

Labeling symbols

	Contains sufficient for < n > tests
	Consult instructions for use
	In vitro diagnostic medical device
	Temperature limitation
	Batch code or Lot number
	Use by…
	Date of manufacture
	Fragile, handle with care
	This icon shows the correct position of the load in space. This side up. Do not turn over or tip on its side a transport packaging with this symbol. Store and transport it vertically only.

Annex B

Designation	Document name
GOST ISO 14971-2011	Medical products. Application of risk management to medical devices.
GOST P 51088-2013	Medical devices for in vitro diagnostics. Reagents, reagent kits, test systems, control materials, culture medium. Requirements to devices and supporting documentation.
GOST R ISO 23640-2015	In vitro medical devices. Evaluation of stability of in vitro diagnostic reagents.
GOST R 51352-2013	In vitro medical devices. Test methods.
GOST R EN 13612-2010	Performance evaluation of in vitro diagnostics medical devices.
GOST R ISO 18113-1-2015	In vitro diagnostic medical devices. Information supplied by the manufacturer (labelling). Part 1. Terms, definitions and general requirements.
GOST R ISO 18113-2-2015	In vitro diagnostic medical devices. Information supplied by the manufacturer (labelling). Part 2. In vitro diagnostic reagents for professional use.
GOST R ISO 15223-1-2020	Medical devices. Symbols to be used with medical device labels, labelling, and information to be supplied. Part 1. Basic requirements.
GOST ISO 13485-2017	Medical devices. Quality management systems. Requirements for regulatory purposes.