Operating Instructions

Helmo-Screen reagent kit is designed for the qualitative detection of helminth DNA (*Ascaris lumbricoides, Enterobius vermicularis, Opisthorchis felineus, Taenia solium, Diphyllobothrium latum*) in clinical samples by polymerase chain reaction (PCR).

Specimens are fecal samples and rectal swabs (for the diagnosis of enterobiosis). Only for *in vitro* tests.

PRINCIPLE OF THE METHOD

Sample analysis includes the following steps:

1. Sample preparation (isolation of DNA from the samples of clinical material);
2. Amplification of specific DNA fragments during PCR reaction with real-time detection.

The reagent kit is intended for use with detection amplifiers for real-time PCR (MiniOpticon, BioRad; DT-96, DNA technology or other amplifiers with similar technical characteristics).

COMPOSITION OF THE REAGENT KIT

Table 1

Kit Component	Purpose	Quantity	
Mixtures for amplification:			
		individual package version	bulk version
Asc PCR mixture	Mixture for detection of DNA of *Ascaris lumbricoides*	50 test tubes (0.2 ml), 10 µl each, sealed with wax	1 test tube (1.5 ml), 520 µl
Ev PCR mixture	Mixture for detection of DNA of *Ascaris lumbricoides*	50 test tubes (0.2 ml), 10 µl each, sealed with wax	1 test tube (1.5 ml), 520 µl
Opis PCR mixture	Mixture for detection of DNA of *Opisthorchis felineus*	50 test tubes (0.2 ml), 10 µl each, sealed with wax	1 test tube (1.5 ml), 520 µl
Tsol PCR mixture	Mixture for detection of DNA of *Taenia solium*	50 test tubes (0.2 ml), 10 µl each, sealed with wax	1 test tube (1.5 ml), 520 µl
DL PCR mixture	Mixture for detection of DNA of *Diphyllobothrium latum*	50 test tubes (0.2 ml), 10 µl each, sealed with wax	1 test tube (1.5 ml), 520 µl
BK PCR mixture	Mixture for testing the quality of DNA isolation procedure	50 test tubes (0.2 ml), 10 µl each, sealed with wax	1 test tube (1.5 ml), 520 µl
Taq Polymerase Solution		6 test tubes of 520 µl each	
Kit of positive control samples (Asc PCS, Ev PCS, Opis PCS, Tsol PCS, DL PCS, BK PCS)		6 test tubes of 30 µl each	
Negative control sample (NCS)		1 test tube (150 µl)	

Each set of reagents is accompanied by an Operating Instruction (1 piece) and a Certificate of the Reagent Kit (1 piece).

Stage 1 - isolation of DNA from the specimen. Reagent kits are used to isolate the DNA from the clinical samples; these kits are recommended for use in clinical laboratory diagnostics for the isolation of DNA from feces.

Stage 2 - PCR amplification and detection of amplification products in real time using the Helmo-Screen reagent kit

SAMPLE PREPARATION

1. The following biological material can be used for the analysis:

– feces (for detection of DNA of *Ascaris lumbricoides, Enterobius vermicularis, Opisthorchis felineus, Taenia solium, Diphyllobothrium latum*);

– rectal smear (the preferred type of material for detecting *Enterobius vermicularis*);

The container with the material should be delivered to the laboratory and stored until the start of the analysis at 2-8°C. The time from the collection of the material to the start of the analysis should not exceed 24 hours. If a longer storage period is required, the material should be placed in a freezer and stored at a temperature of no more than minus 18°C.

2. ***Recommendations for the isolation of DNA from fecal samples and rectal swabs***

It is desirable to isolate the DNA from a liquid material. If the sample is solid (feces), a small amount of the material (no more than 100 mg) is resuspended in a transport medium or saline solution.

When isolating DNA from a solid material, a small amount of the sample must be transferred directly to a test tube with a lysing solution (an approximate amount of the sample being introduced is shown in the figure - at the end of the dispenser tip).

Caution: Using excess material for isolation may result in PCR inhibition.

CONDUCTING AN ANALYSIS

Total volume of the reaction mixture is 25 µl, including the volume of the DNA sample – 5 µl.

ATTENTION! Disposable tips with an aerosol barrier should be used to add reagents, DNA samples, and control samples to the test tubes!

Preparation for PCR (individual package version)

1. Put the required number of test tubes with the mixture for amplification in the test-tube rack (**n + 2** test tubes of each specificity, where **n** is the number of samples to be analyzed, two additional test tubes are intended for the analysis of PCS and NCS;
2. Add 10 µl of thoroughly mixed Taq polymerase solution to each test tube (without damaging the wax layer), close the test tubes. The test tubes are ready for the introduction of DNA samples, PCS and NCS.
3. Add a drop of mineral oil for PCR (approximately 25 µl) to each test tube, plug the test tubes. The test tubes are ready for the introduction of DNA samples, PCS and NCS.

Preparation for PCR (bulk version)

1. Put the required number of empty 0,2 ml test tubes (**n + 2** test tubes of each specificity, where **n** is the number of samples to be analyzed, two additional test tubes are intended for the analysis of PCS and NCS;
2. Thoroughly mix all the amplification mixtures (Asc PCR mixture, Ev PCR mixture, Opis PCR mixture, Tsol PCR mixture, DL PCR mixture, BK PCR mixture), and then precipitate the drops using a vortex;
3. Add 10 µl of the appropriate amplification mixture to the prepared test tubes;
4. Add 10 µl of a thoroughly mixed Taq polymerase solution to each test tube.

Conducting PCR (similar for the individual package and bulk versions)

1. Alternately opening the caps of the test tubes, add the following to the test tubes:
   - To one of the prepared test tubes - 5 µl of NCS;
   - To test tubes intended for the analysis of samples - 5 µl of the analyzed samples;
   - To the remaining test tube-5 µl of PCS.
2. Place all the test tubes to the detection amplifier unit;
3. Run the amplification program according to the parameters specified in Table 2.

ATTENTION! The fluorescent signal is registered via the FAM channel

REGISTRATION OF RESULTS

Table 2. Amplification program

Amplification mode for the MiniOpticon, BioRad device		
Temperature	Time	Number of cycles
94°C	5 min	1
94°C	5 sec	40
58°C	6 sec *	
72°C	10 sec	
10°C - storage		
Amplification mode for the DT96 device, DNA technology		
Temperature	Time	Number of cycles
94°C	5 min	1
94°C	10 sec	40
58°C	10 sec *	
72°C	10 sec	
10°C - storage		

* - detection of the fluorescent signal

ANALYSIS AND INTERPRETATION OF THE RESULTS

The analysis of the results is carried out using the software of the device used for conducting PCR with real-time detection. The results are interpreted based on the presence (or absence) of the intersection of the fluorescence curve with the threshold line, which determines the presence (or absence) of the Ct threshold cycle value for this sample. The analyzed sample is considered positive if:

- the graph of the increase in fluorescence has the form of an exponential curve;
- the Ct threshold cycle value was determined for the sample;
- the fluorescence curve crosses the threshold line at the exponential growth site.

Attention: when amplifying the analyzed sample with Asc and Tsol PCR mixtures, only the result of the Ct threshold cycle not exceeding 38.0 is taken into account (Table 3).

Interpretation principle:

1. Registration of the results should be started with the results of the amplification of the positive (PCS) and negative (NCS) control samples.

2. The results of the analysis <u>are not taken into account</u> if:

- During the reaction, the detecting amplifier **does not register** an exponential increase in the level of fluorescence in a test tube(s) with a positive control sample through the FAM channel; **in this situation, it is necessary to re-examine all the samples using the PCR mixture, which did not pass positive control**;
- An exponential increase in the level of fluorescence **is registered** through the FAM channel in a test tube with NCS. **It is necessary to take measures to eliminate contamination in the PCR laboratory and re-examine all the samples.**
- In the sample being examined in a test tube with BK there is no registered exponential growth of fluorescence level or the Ct threshold cycle value exceeds the permissible value specified in the kit certificate; this indicates a poor quality of DNA extraction; **in this situation, you need to re-analyse this sample, starting from the stage of DNA extraction**. **If the result is repeated, it is concluded that the quality of the collection or storage of clinical material is poor, and the sample should be taken again.**

3. The results of the analysis <u>are taken into account</u> if:

- During the reaction, an exponential increase in the level of fluorescence channel in a test tube with a positive control sample is registered through the FAM channel; the value of the

Ct threshold cycles for each of the PCS does not exceed the permissible values specified in the kit certificate;

- During the amplification process, there is no fluorescent signal in the test tube with the NCS through the FAM channel; for the BK PCR mixture, the value does not exceed the permissible one specified in the kit certificate;
- In the test sample, an exponential increase in the level of fluorescence in the test tube with BK is registered and the value of the Ct threshold cycle for each of the samples does not exceed the permissible value specified in the kit certificate.

4. - The sample is **positive** for the presence of DNA of *Enterobius vermicularis, Opisthorchis felineus, Diphyllobothrium latum*, if the Ct threshold cycle value is determined for this sample during amplification with a mixture of appropriate specificity, and the fluorescence curve of this sample should cross the threshold line at the site of exponential growth of fluorescence (Table 3).

- The sample is **positive** for the presence of DNA of *Ascaris lumbricoides, Taenia solium*, if the samples have determined value of the Ct threshold cycle and fluorescence curve of the sample should cross the threshold line at the site of exponential growth of fluorescence, and the Ct threshold cycle value is less than 38,0 (Table 3).
- The sample is **negative** for the presence of helminth DNA (*Ascaris lumbricoides, Enterobius vermicularis, Opisthorchis felineus, Taenia solium, Diphyllobothrium latum*) if the threshold cycle value is absent (not determined) for this sample (the fluorescence curve of this sample does not cross the threshold line) (Table 3) or for Asc and/or Tsol PCR mixtures the threshold cycle value is greater than or equal to 38.0.

Table 3. The principle of interpretation of the results of the analysis

	The presence of fluorescence (via the FAM channel)	
	The sample is positive for the presence of helminth DNA	The sample is negative for the presence of helminth DNA
	Ascaris lumbricoides	
Asc PCR mixture	+ Ct < 38,0	-
Asc PCS	+	+
NCS	-	-
BK PCR mixture	+	+
	Enterobius vermicularis	
Ev PCR mixture	+	-
Ev PCS	+	+
NCS	-	-
BK PCR mixture	+	+
	Opisthorchis felineus	
Opis PCR mixture	+	-
Opis PCS	+	+
NCS	-	-
BK PCR mixture	+	+
	Taenia solium	
Tsol PCR mixture	+ Ct < 38,0	-
Tsol PCS	+	+
NCS	-	-
BK PCR mixture	+	+
	Diphyllobothrium latum	
DL PCR mixture	+	-
DL PCS	+	+
NCS	-	-

BK PCR mixture	+	+

KIT TRANSPORTATION AND STORAGE CONDITIONS

Shelf life of the reagent kit is 6 months from the date of manufacture.
Conditions of storage of the Helmo-Screen reagent kit and its individual components are stated on the packaging.
Test tubes with mixtures for amplification should be stored in a dark place at a temperature of 2 to 8°C. The solution of Taq polymerase, PCS and NCS must be stored at a temperature of 2 to 8°C.