AmpliSens
biotechnologies

Acute Enteric Infections

Reagent Kits Format and Composition

By detection type:

FRT format – real-time fluorescence detection

The format is intended for use of specialized equipment for real-time PCR. Labeling of reagent kits reflects the adapted equipment:

RG — Rotor-Gene 3000/6000 (Corbett Research)
iQ — iCycler/iQ5 (BioRad)

FEP format – end-point fluorescence detection

The format is intended for amplification in a standard thermal cycler with subsequent detection of the end point fluorescent signal on a specialized fluorescent detector, for example, ALA-1 (BioSan), Jin (DNA-Technology) or a real-time PCR unit with detection of fluorescence end point, for example, Rotor-Gene 6000 (Corbett Research).

EPh format – electrophoretic detection

The format is intended for detection with use of electrophoresis in agarous gel.

By configuration:

Complete Set Reagent Kit format

The kit includes reagents for extraction, amplification and detection.

Amplification Reagent Kit (PCR Kit)

The kit includes only amplification reagents.

By hot start type and filling:

«Wax» format

The kit includes PCR test tubes ready for use with a lower mixture applied under wax;

FRT and FEP Reagent Kits

Reagent Kits for DNA isolation «DNA-sorb-B' or RNA/DNA isolation «RIBO-sorb»

- Lysing solution
- Sorbent (silica)
- Washing solutions
- Eluting solution

"PCR-Set" Amplification Reagent Kits

- PCR-mixture (primers) applied under wax in test tubes for PCR 0.5 ml (R0,5) or 0.2 ml (R0,2)
- PCR-mixture-2 (a mixture of a buffer solution and non-modified polymerase)
- Positive control samples (PCS) of the isolation stage and PCR
- Negative control samples of the PCR isolation stage
- Internal CS (control sample)

of the isolation stage"Reverta-L" Reverse Transcription Reagent Kits

- M-MLV revertase
- Buffer for reverse transcription with random-primers
- RTG-mix reverse transcription facilitator

EPh Reagent Kits

Reagent Kits for DNA isolation «DNA-sorb-B' or RNA/DNA isolation «RIBO-sorb»

- Lysing solution
- Sorbent (silica)
- Washing solutions
- Eluting solution

Electrophoresis Reagent Kit

- TBE-buffer with ethidium bromide
- Agarose

"PCR-Set" Amplification Reagent Kits

- PCR-mixture (primers) applied under wax in test tubes for PCR 0.5 ml (R0,5) or 0.2 ml (R0,2)
- PCR-mixture-2 (a mixture of a buffer solution and non-modified polymerase)
- Positive control samples (PCS) of the isolation stage and PCR
- Internal CS (control sample) of the isolation stage

"Reverta-L" Reverse Transcription Reagent Kits

- M-MLV revertase
- Buffer for reverse transcription with random-primers
- RTG-mix reverse transcription facilitator

INTRODUCTION

Acute Enteric Infections (AEI)

Are one of the primary causes of hospitalization in infectious disease inpatient departments. The idea that doctors of clinical and laboratory practice have of the infectious diseases etiology is often based on information obtained from the traditional medical Russian literature in which partial view on the leading role of several well-studied pathogens plays the predominant role. Such idea is closely connected to use of diagnostic tests that are available for a wide practical application in routine clinical and laboratory practice, failing currently to provide an effective solution with respect to issues on etiology diagnostics of acute enteric infections.

This fact is confirmed by the evidence provided by the official statistics data stating that in the RF up to 65-67 percent in this group of diseases are accounted for the unspecified etiology AEI.

Etiological verification of AEI outbreak requires prompt attention as the volume, character and, finally, effectiveness of epidemic countermeasures largely depends on it.

The task of germ detection in environmental entities is no less difficult and is required for scheduled monitoring of water consumption objects and for investigation of disease outbreaks.

About causative agents

In accordance with the data provided by the contemporary literature the following bacterial and viral agents are the most often detectable and generally spread etiological agents of AEI:

1. Bacterial agents

- Shigella species microorganisms and enteroinvasive E coli (EIEC);
- Salmonella species microorganisms;
- A thermophillic group of Campylobacter species microorganisms;
- Causative agents of colibacilloses as enteropathogenic E coli (EPEC) and enteroaggregative E coli (EAEC).

2. Viral agents (all the viral agents stated further, except for adenoviruses, are RNA-containing viruses)

- Group A rotaviruses;
- Genotype 2 noroviruses;
- Group F adenoviruses (Types 40 and 41);
- Astroviruses.

The following causative agents are less widely or not universally spread but are no less important for epidemic outbreaks:

- V.cholerae;
- Causative agents of typhoid and paratyphoid diseases;
- Yersiniosis and pseudotuberculosis;
- Cl.difficilae;
- enterotoxigenic E. coli (ETEC), enterohemorrhagic E. coli (EHEC);
- Genotype 1 enteroviruses;
- Group C rotaviruses;
- sapoviruses;

The detection frequency ratio of viral to bacterial agents varies in different age categories: 80-90 percent of 3-year old children diseases are of viral etiology whereas bacteria cause about 10-20 percent of diseases; the share of viral causative agents at the adult age reduces to 30 percent.

Molecular AmpliSens® Methods

Application of diagnostic tests based on polymerase chain reaction (PCR) allows achieving the maximum efficiency and information value of conducted tests. The distinctive peculiarities of AmpliSens® branded kits are:

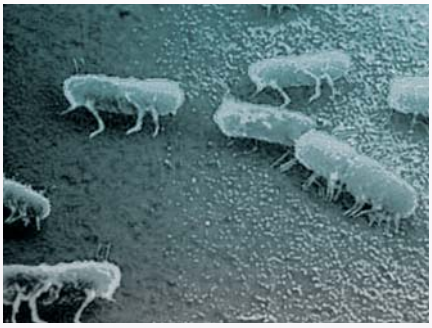
- development of the tests range for the most effective complex solution of AEI etiological verification as a disease group;
- validation on a great amount of the clinical material in the territory of the RF and CIS states;
- accurate verification of analytical characteristics;
- universal nature of test algorithms;
- reliable prevention of falsely negative test results due to use of control samples on all stages of the analysis.

At present the Federal State Scientific Institution Central Scientific and Research Institute of Epidemiology is the only producer of reagent kits for PCR-based diagnostics of AEI with marketing authorizations in the territory of the RF.

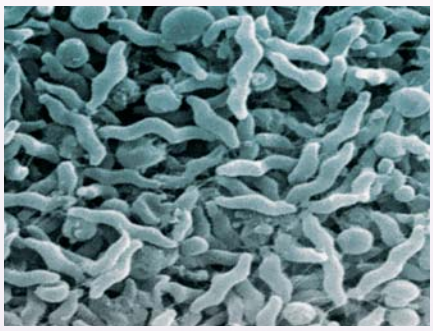
Trials of the kits in the territory of the RF and countries of the former USSR that have been conducted for many years allowed developing of diagnostics kits with a multi-prime hybridization-fluorescent format of results analysis, additional distinctive features of which include:

- a high level of users protection from falsely negative results of tests (contamination protection of the test);
- the optimum range of tests developed by the producer in the multi-prime kit format;
- higher effectiveness and lower labour intensity of tests;
- adaptation to the commercially available equipment (a possibility of end-point detection);
- successful validation of kits in the Center for Disease Control and Prevention (CDC)) (USA).

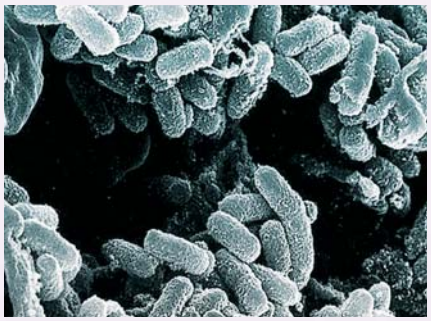
Laboratory Diagnostics of Acute Enteric Infections



Enteroinvasive ischerichia



Campilobacteria



Yersinia

Cultural methods of diagnostics are a conventional direct method of bacterial-etiology AEI activator detection. In certain cases use of such methods is indispensable as it allows isolating a pure culture of the microorganism but with regard to certain pathogenic agents their application is associated with difficulties or has a limited informative value.

Bacterial Pathogenic Agents

Diagnostics of diseases similar to dysentery

Such a disease entity as “bacteriologically unconfirmed dysentery” listed in official sources of information about the infectious and parasite disease incidence illustrates difficulties of bacteriological diagnostics of this pathology. The greater share of similar cases of “bacteriologically unconfirmed dysentery”, especially in children, is caused by enteroinvasive escherichiosis. Enteroinvasive E. coli (ETEC) by taxonomic properties are close to Schigellas, cause clinically identical diseases, have similar factors of virulence and are able to exchange with them in plasmids bearing encoding genes. These facts give the reason to some authors (L. Wang et al, 2001) to call to review of the existing classification of these groups of microorganisms with their categorization as a single class.

In addition to reduction of the term of analysis, PCR application for diagnostics of diseases similar to dysentery allows detecting the whole range of microorganisms (Shigella spp + EIEC), responsible for their development.

Campylobacteriosis

Campylobacteria are a group of the microorganisms most difficult for cultivation. This can be accounted for their micro aerophilic character and a possibility to inhibit their growth by the concomitant flora. The campylobacteria species unite AEI causative agents (thermophilic species) and saprophytic and opportunistic types, which should be born in mind while detecting these microorganisms in the clinical material. Application of PCR-based kits for detection of the thermophilic group of campylobacteria allows not only preventing labour intense and costs-churning bacteriological works but preventing detection of those species of campylobacteria that have the etiological connection with the acute enteric infection (C. jejuni, C. coli, C. lari, C. upsaliensis).

Yersiniosis and pseudotuberculosis

Isolation of Yersinia with application of bacteriological methods requires use of specialized media as well as the necessity to verify in relation to Y. enterocolitica of viral properties of the isolated strain. The biochemical and serological tests of identification of viral Yersinia applied in the bacteriological practice very often lead to discordant results obtained on the same studied strains. Taking into account peculiarities of these microorganisms many methods of viral Yersinia detection are developed with use of PCR with amplification of gene parts encoding various virulence factors. At the same time it's necessary to note that the diagnostic value of methods with amplification of various target genes might be variable to a great degree. The tests with amplification of genes invasion and plasmid factors of Y.enterocolitica virulence are the most informative. At detection of Y. pseudotuberculosis it’s necessary to avoid using tests providing no differentiation between Y. pseudotuberculosis и Y.pestis (the so-called tests for detection of viral Yersinia) as preventing unambiguous interpretation of the study results.

Typhoid-paratyphoid diseases

In accordance with the official statistics each year about two hundred typhoid-paratyphoid diseases are registered in our country. The necessity of urgent epidemic countermeasures in the event of their detection requires application of quick tests of laboratory diagnostics. Molecular-genetic methods of typhoid and paratyphoid fever allows reducing the term of laboratory examinations and facilitating detection of the causative agent in environmental entities, completing classical schemes of microbiological diagnostics.

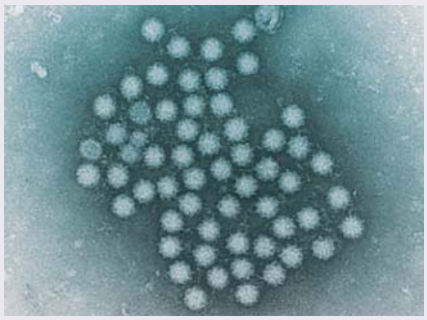
Eschirechia

A group of diarrhogenic Eschirechia differentiated by the presence of various virulence factors unites causative agents with different epidemiology and syndromic manifestations of the GI tract affection. In addition to enteroinvasive Eschirechia (EIEC), described above, enterohemorrhagic E. coli (EHEC) are most widely spread and known, that in some instances are capable of causing such grave pathology as hemolytic-uremic syndrome, enteropathogenic (EPEC) and enteroaggregative (EAEC) E. Coli, widely spread activators of diarrheic diseases in children, as well as enterotoxigenic E. coli (ETEC). A traditional method of isolation of these groups of causative agents is isolation of their cultures with subsequent determination of the serogroup the isolated strains belong to, which can be an implicit indicator of certain virulent factors they bear. Correlation between the serogoup of the strain and their classification to this or that group of diarrhogenic E. coli is rather notional and due to this methods of molecular and genetic analysis with detection of genes encoding key virulence factors of different Eschirechia groups have become widely spread.

Eschirechia

Virus pathogenic agents

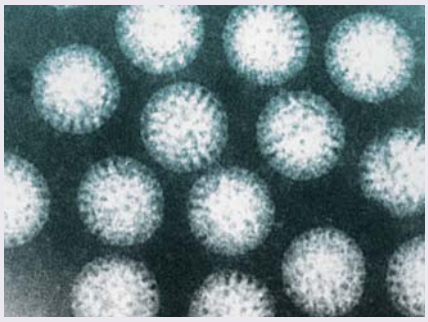
For diagnostics of AEI promoted by viral etiology causative agents diagnostic test systems only for Group A rotaviruses are widely used. In relation to the whole complex of other viral etiology AEI activators a choice of diagnostic tests is rather limited or is lacking.



Noroviruses

Noroviruses

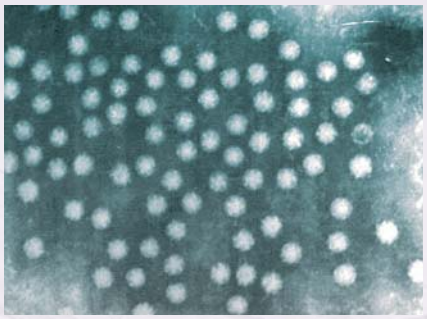
In accordance with the foreign literature sources norovirus is the most frequent activator of nonbacterial etiology AEI. This peculiarity is associated with a low infecting dose and high resistance of the norovirus in the environment. These causative agents have variable antigenic characteristics encumbering development of effectively working IFA test-systems and are not cultivated on tissue cultures. Due to these reasons PCR is a golden standard as in the clinical diagnostics of diseases caused by them as well as in detection of these viruses in the environmental entities.



Rotaviruses

Rotaviruses

Group A rotaviruses are the most frequent cause of sporadic AEI diseases in children. In Russian literature the rotavirus infection means the disease caused by Group A rotaviruses. But it should be remembered that up to 2-3 percent of rotavirus infection is associated with Group C rotaviruses that serologic tests used for diagnostics of rotavirus infection. IFA test systems for detection of rotavirus antigens in faeces have been most extensively used. Unfortunately, the specificity of some test-systems is rather low, which leads to a great amount of invalid test results. Due to this WHO recommends application of a limited number of test systems for diagnostics of rotavirus infection («Basic protocols of burden of rotavirus gastroenteritis by results of hospitalization and study of children application for medical assistance for gastroenteritis on the level of the serviced population»). IFA test-systems do not provide the sensitivity required for detection of rotaviruses in the environment.

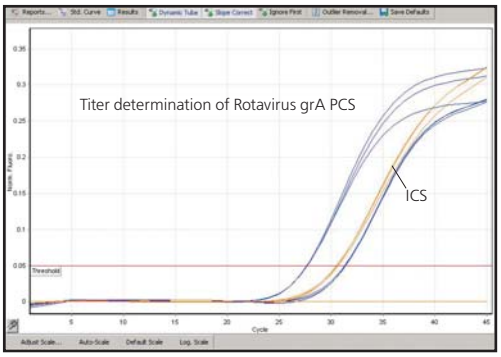


Astroviruses

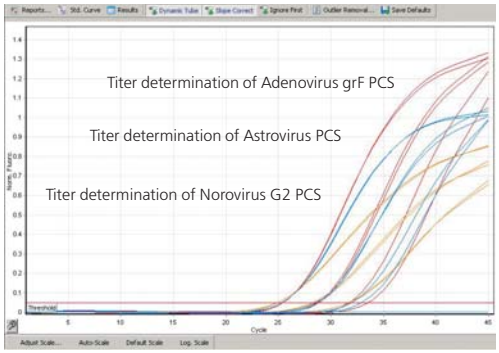
Astroviruses

Despite the lower incidence of astroviruses as compared to rota- and norovarises, they are responsible for a great amount of enteric infections, third of patients being affected by colitis signs. Tests based on IFA and PCR have been most widely spread methods for diagnostics of astrovirus infection in the world. Commercially available IFA test systems for detection of astrovirus antigen detection are lacking in our country.

Reagent kits for detection of viral AEI: rota-, noro- and astroviruses



Green/Fam channel – reaction mixtures 1 and 2



Yellow/Joe channel – reaction mixtures 1 and 2

Advantages of reagent kits

- Kits are tested on a great amount of clinical material samples (more than 14 thousand samples) in the course of disease outbreak decoding and at examination of environmental entities, which provided express proof of specificity, development and recommendation of the optimum algorithm of their application for AEI diagnostics.
- A number of kits were validated in the Center for Disease Control and Prevention (CDC) (USA).
- Availability of the recombinant internal control samples allows control of all stages of analysis (isolation of nucleic acids, reverse transcription, PCR) and evaluation of effect of PCR inhibitors on test results.

Clinical material for examination

Clinical Material	Recommended kits for extraction
Faeces	"RIBO-sorb", material preprocessing is required
Environmental entities (concentrates of water samples, food products)	"RIBO-sorb", material preprocessing is required

FRT format. Fluorescence Detection in Real-Time Regime

Cat.No.	Name	Set	No. of tests	Type	Mark	Special equipment
R-V40 (RG)	AmpliSens® Rotavirus/ Norovirus/ Astrovirus-FL*		50		Green/FAM, Yellow/JOE	Rotor-Gene 6000 (Corbett Research, Australia)
R-V40 (iQ)	AmpliSens® Rotavirus/ Norovirus/ Astrovirus-FL*		50		FAM, HEX	iCycler/iQ5 (BioRad, USA)

«Reverta-L" kit is required for reverse transcription.

FEP Format. End Point Fluorescence Detection

Cat.No.	Name	Set	No. of tests	Type	Mark	Special equipment
V40-50-R0,5-FEP	AmpliSens® Rotavirus/ Norovirus/ Astrovirus-FL*		50		FAM, HEX	Jin (DNA-Technology, Russia), ALA-1/4 (BioSan, Latvia)
V40-50-R0,2-FEP	AmpliSens® Rotavirus/ Norovirus/ Astrovirus-FL*		50		FAM, HEX	ALA-1/4 (BioSan, Latvia)

«Reverta-L" kit is required for reverse transcription.

By configuration:

an "amplification reagent kit" format (PCR-set)
includes only amplification reagents.

By a hot start type and filling

"Wax" format
"Hot Start" is provided by a wax layer:

- a set includes ready to use PCR test tubes with the lower mixture applied under the wax

Advantages of FRT and FEP formats

- A range of causative agents included in the test was primarily selected for the maximum effective decoding of AEI.
- Validation protection (contamination protection and availability of internal control).
- Adaptation to the commercially available equipment.
- Reagent kits were validated in the Center for Disease Control and Prevention CDC (USA) in 2007.
- State testing in the L.A. Tarasevich State Medicinal Biological Products Standardization and Control Institute and have marketing authorizations issued by the Ministry of Health of the RF.

Analytical properties for FRT and FEP formats

Sensitivity	Group A rotaviruses, adenoviruses, astroviruses - 1x10^4 GE per ml, 2 genotype noroviruses - 5 x10^3 GE per ml (by results of state testing in the L.A. Tarasevich State Medicinal Biological Products Standardization and Control Institute).
Specificity	Specificity in the presented panel* made 100%.

** — Intralaboratory specificity examinations were conducted on enteric virus strains (Coxsakie B1, B2, B3, B4, B5, B6; Polio (Sabin) I, II, III). Adenoviruses of 5 and 7 serogroup, Group A flu viruses (H13N2, H9N2, H8N4, H2N3, H4N6, H11N6, H12N5, H3N8, H1N1, H6N2, H10N7, H5N1), Group B flu viruses, rhinoviruses, RS viruses, human adenoviruses — types 3, 5, 7, 37, 40, faeces samples containing genotype I noroviruses (GI.1 - GI.4, GI.14), genotype II noroviruses (GII.1- GII.7, GII.10, GII.12, GII.14, GII.17), Group A rotaviruses (G1-9), astroviruses (1,2,4,5,8 genotypes), adenoviruses (types 39, 40, 41) were tested too..*

EPh Format - Electrophoretic Detection

Attention! The technology presents danger of contamination!
A separate room and personnel are required for the detection!

Cat.No.	Name	Set	No. of tests	Type	Special equipment
V19-50-R0,5	AmpliSens® Astrovirus-EPh		55		Electrophoretic chamber, transilluminator, gel-documentation system
V19-50-R0,2	AmpliSens® Astrovirus-EPh		55		
V15-50-R0,5	AmpliSens® Astrovirus-EPh		55		
V15-50-R0,2	AmpliSens® Astrovirus-EPh		55		
V27-50-R0,5	AmpliSens® Norovirus 1,2 genotypes Eph		55		
V27-50-R0,2	AmpliSens® Norovirus 1,2 genotypes Eph		55		

Analytical properties

Sensitivity	Group A rotaviruses, adenoviruses, astroviruses - 1x10^4 GE per ml, 2 genotype noroviruses - 5 x10^3 GE per ml.
Specificity	Specificity in the presented panel* made 100%.

Results of FEP and FRT clinical tests

Reagent kits were tested on the decoded panel of clinical samples, from patients with different etiology AEI that was preliminary characterized on test systems used in the center of the Center for Disease Control and Prevention (CDC). Diagnostic sensitivity made 100 percent, the diagnostic specificity at deletion of adenoviruses of Group F – 97 percent, in relation to other pathogenic agents – 100 percent.

	Sample	Tests	1	2
1	1	AEI NoroIC	IC+	Noro - detected
2	2	AEI NoroIC	IC+	Noro - detected
3	3	AEI NoroIC	IC+	Noro - detected
4	4	AEI NoroIC	IC+	Noro - detected
5	5	AEI NoroIC	IC+	Noro - detected
6	6	AEI NoroIC	IC+	Noro – not detected
7	7	AEI NoroIC	IC+	Noro – not detected
8	8	AEI NoroIC	IC+	Noro – not detected
9	9	AEI NoroIC	IC+	Noro – not detected
10	10	AEI NoroIC	IC+	Noro – not detected
11	background	AEI NoroIC	45.32	76.34
12	background	AEI NoroIC	44.64	75.06

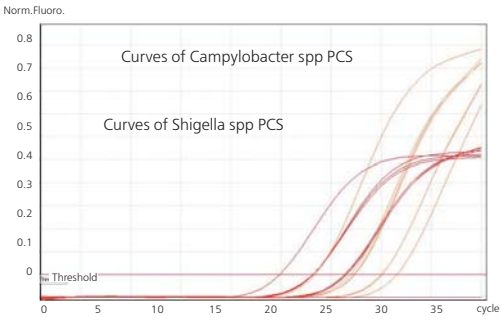
Reaction mixture 1:
detection of Norovirus and internal control

	Sample	Tests	1	2
1	1	AEI RotaAst	Rota - detected	Astro - not detected
2	2	AEI RotaAst	Rota - detected	Astro - not detected
3	3	AEI RotaAst	Rota - detected	Astro - not detected
4	4	AEI RotaAst	Rota - detected	Astro - not detected
5	5	AEI RotaAst	Rota - detected	Astro - not detected
6	6	AEI RotaAst	Rota - not detected	Astro - detected
7	7	AEI RotaAst	Rota - not detected	Astro - detected
8	8	AEI RotaAst	Rota - not detected	Astro - detected
9	9	AEI RotaAst	Rota - not detected	Astro - detected
10	10	AEI RotaAst	Rota - not detected	Astro - detected
11	background	AEI RotaAst	85.09	35.52
12	background	AEI RotaAst	83.77	35.61

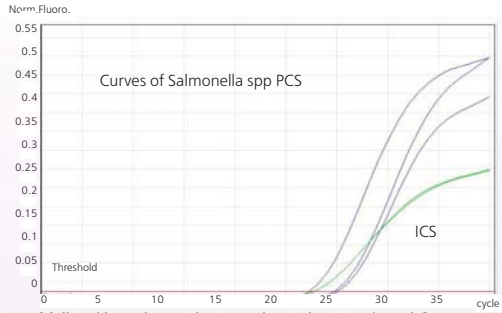
Reaction mixture 2: detection of Astrovirus and Rotovirus.

Reagent kits for detection of viral AEI: Schigella, EIEC, Salmonella, Campylobacter, Yersinia, Clostridium

Representative works. FRT format



Green/Fam channel – reaction mixtures 1 and 2



Yellow/Joe channel – reaction mixtures 1 and 2

Explanation:

By configuration:

an "amplification reagent kit" (PCR-set) includes only amplification reagents

By hot start type and filling:

"Wax" format

"Hot Start" is provided by a wax layer:

- a set includes ready to use PCR test tubes with the lower mixture applied under the wax

Advantages of reagent kits

- Kits are tested on a great amount of clinical material samples (more than 14 thousand samples) in the course of disease outbreak decoding and at examination of environmental entities, which provided express proof of specificity, development and recommendation of the optimum algorithm of their application for AEI diagnostics.
- The only reagent kits produced in Russia that passed state tests in the L.A. Tarasevich State Medicinal Biological Products Standardization and Control Institute and registered in the Russian Federation.
- A number of kits were validated in the Center for Disease Control and Prevention (CDC) (USA).
- Availability of the recombinant internal control samples allows control of all stages of analysis (isolation of nucleic acids, reverse transcription, PCR) and evaluation of effect of PCR inhibitors on test results.

Clinical material for examination

Clinical Material	Recommended kits for extraction
Faeces	"RIBO-sorb", material preprocessing is required
Environmental entities (concentrates of water samples, food products)	"RIBO-sorb", material preprocessing is required

FRT format. Fluorescence Detection in Real-Time Regime

Cat.No.	Name	Set	No. of tests	Type	Mark	Special equipment
R-B44 (RG)	AmpliSens® Shigella spp and EIEC/ Salmonella spp./ Campylobacter spp.-FL		50		Green/FAM, Yellow/JOE	Rotor-Gene 6000 (Corbett Research, Australia)
R-B44 (iQ)	AmpliSens® Shigella spp and EIEC/ Salmonella spp./ Campylobacter spp.-FL		50		Green/FAM, Yellow/JOE	iCycler/iQ5 (BioRad)

FEP Format. End Point Fluorescence Detection

Cat.No.	Name	Set	No. of tests	Type	Mark	Special equipment
B44-50-R0,5-FEP	AmpliSens® Shigella spp and EIEC/ Salmonella spp./ Campylobacter spp.-FL		50		FAM, HEX	ALA-1/4 (BioSan, Latvia), Jin (DNA-Technology, Russia)
B44-50-R0,2-FEP	AmpliSens® Shigella spp and EIEC/ Salmonella spp./ Campylobacter spp.-FL		50		FAM, HEX	ALA-1/4 (BioSan, Latvia), Jin (DNA-Technology, Russia)

Analytical properties for FRT and FEP formats

Sensitivity	Bacterial pathogens - 1x10⁴ GE per ml (by results of state testing in the L.A. Tarasevich State Medicinal Biological Products Standardization and Control Institute).
Specificity	Specificity in the presented panel* made 100%.

** – Intralaboratory testing of specificity was conducted on strains: Salmonella Ser. grumpensis, newport, enteriditis, typhimurium, kentucky, oranienburg, anatum, heidelberg; Shigella dysenteriae type 1, 2, fl exneri, boydii, sonnei; E. coli enterotoxigenic, Shiga-toxin, O6:H1; Campylobacter spp.; Serratia marcescens; Edwardsiella spp.; Arcobacter butzleri; Proteus vulgaris; Helicobacter cineadi, pullorum, pylori; Vibrio vulnificus, cholerae, parahaemolyticus; Yersinia enterocolitica; Citrobacter freundii; Aeromonas spp.; Providencia stuartii; Pseudomonas aeruginosa and others.*

EPh Format – Electrophoretic Detection

Attention! The technology presents danger of contamination!
A separate room and personnel are required for the detection!

Cat.No.	Name	Set	No. of tests	Type	Special equipment
TB12-50-R0,5	AmpliSens Shigella species and EIEC		50		
TB12-50-R0,2	AmpliSens Shigella species and EIEC		50		
B12-50-R0,5	AmpliSens Shigella species and EIEC		55		
B12-50-R0,2	AmpliSens Shigella species and EIEC		55		
B11-50-R0,5	AmpliSens Salmonella species-EPh		55		
B11-50-R0,2	AmpliSens Salmonella species-EPh		55		
TB35-50-R0,5	AmpliSens Campylobacter species		50		
TB35-50-R0,2	AmpliSens Campylobacter species		50		Electrophoretic chamber, transilluminator, gel-documentation system
B35-50-R0,5	AmpliSens Campylobacter species		55		
B35-50-R0,2	AmpliSens Campylobacter species		55		
B22-50-R0,5	AmpliSens Yersinia enterocolitica-EPH		55		
B22-50-R0,2	AmpliSens Yersinia enterocolitica-EPH		55		
B39-50-R0,5	AmpliSens Yersinia pseudotuberculosis-EPh		55		
B39-50-R0,2	AmpliSens Yersinia pseudotuberculosis-EPh		55		
B23-50-R0,5	AmpliSens Clostridium difficile-EPh		55		
B23-50-R0,2	AmpliSens Clostridium difficile-EPh		55		

Analytical properties

Sensitivity	Bacterial pathogens - 1x10⁴ GE per ml (by results of state testing in the L.A. Tarasevich State Medicinal Biological Products Standardization and Control Institute).
Specificity:	Specificity in the presented panel* made 100%.

** – Intralaboratory testing of specificity was conducted on strains: Salmonella Ser. grumpensis, newport, enteriditis, typhimurium, kentucky, oranienburg, anatum, heidelberg; Shigella dysenteriae type 1, 2, flexneri, boydii, sonnei; E. coli enterotoxigenic, Shiga-toxin, O6:H1; Campylobacter spp.; Serratia marcescens; Edwardsiella spp.; Arcobacter butzleri; Proteus vulgaris; Helicobacter cineadi, pullorum, pylori; Vibrio vulnificus, cholerae, parahaemolyticus; Yersinia enterocolitica; Citrobacter freundii; Aeromonas spp.; Providencia stuartii; Pseudomonas aeruginosa and others.*

Advantages of FRT and FEP formats

- A range of causative agents included in the test was primarily selected for the maximum effective decoding of AEI.
- Validation protection (contamination protection and availability of internal control).
- Adaptation to the commercially available equipment.
- Reagent kits were validated in the CDC (USA) in 2007.
- State testing in the L.A. Tarasevich State Medicinal Biological Products Standardization and Control Institute and marketing authorizations issued by the Ministry of Health of the RF.

Results of FEP and FRT clinical tests

Reagent kits were tested on the decoded panel of clinical samples, from patients with different etiology AEI that was preliminary characterized on test systems used in the center of the Center for Disease Control and Prevention (CDC). Diagnostic sensitivity and diagnostic specificity made 100%.

	Sample	Tests	1	2
1	1	AEI CspIC	Csp - detected	IC+
2	2	AEI CspIC	Csp - detected	IC+
3	3	AEI CspIC	Csp - detected	IC+
4	4	AEI CspIC	Csp - detected	IC+
5	5	AEI CspIC	Csp - detected	IC+
6	6	AEI CspIC	Csp - not detected	IC+
7	7	AEI CspIC	Csp - not detected	IC+
8	8	AEI CspIC	Csp - not detected	IC+
9	9	AEI CspIC	Csp - not detected	IC+
10	10	AEI CspIC	Csp - not detected	IC+
11	background	AEI CspIC	64.12	35.17
12	background	AEI CspIC	63.83	34.87

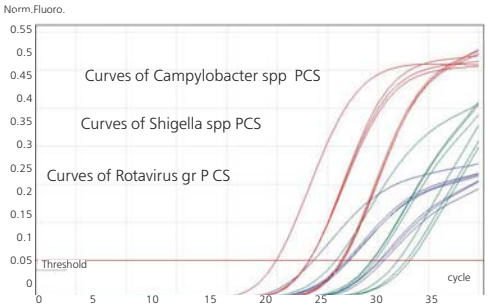
Reaction mixture 1: detection of Campylobacter species and internal control

	Sample	Tests	1	2
1	1	AEI ShSal	Shig - detected	Salm - not detected
2	2	AEI ShSal	Shig - detected	Salm - not detected
3	3	AEI ShSal	Shig - detected	Salm - not detected
4	4	AEI ShSal	Shig - detected	Salm - not detected
5	5	AEI ShSal	Shig - detected	Salm - not detected
6	6	AEI ShSal	Shig - not detected	Salm - detected
7	7	AEI ShSal	Shig - not detected	Salm - detected
8	8	AEI ShSal	Shig - not detected	Salm - detected

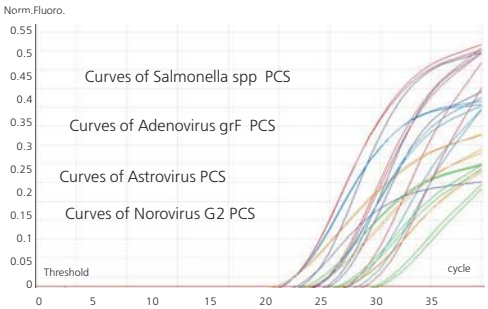
Reaction mixture 2: detection of Salmonella species+EIEC.

Reagent kits for AEI diagnostics in MultiPrime format

Representative works



Green/Fam channel



Yellow /Joe channel

Results of FEP and FRT clinical tests

Reagent kits were tested on the decoded panel of clinical samples, from patients with different etiology AEI that was preliminary characterized on test systems used in the center of the Center for Disease Control and Prevention (CDC). Diagnostic sensitivity made 100 percent, the diagnostic specificity at deletion of adenoviruses of Group F - 97%, in relation to other pathogenic agents - 100%.

Explanation:

By configuration:

- a "complete set reagent kit» includes reagents for extraction, amplification and detection;

- an "amplification reagent kit" (PCR-set) includes only amplification reagents.

By a hot start type and filling

"Wax" format

"Hot Start" is provided by a wax layer:

- a set includes ready to use PCR test tubes with the lower mixture applied under the wax

Advantages of reagent kits

- Kits protect the user from the non-optimum selection of diagnostic kits for AEI diagnostics, i.e. are developed with due account of the optimum algorithm of the diagnostic search.
- Kits are developed with due account of the optimum algorithm of the diagnostic search.
- Reagent kits "AmpliSens® AEI screen-FL» allow detecting 7 AEI activators: Rotavirus, Norovirus, Astrovirus, Adenovirus, Shigella spp. и EIEC, Salmonella spp., Campylobacter spp.
- The multi-prime format ensures work-effectiveness, time-saving and reduction of the equipment load.
- The kits were tested on a great amount of clinical material samples, passed state tests in the L.A. Tarasevich State Medicinal Biological Products Standardization and Control Institute and have marketing authorizations issued by the Ministry of Health of the RF.

Clinical material for examination

Clinical Material	Recommended kits for extraction
Faeces	"RIBO-sorb", "Reverta-L», «DNA-sorb-B"
Environmental entities	"RIBO-sorb"

➦ — a kit is included in the complete set reagent kit ( )

FRT format. Fluorescence Detection in Real-Time Regime

Cat.No.	Name	Set	No. of tests	Type	Mark	Special equipment
R-B45(RG)	AmpliSens® AEI screen-FL		50		Green/FAM, Yellow/JOE	Rotor-Gene 6000 (Corbett Research, Australia)
R-B45(iQ)	AmpliSens® AEI screen-FL		50		FAM, HEX	iCycler/iQ5 (BioRad)

FEP Format. End Point Fluorescence Detection

Cat.No.	Name	Set	No. of tests	Type	Mark	Special equipment
B45-50-R0,5-FEP	AmpliSens® AEI screen-FL		50		FAM, HEX	ALA-1/4 (BioSan, Latvia), Jin (DNA-Technology, Russia)
B45-50-R0,2-FEP	AmpliSens® AEI screen-FL		50		FAM, HEX	

Analytical properties for FRT and FEP formats

Sensitivity	GE per ml (IE per ml) Group A rotaviruses, adenoviruses, astroviruses - $1x10^4$ GE per ml, 2 genotype noroviruses - 5 $x10^3$ GE per ml, bacterial pathogens - $1x10^4$ GE per ml (by results of state testing in the L.A. Tarasevich State Medicinal Biological Products Standardization and Control Institute).
Specificity	Specificity in the presented panel* made 100%.

** — Intralaboratory specificity examinations were conducted on strains: Salmonella Ser. grumpensis, newport, enteriditis, typhimurium, kentucky, oranienburg, anatum, heidelberg; Shigella dysenteriae type 1, 2, fl exneri, boydii, sonnei; E. coli enterotoxigenic, Shiga-toxin, O6:H1; Campylobacter spp.; Serratia marcescens; Edwardsiella spp.; Arcobacter butzleri; Proteus vulgaris; Helicobacter cineadi, pullorum, pylori; Vibrio vulnifi cus, cholerae, parahaemolyticus; Yersinia enterocolitica; Citrobacter freundii; Aeromonas spp.; Providencia stuartii; Pseudomonas aeruginosa and others. Specificity was tested on enteric virus strains (Coxsakie B1, B2, B3, B4, B5, B6; Polio (Sabin) I, II, III). Adenoviruses of 5 and 7 serogroup, Group A flu viruses (H13N2, H9N2, H8N4, H2N3, H4N6, H11N6, H12N5, H3N8, H1N1, H6N2, H10N7, H5N1), Group B flu viruses, rhinoviruses, RS viruses, human adenoviruses — types 3, 5, 7, 37, 40, faeces samples containing 1 genotype noroviruses (GI.1 - GI.4, GI.14), II genotype noroviruses (GII.1- GII.7, GII.10, GII.12, GII.14, GII.17), Group A rotaviruses (G1-9), astroviruses (1,2,4,5,8 genotypes), adenoviruses (types 39, 40, 41) were tested too.*

Enterovirus reagent kits

Reagent kits for Enteric Viruses Detection

- AmpliSens® Enterovirus reagent kits allow conducting operative diagnostics of various enteric viral diseases without the long and labour intensive stage of virusological examinations

Clinical material for examination

Clinical Material	Recommended kits for extraction
Cerebrospinal fluid	"RIBO-sorb" ➦ ▤
Environmental entities	"RIBO-sorb" ➦ ▤ preprocessing of material is required

➦ ▤ — a kit is included in the complete set reagent kit (🗐)

FRT and FEP formats. Fluorescence detection in the real-time regime and by the end point.

Cat.No.	Name	Set	No. of tests	Type	Mark	Special equipment
TR-V16(RG)	AmpliSens® Enterovirus	🗐	50	⇊	Green/FAM, Yellow/JOE	Rotor-Gene 6000 (Corbett Research, Australia)
TV16-50-R0,5 FEP	AmpliSens® Enterovirus	🗐	50	⇊	FAM, HEX	Jin (DNA-Technology, Russia), ALA-1/4 (BioSan, Latvia)
TV16-50-R0,2 FEP	AmpliSens® Enterovirus	🗐	50	⇊	FAM, HEX	

Analytical properties

Sensitivity	5x10⁴ GE per ml (by results of state testing in the L.A. Tarasevich State Medicinal Biological Products Standardization and Control Institute).
Specificity	Specificity in the presented panel* made 100%.

** — Strains of enteric viruses are the courtesy of the L.A. Tarasevich State Medicinal Biological Products Standardization and Control Institute (Coxsakie B1, B2, B3, B4, B5, B6; Polio (Sabin) I, II, III). Adenoviruses of 5 and 7 serogroups; GroupA flu viruses (H13N2, H9N2, H8N4, H2N3, H4N6, H11N6, H12N5, H3N8, H1N1, H6N2, H10N7, H5N1), Group B flu viruses, rhinoviruses, RS viruses, human adenoviruses — types 3, 5, 7, 37, 40 were evaluated too. Specificity was assessed on strains N meningitides, St pneumoniae, H influenzae, Clebsiella K 65 SW4, Listeria monocitogenes USHC 19, Listeria monocitogenes USHC 52, Proteus vulgaris 115/98, Pseudomonas aeruginosa DN c1, Staphilococcus aureus 653, Staphilococcus aureus 29112, Morganella Morganii 619 c 01, Enterobacter faecalis 356.*

EPh Format - Electrophoretic Detection

Attention! The technology presents danger of contamination! A separate room and personnel are required for the detection!

Cat.No.	Name	Set	No. of tests	Type	Special equipment
TV16-50-R0,5	AmpliSens® Enterovirus	🗐	50	⇊	Electrophoretic chamber, transilluminator, gel-documentation system
TV16-50-R0,2	AmpliSens® Enterovirus	🗐	50	⇊	
V16-50-R0,5*	AmpliSens® Enterovirus	▤	50	⇊	
V16-50-R0,2*	AmpliSens® Enterovirus	▤	50	⇊	

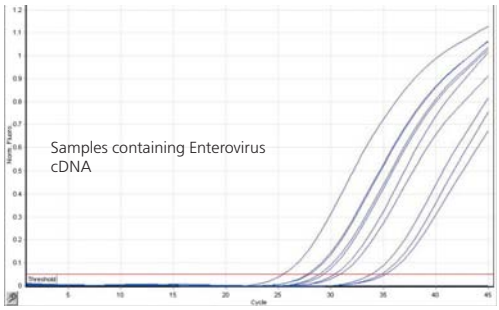
** "Reverta L" is used for reverse transcription.*

Analytical properties

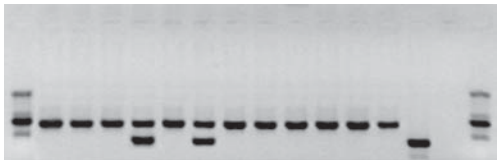
Sensitivity	5x10⁴ GE per ml (by results of state testing in the L.A. Tarasevich State Medicinal Biological Products Standardization and Control Institute).
Specificity	Specificity in the presented panel* made 100%.

** — Specificity was assessed on strains of enteric viruses Coxsakie B1, B2, B3, B4, B5, B6; Polio (Sabin) I, II, III, adenoviruses of 5 and 7 serogroup, A Group flu viruses (H13N2, H9N2, H8N4, H2N3, H4N6, H11N6, H12N5, H3N8, H1N1, H6N2, H10N7, H5N1), Group B flu viruses, rhinoviruses, RS viruses, human adenoviruses — types 3, 5, 7, 37, 40, as well as on strains N meningitides, St pneumoniae, H infl uenzae, Clebsiella K 65 SW4, Listeria monocitogenes USHC 19, Listeria monocitogenes USHC 52, Proteus vulgaris 115/98, Pseudomonas aeruginosa DN c1, Staphilococcus aureus 653, Staphilococcus aureus 29112, Morganella Morganii 619 c 01, Enterobacter faecalis 356.*

Representative works



Sample	1	2
1	PC+	Enterovirus - detected
2	IC+	Enterovirus - detected
3	IC+	Enterovirus - detected
4	PC+	Enterovirus - not detected
5	IC+	Enterovirus - not detected
v-	IC+	Enterovirus - not detected
k-	IC-	Enterovirus - nd
k+	IC-	Enterovirus - detected
background	56.04	52.30
background	56.68	53.24



FRT and FEP format advantages

- High level of use protection from falsely-negative test results (contamination protection of a test).
- Higher operational efficiency and lower labour intensity of studies.

Results of clinical tests (all formats: FRT, FEP, EPh)

Reagent kits passed tests on the clinical material (cerebrospinal fluid of patients affected by meningitis of various etiology and patients with CNS afflictions of non-inflammatory etiology) in the period from 01.02.1998 to 01.03.2000 and from 16.02.1998 to 08.08.2007. The study allowed detection of 39 positive (by RNA content) enteroviruses of samples. Specificity of their detection was confirmed by a direct sequenation of nucleotide sequences. No unspecific reactions were detected, specificity made 100 percent. The state testing in the L.A. Tarasevich State Medicinal Biological Products Standardization and Control Institute included testing of the panel containing cerebrospinal liquid of patients affected by tick-borne encephalitis with confirmation of diagnosis by serological indicators (IgM and IgG), determination of the tick-borne encephalitis in blood and/or CSF of patients with somatic diseases, patients with purulent meningitis of different etiology, liquor samples containing RNA virus of parotitis and enteric viruses of various groups. Diagnostic sensitivity and specificity made 100 percent

ИЛС
InterLabService

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