

gical, immunological). Moreover, the latter do not allow to establish the disease etiology in 20–40% of cases due to the variability of morphological, cultural properties and antigenic structure of the pathogen [Gushchin A.E. et al., 2014]. The use of MGT had to improve the laboratory diagnostics quality and, as a consequence, to provide a high level of GI detection. According to official statistics, in the Perm region there is a stable reduction of GI incidences. Meanwhile, PCR is used no more than in 25% cases of GI and is carried out exclusively by commercial laboratories. The PCR modifications used in such institutions do not exclude the appearance of both false-negative and false-positive results due to a number of objective and subjective reasons. Thus, a high degree of nucleotide sequence homology and frequent genetic exchange between gonococci and other species of the genus *Neisseria*, as well as a high level of genetic polymorphism of different strains of *Neisseria gonorrhoeae* are the serious problem of molecular diagnosis of GI [Palmer H.M. et al., 2003; Whiley D.M. et al., 2006; Tabrizi S.N. et al., 2011]. The cross-reaction with other microorganisms leads to a false positive results. MGT, which does not involve analysis of strain characteristics (presence of “pathogenicity islands”, genetic markers of resistance, etc.), does not provide qualitative monitoring of *N. gonorrhoeae*. In addition, the detectable fragments of nucleic acids do not always belong to living pathogens. Thus, microbiological diagnostics of GI, including the use of PCR, remains an unsolved problem. The needs of practical health care require the improvement of existing approaches and the development of new ones.

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EXPERIENCE OF EXPANDED PRIMER SET USING FOR DETECTION OF THE PATHOGENIC POTENTIAL OF *ESCHERICHIA COLI*

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The purpose of the study to reveal the pathogenic potential of *E.coli* in routine intestinal dysbiosis diagnostics in children are under regular medical check-up because of chronic conditions by detection of genes encoding pathogenicity factors by polymerase chain reaction (PCR).

9–17 years old children were examined with standard procedure of bacteriological diagnostics of intestinal dysbiosis and subdivided into groups: 1) with chronic gastroduodenitis – 22 patients; 2) with atopic dermatitis (AtD) – 19; 3) with bronchial asthma – 7. *E.coli* DNA was recovered with assay reagent set “Ribo-prep” (Russia). Specific gene sequences of *ETEC*, *EPEC*, *EHEC*, *EAEC* and *EIEC* were found by real-time PCR (CFX 96, BioRad, USA) with assay reagent set “Amplisens-Escherichioses” (Russia). Also, individual primers (BioBeagle, Russia) were used to identify genes of pathogenicity factors of *E. coli*, specifically *eaeA*, *bfpA* – of *EPEC*, *stl*, *lt* – of *ETEC*, *cnf1* – *NMEC*, *afa* – *DAEC*, *ipaH*, *ial* – *EIEC*, *aggA*, *east1* – *EAEC*, *stx1*, *stx2* – *EHEC*, *chuA* – of *AIEC* (so-called Adherent-Invasive *E. coli*, associated with Crohn's disease) correspondingly.

In the group of chronic gastroduodenitis *E. coli* stains with pathogenicity coding genes were detected in 7 of 22 tested strains (genes *eaeA*, *bfpA*, *aggA*, *afa*, *chuA*), in the group with AtD – 3 of 19 (genes *eaeA*, *bfpA*, *aggA*, *east*). Among the children with bronchial asthma, pathogenic *Escherichia* was not found. In routine intestinal dysbiosis diagnostics virulence coding genes of diarrheagenic *E. coli* were detected in 11 cases (23.9%).

In order to reveal the etiological significance of *Escherichia* in children with chronic diseases, as well as to determine their probabilistic role in the manifestation of diseases, the infectious component of which remains poorly studied, the multiplex real-time PCR should be introduced in the laboratory procedure specifications at very least, as well as the creation of primer set based on updated data on diarrheagenic *E. coli* or sighting of genes, as in the case of Crohn's disease.

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HEPATITIS B VIRUS IDENTIFICATION IN THE ENSURING INFECTIOUS SAFETY OF BLOOD TRANSFUSIONS

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Ensuring the infectious safety of blood transfusions during planned and urgent surgical operations is a topical medical problem and should be implemented first of all in order to prevent the transmission of viruses. Hepatitis B virus (HBV) is one of the most common hepatotropic viruses that can cause both acute and chronic course of the disease. One form of chronic viral hepatitis B is occult hepatitis B, characterized by the presence of HBV DNA in the liver and undetectable levels of HBsAg and HBV DNA in the peripheral blood. Then in most cases, virus replication and gene expression can be suppressed so much that the viral load in the peripheral blood of the patient is extremely low, up to the impossibility of detecting HBV DNA by standard methods, but no elimination of the virus.

The aim of our study was the identification and genotyping of HBV in blood donors.

The material was blood plasma of 1003 blood donors from two transfusion centers. A method for detecting HBV DNA with a low viral load based on a two-step PCR, followed by sequencing was used.

HBV was detected in 6.14% of donors. In the region with a high prevalence of HBV, the incidence of occult HBV in blood donors was 9.4%. In the region with a relatively lower prevalence of HBV, the incidence of occult HB in blood donors was 4.23%. In phylogenetic analysis among the HBV samples obtained from HBsAg-negative blood donors in a region with a high prevalence of HBV, the following subgenotypes are represented: D1 – 46.8%, D2 – 17.05%, D3 – 31.9%, A2 – 4.25%, respectively. In the region with a relatively lower prevalence of HBV subgenotypes are presented: D1 – 22.73%, D3 – 72.73%, C – 4.54%. In the case of donors with detected HBV DNA, HBcor IgG antibodies were detected in 34.7% of cases. At the same time, in the analysis for serological markers of the whole group, HBcor IgG antibodies were detected in 21.2% of cases, of which HBV DNA was detected only in 11.3%, which is in agreement with the data on the hyperdiagnosticity of this marker.

The use of molecular diagnostic methods in the blood safety algorithm to detect HBV with low viral load can ensure the viral safety of blood transfusions, especially where viral hepatitis is widespread.