Volume 3, Issue 3, March 2025 ISSN (E): 2938-3765

FEATURES OF USING MOLECULAR BIOLOGICAL RESEARCH METHODS FOR DIAGNOSTICS OF SEXUALLY TRANSMITTED INFECTIONS

Muxamadiyeva L. A.
DSc, Professor, Head of the Department of 3-Pediatrics and Medical Genetics of Samarkand State Medical University

Ashirova E.
Student of the Pediatric Department of Samarkand State Medical University
Samarkand State Medical University Samarkand, Uzbekistan

Abstract
The PCR method, as applied to clinical diagnostic purposes, is classified as a direct diagnostic method, i.e. it is aimed at directly identifying the pathogen (its genetic material) in the sample. Direct methods also include bacterioscopic, virological, bacteriological and immunofluorescent methods. In contrast, indirect (serological) methods are aimed at identifying the body's response to pathological exposure and/or therapy (most often, enzyme-linked immunosorbent assay (ELISA)). In this regard, the PCR method in most of its modifications (if DNA is used as the target) does not allow one to evaluate the effectiveness of the therapy. This is due to the fact that DNA is a fairly stable product, is slowly destroyed by DNases and remains in the body for a long time (for several weeks in the case of a pronounced infectious process), even when antibacterial therapy has proven effective.

Keywords: Real-time PCR, antibacterial therapy, false positive results, false negative results, analytical and post-analytical quality control stage.

INTRODUCTION

To obtain correct research results, especially when conducting quantitative analysis using the RT-PCR method, it is important to take into account a number of requirements that will help avoid typical errors both at the stage of technology implementation and when interpreting the obtained data.

There are three main stages in the preparation and implementation of PCR analysis, in which errors are most often made, leading to false positive and false negative results: y pre-analytical stage; y analytical stage; y post-analytical stage.

Preanalytical stage errors. Compliance with preanalytical rules should exclude or limit the influence of extra-laboratory factors on the results of laboratory tests. Despite the existence of

Web of Medicine: Journal of Medicine, Practice and Nursing
webofjournals.com/index.php/5

general recommendations for the correct conduct of the preanalytical stage, the PCR method has a number of features that most often manifest themselves in typical preanalytical errors [3].

Obtaining a clinical sample for research using direct diagnostic methods. The biomaterial taken must strictly correspond to the localization of the infectious process - this is a mandatory condition for direct diagnostic methods. First of all, this concerns those microorganisms for which tropism to many types of tissue is known.

For example, C. trachomatis causes UGT infections, inflammatory diseases of extragenital localization, and causes an ascending infectious process, therefore, taking a scraping from the vagina or cervical canal as part of laboratory diagnostics of salpingitis or oophoritis against the background of chlamydial infection can lead to false-negative PCR results. Quality of collection and processing of biological material The second common error of the preanalytical stage is incorrect collection of material for testing. Even with the correct determination of the localization of the process, it is necessary to take into account the fact that the biomaterial must contain the maximum concentration of the desired microorganisms, and must also be free of unwanted impurities that inhibit PCR. Modern reagent kits for PCR analysis allow monitoring the number of epithelial cells in a biomaterial sample by introducing a material collection control (MCC) into their composition, which is fundamentally important for identifying STI pathogens capable of adhesion to the surface of the epithelium and its invasion, as well as parasitism in the interepithelial space. The results of the PCR test may be affected by transient flora from a sexual partner during unprotected sexual contact or microorganisms entering the body when taking probiotic or symbiotic drugs. This factor is especially important to consider when conducting quantitative PCR analysis to diagnose dysbiotic disorders in the UGT. In addition, it is important to minimize the amount of unwanted impurities in the sample, such as mucus, pus and blood, as well as chemical impurities - PCR inhibitors, which can get into the biomaterial if certain manipulations were carried out the day before it was taken. If necessary, urine (the cellular sediment of the first portion of morning urine) can be used as a material for research. It is important to thoroughly rinse the sample with saline, as the sediment contains large amounts of salts and urea, which denature the probes and lead to false positive results when using fluorescence detection technologies.

Errors in the analytical stage

Conducting the actual laboratory study may also be accompanied by a number of errors, one of the main ones being the incorrect choice of the method for isolating nucleic acids (NA). The choice of the NA isolation method should be determined by the nature of the biomaterial, the degree of its contamination with potential PCR inhibitors. Three most common approaches are used to isolate nucleic acids in routine laboratory practice:

1. Express method, which is based on temperature lysis of cells. The method is easy to use, takes no more than 10-15 minutes, provides maximum DNA yield, but is ineffective when working with material containing a high amount of impurities and PCR inhibitors. If it is necessary to use express methods with "complex" samples, it is possible to introduce additional methods of purification and concentration of the material, for example, in the case of excess mucus in the sample, preliminary washing and treatment of the biomaterial with mucolytics are advisable.

Web of Medicine: Journal of Medicine, Practice and Nursing
webofjournals.com/index.php/5

2. Sorbent extraction methods - differential sorption of nucleic acids on a solid carrier. Provide a high degree of purification of nucleic acids, but may be associated with losses of NA (especially in the case of their low content in the sample - "low-copy sample") due to irreversible sorption on the carrier or during several washes. In addition, the residual amount of sorbent in the final DNA solution can inhibit PCR.
3. Alcohol precipitation - aggregation of NC in the presence of salt and alcohol. The advantage of this technology is the ability to work with "complex" samples, to isolate both DNA and RNA equally. Like sorbent methods, it provides a high degree of purification of nucleic acids, but may be associated with NC losses.

Genetic variability of microorganisms. The probability of obtaining a false negative result may be due to the genetic variability of microorganisms: some genotypes or strains of the sought pathogen may acquire mutations in the amplified DNA region and become inaccessible for analysis by this test system. Therefore, if questions arise regarding the results of the PCR study, it is recommended to contact the manufacturer of the reagent kits.

Errors of the post-analytical stage. The main problem of the post-analytical stage is the incorrect interpretation of PCR analysis results due to erroneous ideas about the infectious agent or the capabilities of the method. One of the most common errors in interpreting PCR analysis results is failure to take into account the specifics of eliminating the STI pathogen, for example, prescribing a control study one week after the end of the antibiotic therapy course. In the vast majority of cases, the test result for the detection of the microorganism - the causative agent of the infection - will be positive. From this, one can conclude that the therapy performed is ineffective. This conclusion is erroneous due to the fact that the DNA of the microorganism can persist for several weeks after its elimination and does not indicate viability. If we are talking about microorganisms associated with the epithelium, the final conclusion about recovery can be made no earlier than 21 days - 1 month after the course of antibiotic therapy [4,12]. On the other hand, if the purpose of the analysis is to assess the state of the UGT microflora, then prescribing a study in 1-2 weeks against the background of the conducted antibacterial therapy will most likely lead to a negative PCR result. In the case of a balanced and stable system, its self-restoration will occur no earlier than 2 weeks - 1 month, then the PCR analysis will be informative and will allow you to assess the dynamics of the condition and determine correction measures. Another mistake is the incorrect interpretation of the results of quantitative PCR analysis. For example, quantitative analysis of individual representatives of opportunistic flora of the urogenital tract (Ureaplasma, Mycoplasma) in the absence of clinical manifestations of inflammation and patient complaints may lead to hyperdiagnosis and unjustified prescription of antibacterial therapy. As a consequence, there is a rapid growth of antibiotic resistance, an increase in the number of cases of recurrent bacterial vaginosis and dysbiosis. An indication for treatment in the absence of clinical signs of an inflammatory process is the detection of Ureaplasmaspp. and/or M. hominis in sperm donors, individuals diagnosed with infertility, and women with a history of miscarriage and perinatal losses.

Web of Medicine: Journal of Medicine, Practice and Nursing
webofjournals.com/index.php/5

Thus, at the stage of analysis and interpretation of PCR results, other problems arise, which can be described as discrepancies in results when using different research methods (for example, PCR and ELISA; PCR and bacteriological examination, PCR and microscopic examination).

References

1. Кудратова З.Е. и др. Роль цитокиновой регуляции при обструктивном синдроме атипичного генеза у детей // Анналы Румынского общества клеточной биологии. – 2021. – Т. 25. – №. 1. – С. 6279-6291.
2. Erkinovna K. Z. et al. Bronchial obstruction syndrome in young children with respiratory infections of different etiology: features of clinical manifestations and immune response //Проблемы науки. – 2021. – №. 1 (60). – С. 60-62.
3. Кудратова З.Е. и др. Хламидийные инфекции (внутриклеточная инфекция) в развитии бронхита // TJE-Tematics journal of Education ISSN. – 2021. – С. 2249-9822.
4. Kudratova Z. E. et al. Principles of therapy of chlamydial and mycoplasma infections at the present stage //Вопросы науки и образования. – 2021. – №. 28 (153). – С. 23-26.
5. Rustamova G. R., Kudratova Z. E. CHRONIC ENDOMETRITIS OLD ISSUES NEW POSSIBILITIES //Western European Journal of Medicine and Medical Science. – 2024. – Т. 2. – №. 5. – С. 12-14.
6. Erkinovna K. Z., Rustamovna R. G., Suratovna H. F. LABORATORY MARKERS OF PERINATAL HYPOXIC DAMAGE TO THE CENTRAL NERVOUS SYSTEM IN NEWBORNS //Наука, техника и образование. – 2020. – №. 10 (74). – С. 102-104.
7. Mukhamadieva L. A., Rustamova G. R., Kudratova Z. E. IMMEDIATE RESULTS OF COMPLEX TREATMENT OF CHILDREN WITH CHRONIC TONSILLITIS AND CHRONIC ADENOIDITIS ASSOCIATED WITH CMV AND EBV //Western European Journal of Medicine and Medical Science. – 2024. – Т. 2. – №. 5. – С. 20-24.
8. Азимова К.Т., Гарифулина Л.М. Факторы риска развития острого бронхиолита у детей //Журнал гепато-гастроэнтерологических исследований-2022. Материалы международной научно-практической конференции с международным участием. Специальный выпуск.-№2(том1).-С. 61-64.
9. Азимова К.Т., Гарифулина Л.М. Клинические особенности течения вирусных бронхиолитов у детей //Журнал гепато-гастроэнтерологических исследований-2022.-№2(том3).-С.13-16.
10. Азимова К.Т., Гарифулина Л.М. Особенности течения респираторно-синцитиального вирусного бронхиолита у детей // Проблемы биологии и медицины. Материалы 76-й международной научно-практической конференции студентов медицинских вузов и молодых ученных-2022. - С.202.
11. Азимова К.Т., Гарифулина Л.М. Показатели цитокинового статуса при бронхиолите у детей // Журнал кардиореспираторных исследований -2023.- №2(том4).-С.45-47
12. Азимова К.Т. Корреляционная взаимосвязь клинических показателей и тяжелого течения острого бронхиолита у детей //Актуальные вопросы современной науки и инноватики Уфа -2023. - С.- 24-29.

Web of Medicine: Journal of Medicine, Practice and Nursing
webofjournals.com/index.php/5