ANALYSIS WITH PCR METHOD IN STIP

Biljana Dodevska
PHI Clinical Hospital Stip, R. N. Macedonia, bdodevskaya@hotmail.com
Marija Dimitrova
PHI Public Health Center Stip, R. N. Macedonia, marijadim@yahoo.com

Abstract: The daily exposure of the body to various biological and chemical agents, as well as to atmospheric (climate) changes, contributes to the deterioration of general health, ie the decline of the immune system as a whole or of certain organs. Due to the relatively rapid lifestyles, not always controlled use of medicines, and especially antibiotics, many causes of diseases (bacteria, viruses, parasites) undergo a transformation and develop resistance to the drugs given in the treatment of a particular disease. As such, the causative agents can be isolated from different regions and biological substances (blood, urine, cerebrospinal fluid, swab, amniotic fluid, faeces, etc.) from the human body, depending on the type that manages to settle in the given environment. This imposes the need for more detailed and specific identification of the cause, for better diagnostics, enabling better therapy and successful healing. Our research is based on the qualitative identification of the DNA molecule in sexually transmitted diseases, more precisely the detection of Chlamydia trachomatis intracellular bacteria with the Polymerase Chain Reaction - method, introduced in July 2019 in microbiological laboratory of PHI Public health center Stip - R. Macedonia. The type of Chlamydia trachomatis causes a sexually transmitted disease of the genito-urethral tract in both sexes, with the highest risk and percentage in reproductive and sexually active periods. As one of the most common culprits for sterility, the bacterium can be isolated from urine and swabs from a particular region (cervical in female patients). This study was performed using the *in vitro RT-PCR* method of *ELITe InGenius® CE-IVD* over a four-month period from July to October 2019, which included a total of 94 patient swabs, 2 conjunctival smears on both eyes of them and 92 cervical. For the qualitative proof of Chlamydia infection, can be used chromatographic so-called quick tests too, but the result is far less-specific. In our research 2 of the cervical smears were initially tested by rapid tests and yielded positive results, but with the polymerase chain reaction of ELITe InGenius® CE-IVD, they were negative. From all the possibilities offered by the menu, combining in real-time 43 different PCR assays from all disease panels (for transplant pathogens, antibiotic resistance testing, sexually transmitted diseases, respiratory infections, meningitis, gastro-infections, genetic disorders), to detect Chlamydia we used the STI ELITe MGB® Panel, and the Chlamydia ELITe MGB® Kit, as well as the appropriate calibrations and controls. According to the referral diagnosis of patients and/or gynecological pathology, by the assessment of the gynecologist or urologist, of all cervical swabs in the study period, three gave a positive result for the DNA molecule identification of Chlamydia trachomatis. The patients whose samples were examined were aged from 20 to 45 years, the same sexually active and with pre-treated infections, infertility and pathological pregnancies, but with different geographical origin from rural to urban areas in the eastern region of R. Macedonia. The three positive Chlamydia detections by ELITe InGenius® RT-PCR method were reported in patients aged 25-30 years, two of whom were from urban and one from less developed socio-economic environment, expressing the fact of the large availability and highest quality in detection to the cause of infection with this kind of analysis. In this way, patients have the opportunity to get the most accurate result in the shortest time possible and thanks to modern medicine to be successfully cured, improving their health as well.
Keywords: PCR, Microbiology, Chlamydia trachomatis.

1. INTRODUCTION

The study, which covers the analysis of Chlamydia trachomatis made in the PHI Center for Public Health Stip, R. Macedonia, thanks to the PCR method of ELITe InGenius® CE-IVD. Namely, ELITech Group is a private company of worldwide manufacturers and distributors of reagents and in vitro diagnostic equipment, which provides innovative products and solutions to serve the laboratories that work closest to the patient, contributing greatly to the improvement of clinical diagnostics in the laboratories. ELITech Group manufactures and distributes diagnostic products for clinical chemistry, microbiology, immunology and molecular biology, with direct sales and distribution network spanning more than 100 countries. ELITe InGenius® is the best sample result solution dedicated to molecular diagnostics that integrates extraction, amplification and interpretation of results with unprecedented flexibility and menu capability. ELITe InGenius® processes 1 to 12 samples in parallel and independent paths. It features a universal extraction process with multiple independent PCR assays, allowing laboratories to create and operate their own analytical panels as needed. ELITe InGenius® offers the labs an unlimited menu option,

combining the largest CE-IVD (cell in vitro diagnostic) infectious disease menu available from a sample to a result instrument with a real-time open opportunity.

Chlamydia trachomatis is the most common bacterium involved in sexually transmitted infections (STIs) and is responsible for serious health outcomes. If left unidentified, this pathogen can cause serious complications, including ectopic pregnancy and infertility. Detection of Chlamydia trachomatis by molecular testing methods is important to identify the pathogen and provide adaptive treatment. Chlamydia kit ELITe MGB® is a qualitative PCR assay for detecting the DNA presence of Chlamydia trachomatis. In Vitro analysis is confirmed on urine samples and smears, in combination with automated extraction systems and PCR.

Figure 1. RT-PCR ELITe InGenius® CE-IVD, exhibited at the Medical Fair 2016 - Düsseldorf, Germany.

2. METHODS AND RESULTS

The polymerase chain reaction (PCR) method is a biochemical technique developed by Curry Mullis in 1983, which is used to generate large amounts of DNA sequences. It is a common laboratory technique used to make many copies (millions and billions) of a particular region of a DNA molecule that can respond to anything that would be examined. Usually, the purpose of PCR is to make enough of the target region of that DNA that it can be analyzed or otherwise used. For example, if the DNA molecule is amplified by PCR it can be sequenced, visualized by gel electrophoresis or cloned into a plasmid for further experiments.[2] The PCR method has been used in many fields of biology and medicine, including molecular biology research, medical diagnostics, and even some branches of ecology. The whole reaction takes place in several stages (steps), with the major role in all of them: Taq-polymerase, primers, DNA templates and nucleotides (DNA-building blocks). The ingredients are assembled together with the cofactors that the enzyme needs, and through repeated cycles of heating and cooling, DNA synthesis is enabled. The three basic stages are:

- Denaturation (96 ° C): Heating the reaction to allow DNA strands to separate, providing a single-stranded template for the next step;
- Annealing (55-65 ° C): Cooling the reaction so that primers can bind to their complementary single-template DNA sequences;
- Extension (72 ° C): The reaction temperature is increased, so that Taq polymerase extends the primers, synthesizing new strands of DNA.[1,4]

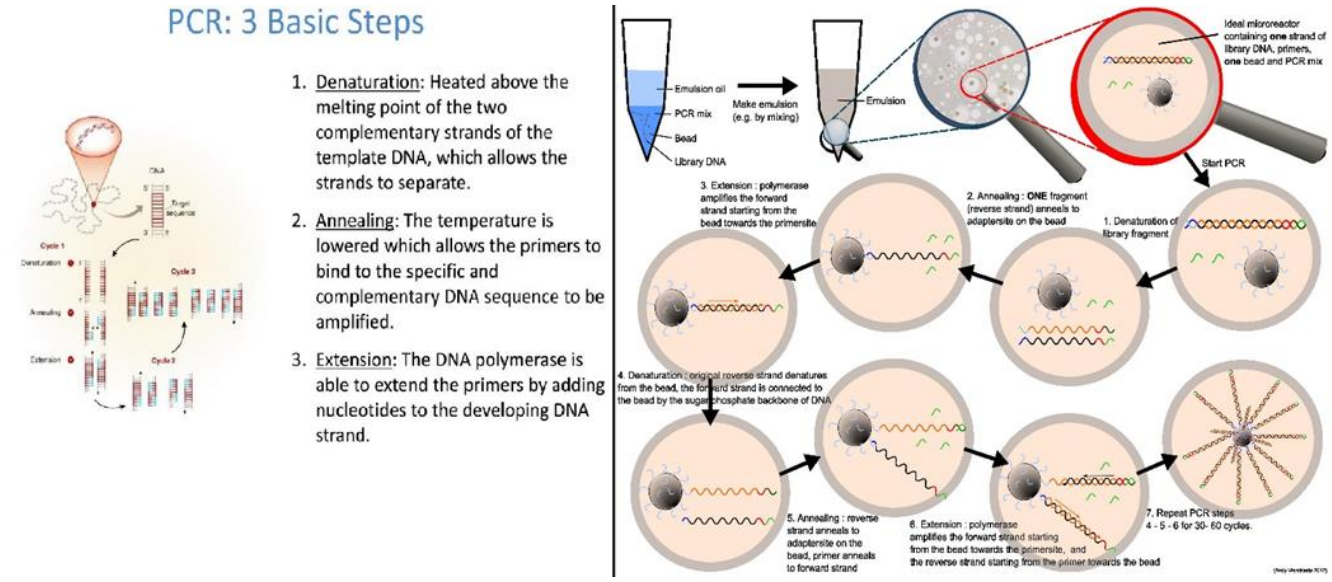

Figure 2. Polymerase chain reaction (PCR) phases

This cycle is repeated 25 - 35 times in a typical polymerase chain reaction, which usually takes 2 - 4 hours, depending on the length of the DNA region being copied. If the response is effective, the target region can range from just one or several copies to billions. This is because not only the original DNA used as a template each time, but also the new DNA that is made, can at one stage serve as a template for the next stage of DNA synthesis. In the reaction, there are many copies of primers and many molecules of Taq-polysomarase, so that the DNA molecules can also be duplicated in each new cycle.[7,8]

PCR steps	Temperature	Time	Cycle
First denaturation	94	5 min	1
Denaturation	94	30 sec	30
Annealing	64	30 sec	
Extension	72	30 sec	
Final extension	72	5 min	1

Figure 3. Duration of the PCR phases.

This test method was introduced in the Microbiological Laboratory at PHI Public Health Center Stip, R. Macedonia, from July 2019, with the RT-PCR apparatus of ELITe InGenius® CE-IVD, which integrates all the steps of molecular diagnostics: automatic nucleic acid amplification, real-time PCR amplification and analysis of results. With a high extraction yield, even a small sample volume, and a unitary tape format, the apparatus performs efficiently in qualitative and quantitative applications. The CE-IVD real-time PCR menu is based on MGB technology,
reducing downtime to less than 2 minutes per sample, or total processing time to a final output of 2 hours and 30 minutes, offering two-way connectivity with integrated LIS system. It has non-rival flexibility, simultaneously processing DNA or RNA from 1 to 12 samples simultaneously in different parallel panels for various pathogens and capable of mixing any type of sample matrix at the same time. Multiple independent PCR analyzes performed on a single sample allow the laboratory to define a comprehensive panel of tests according to the needs of each patient. It offers several operating modes available: extraction-only, amplification-only, or extraction-amplification, as well as analysis of results, as well as storage of extracted nucleic acid allowing multiple PCR testing, further replication and / or archiving.[6]

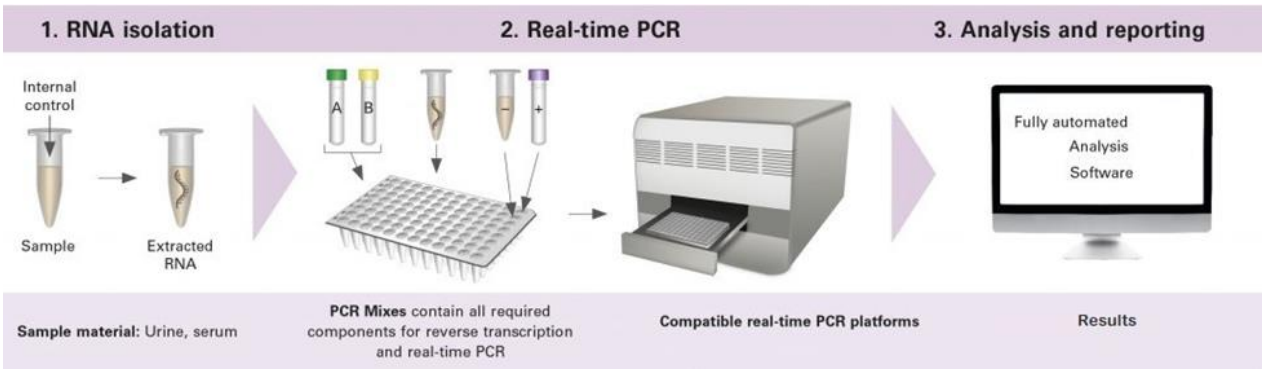

Figure 4. Identification of RNA by PCR method.

The complete real-time PCR menu of ELITe InGenius® CE-IVD is practical and unlimited; pathogen monitoring for transplantation, combined infections, antibiotic resistance testing, sexually transmitted diseases, respiratory infections, meningitis, gastro-intestinal infections, genetic abnormalities, 98% clinical and highest 98% clinical performance. specificity, and a personalized menu by saving the test panel. Studies of sexually transmitted infections, or identification of Chlamydia trachomatis, were conducted over a four-month period, from July to October 2019, with a total of 94 patient swabs, 2 of which were conjunctival on both eyes and 92 on cervical smears. We used the STI ELITe MGB® Panel, ie the Chl ELITe MGB® Kit to detect a specific region of the Chlamydia trach dna-B endogenous plasmid. and a specific region of the Chlam omp-A gene. trach. chromosome, as well as appropriate calibration and internal controls of human beta-globin. According to the referral diagnosis of patients and / or gynecological pathology, by the assessment of the gynecologist or urologist, of all the cervical smears in the study period, three gave a positive result for the DNA molecule identification of Chlamydia trachomatis.[5,10]

Figure 5. Swab and reagent kit for STI ELITe MGB® Panel.

Patients whose samples were sent to us for identification are aged 20 to 45 years, the same sexually active and with pre-treated infections, (some) infertility and pathological pregnancies, but of different geographical origin from rural to urban areas in the east region of r. Macedonia. Three positive detections of Chlamydia by RT-PCR method of ELITe InGenius® device were recorded in patients aged 25-30 years. Extraction of the DNA molecule is performed using the NucliSENS lightweight MAG principle, and the heat cycles with the ABI 7300 real-time PCR system and the ABI 7500 fast DX PCR instrument in real time.[10]

Chromatographic so-called chromatographic assays can be used for qualitative proof of Chlamydia infection. quick tests, but the result is far less specific. In our study 2 cervical smears were initially tested by rapid tests and yielded positive results, but by ELITe InGenius® CE-IVD RT-PCR, they were negative.[3,9]

3. PURPOSE

Ensure easy accessibility and the highest quality in proving the cause of infections with clinical trials by PCR method, and at the same time promote and improve the health of the population.

4. CONCLUSION

RT-PCR ELITe InGenius® CE-IVD, with its ELITe MGB® analyses designed with a small ligation band, Superbase® and Eclipse® Dark Quencher, offers the highest quality DNA molecule identification, making it the best apparatus in its class of PCR molecular diagnostics.

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