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Title of Thesis
Investigation of specific and non-specific detection methods for isothermal amplification of *Morganellamorganii*

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Abstract

Introduction: The first key in identifying an isolated colony is the environmental nature of which the bacterial colony on it grows.Has given The choice of method depends on the type of sample, laboratory facilities, patient conditions, the possible time to provide the final answer, and the use of a suitable primer that can identify and identify the species of the species of interest is important. An anaerobic gram negative bacteria is the only species in the genus Morganella .There belongs to the Proteeae of the Enterobacteriaceae family. The molecular method of selecting the method depends on the type of sample, the laboratory facilities, the patient's condition, the possible time to provide the final answer, and the use of a suitable primer that can be identified with the sensitivity and the high specificity of the species is important. LAMP is one of the methods that, without the need for temperature change, is a proliferation of LAMP based on proliferation of nucleic acids at constant temperature of C65-62. In this method, without the need for thermal evolution, with the aid of the Bst polymerase DNA enzyme, it is possible simultaneously to separate the two strands of DNA and proliferation. Therefore, unlike other conventional propagation methods, it is possible to do without the need for thermocycles and thermocyclers, using inexpensive equipment such as a hot water bath or a thermal block.

Materials and Methods: In this research, six exclusive primers were designed in order to propagate specific sequences of MorganelaMorgani bacteria. The temperature Tm for the regions Fc1 and Bc1 was about C65 (66-64) and for F2 B2, F3 and B3, it was about C60 (between 61-59). When double stranded DNA was exposed at a temperature of about 65-65 ° C, specific amplification occurred. In non-specific method, the mechanism of detection of bacterial proteins was performed by ELISA method.

Results: The amplification result was obtained by using the LAMP simulation and observation spectrum for positive electrophoresis of the band and was not negative. The amplification result was obtained by using a LAMP simulation and observational detection of the positive electrophoresis of the band and a positive specimen of pinpointing all negative bonding samples.

Discussion and Conclusion: According to the results, the use of specific methods in propagation of different species and strains of bacteria is useful and makes it possible to detect them to the extent possible

Morganella Morgani an Anaerobic gram negative bacterium is the only species in the Morganella species and belongs to the Praft ceae of the Ertervhxar tricaccae family. In the molecular method, the choice of method depends on the type of sample, the laboratory facilities, the patient's condition, the time it takes to provide the final answer .The use of appropriate tools and methods that can identify the species with high sensitivity and specificity is important. The LAMP is one of the methods that, without the need for temperature change, the LAMP is based on the propagation of nucleic acid at a constant temperature of 65 ° C. In this method, without the need for thermal fission, with the help of the Bst polysorbate DNA enzyme, simultaneously, it is possible to separate the two strands of DNA and multiplication. Therefore, unlike other common propagation methods, it is possible to do without the need for temperature cycles and thermo cycler devices by using inexpensive equipment such as a hot water bath or a thermal block.

Materials and Methods: In this research, non-specific and specific methods for identification were investigated. While there is no proper diagnostic method for product identification, there are six specific primers designed to proliferate the specific sequences of the Morganella Morganbi bacteria. The temperature Tm for the areas F1 and B1 was about 65 (66-64), and for F3 F2B2 and B3, it was approximately 160 between 59 and 41. When double stranded DNA was at a temperature about 65-62, a specific amplification occurred. Spectrophotometry and electrophoresis were used to evaluate the reproduction product by non-specific method, for a specific study, a nuclide amine probe was designed and examined by the nano probe method. Since most clinical specimens are inoculated on or in several environments, such as selective or differential agarose, the first key in identifying an isolated colony is the nature of the environment in which the colony bacteria has grown. For example, with rare exceptions, such as Enterococcus, only gram-negative bacteria can well grow on the McCanky agar medium, and the other medium containing growth-inhibiting

materials for the growth of gram-negative bacteria. Like Columbia Agar, which has colistin and nalidixic acid, it causes the growth of warm-positive bacteria. Most bacteria and fungi grow well on supplementary food environments such as agar reader with 5% sheep and BHI agar. Investigating and identifying the molecular causes of many inherited and acquired diseases by new cellular biologists has opened up therapeutic strategies (such as cell therapy and gene therapy) to patients. The vision of these technologies is to diagnose, prevent and control life-threatening illnesses such as cancer, which has not yet been diagnosed with cure. The production of mass, cheap and safe products of many biological products such as vaccines and hormones using transgenic organisms and prenatal diagnosis of genetic diseases is another achievement of cellular biology in the medical field.

At the moment, the importance of bacteria is not considered to be medically humanized as human pathogens in the world. Molecular identification is one of the newest parts of the laboratory that uses methods and automated methods to detect molecules of various

infectious diseases or illnesses of the RFLP Real Time using a device and some genetic tests. Accordingly, many answers can be made quantitatively and qualitatively to diagnose the disease as well as for other specialized and research assistance. The common laboratory diagnostic method for identifying bacteria is observation of patients' samples. Although samples observation is the correct method for the bacteria detection, but it's not specific and sensitive. There may even be up to 15% false positives. Although the use of the culture medium is specific, it is very slow and may last for 8 weeks.

Current diagnosis methods of microscopic and cultivar testing have major and important disadvantages, such as the need for a long time to diagnose fungal and bacterial infections, and consequently heavy costs for frequent diagnosis and treatment.

Molecular biology has now been able to open new horizons in various fields of mycology and bacteriology, including in their medical field. The expansion of molecular biology in diagnostic and clinical settings has saved the lives of more from the danger of

death, and another government looks to the future. In the past, due to the lack of rapid and timely diagnostic methods, many patients with a weak immune system who were attacked by fungal infectious agents, after a while, with the spread of infection to various parts of the body, there was no possibility of patient treatment, and after such a long time, death was the end of such people.

But nowadays, with the influence of molecular biology in the field of diagnosis, the patient can be fully treated with rapid diagnosis of the infection. Regarding the problems with conventional and traditional clinical diagnostic methods, researchers are constantly looking for more accurate, easier and faster methods. For this reason, in recent years, molecular biology has entered the field of medicine, especially in the field of mycology and bacteriology, and offers very promising horizons for professionals, scientists, researchers and all those who seek the technology of the day.

Current methods of diagnosis, such as direct microscopic testing and culture, have major and important disadvantages, such as the

need for a long time to detect fungal and bacterial infections, and consequently heavy costs for frequent diagnosis and treatment. This requirement proves the necessity of alternative and suitable methods in terms of speed, accuracy, and sensitivity. If the advantages mentioned in modern and molecular researches are remarkable and not comparable with the usual methods. Therefore, today researchers are trying to identify, by the various studies, the best, most correct and at the same time the easiest and cheapest molecular methods and introduce them to the clinical labs. The most important task ahead is how to use molecular methods based on new classification systems to prevent and treat fungal and bacterial diseases and management of patients.

Morganielta norganii is a gram negative bacterium as a natural flora in the human intestines, mammals and reptiles or as an environmental bacterium. Some cases (such as Ricketzia species) are difficult or impossible to recycle infectious organisms, in which case they should be trusted in serological or other methods. But as long as the biology techniques reach the point of

development that can easily be identified by the genetic index of infectious agents, the definitive diagnosis of infectious agents will be described.

The molecular method of selecting a method depends on the type of sample, the laboratory facilities, the patient's condition, the time it takes to provide the final answer, and the use of a suitable primer that can identify the species with the specific sensitivity and specificity of the species is important. In fact, it may not be possible to use it for any specific primer to detect RFLP or PCR species, but it is better to detect more classic PCR with a primer or two primers or identify one or two Multipex PCR reactions.

The expansion of gene matching methods in the 1970s brought new impetus to research using the ability of genes and gene activity to be done in ways that were previously impossible. The same thing happened in the late 1980s with the introduction of the second revolutionary method (Polymerase Chain Reaction PCR). PCR is a quite simple technique, and what happens is that a short region of a DNA molecule, such as a gene, is often multiplied by

the DNA polymerase enzyme. This approach may seem like a technique, but it has a multiple application in genetic research and is widely used in biological research.

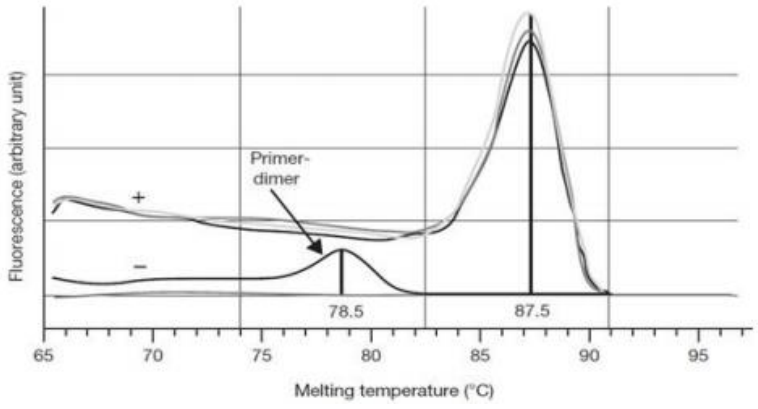
PCR is the selective duplication of a desired region of a DNA molecule. Each region of each DNA molecule provided that the sequences on both sides of that region are known. Sequences around the pieces should be known for the reason that they must be hybridized to the DNA molecule in a short oligo nucleotide, to each DNA strand of a double DNA helix.

A quantitative study of the levels of gene expression in addition to novices, extensions, deletions, or spontaneous mutations of the gene has been well established by the Real Time PCR method. This method has wide applications in clinical and research cases and is based on the detection of fluorescent messages.

The Real-Time PCR technique is thousands of times more sensitive than other methods of measuring and expressing the gene. In addition, this method can differentiate between mRNAs that are similar to each other and also require very little mRNA for gene

expression testing. There are three basic methods based on the use of fluorescent colors that are commonly used to detect Real Time PCR products.

During each cycle of the PCR, the fluorescence signal "is proportional to the increase in manufactured products, so that it is possible to determine the amount of PCR product in each cycle of the reaction using these messages." And during the reaction, the development of the expansion curve in each well can be seen on the device screen. In the easiest way, a free fluorescence color is used which, during the reaction, is nonspecifically placed in the structure of synthesized DNA molecules. CyberGrin is a color that is usually used in these cases and is less costly than other methods.



The specificity of the primers designed for the genes of interest due to the use of SG in order to prevent the formation of non-specific products is of particular importance. Due to the optimal conditions of the enzyme activity of the Taq polymerase, the binding temperature of the primers is limited to 60-60 ° C. The length, sequence, and concentration of primers should be such as to minimize the formation of primer-dimers. The length of the primers should be between 18 to 23 nucleotides and G-C is between 40 and 60. For best results, one or close to one, primers should be designed in such a way that the length of the reproduced piece is less than 200 bp.

To calculate the reaction efficiency, a standard curve is used. For this purpose, we provide different dilutions of the desired gene. We make real time PCR for dilutions. The resulting gradient device then calculates the resulting gradient, which should not exceed the standard limit of the device.

Melting curve

Due to the non-specific color coupling, each double-stranded DNA molecule produced in the reaction, such as primer-dimers, will produce non-specific products of a fluorescent message. Since the melting temperature of the PCR products varies with lengths and sequences, it is possible to identify the reaction using the specific melting charts of the product. At the end of the PCR reaction, the resulting products are heated and, with their single strand, as a result of the temperature increase, the paint is released into the reaction solution and the fluorescence intensity decreases.

The fluorescence curve - temperature is plotted by the device software, and in this curve, the curve peaks should be close to Tm of the predicted PCR product for the proprietary product. If there are non-specific products or primers-dimers, then more than one peak in the curve will be observed.

LAMP Isothermal methods

In this method, without the need for a heat stroke phase, with the help of the Bst polysorbate DNA enzyme, simultaneous separation of two strands of DNA and proliferation is possible. Therefore,

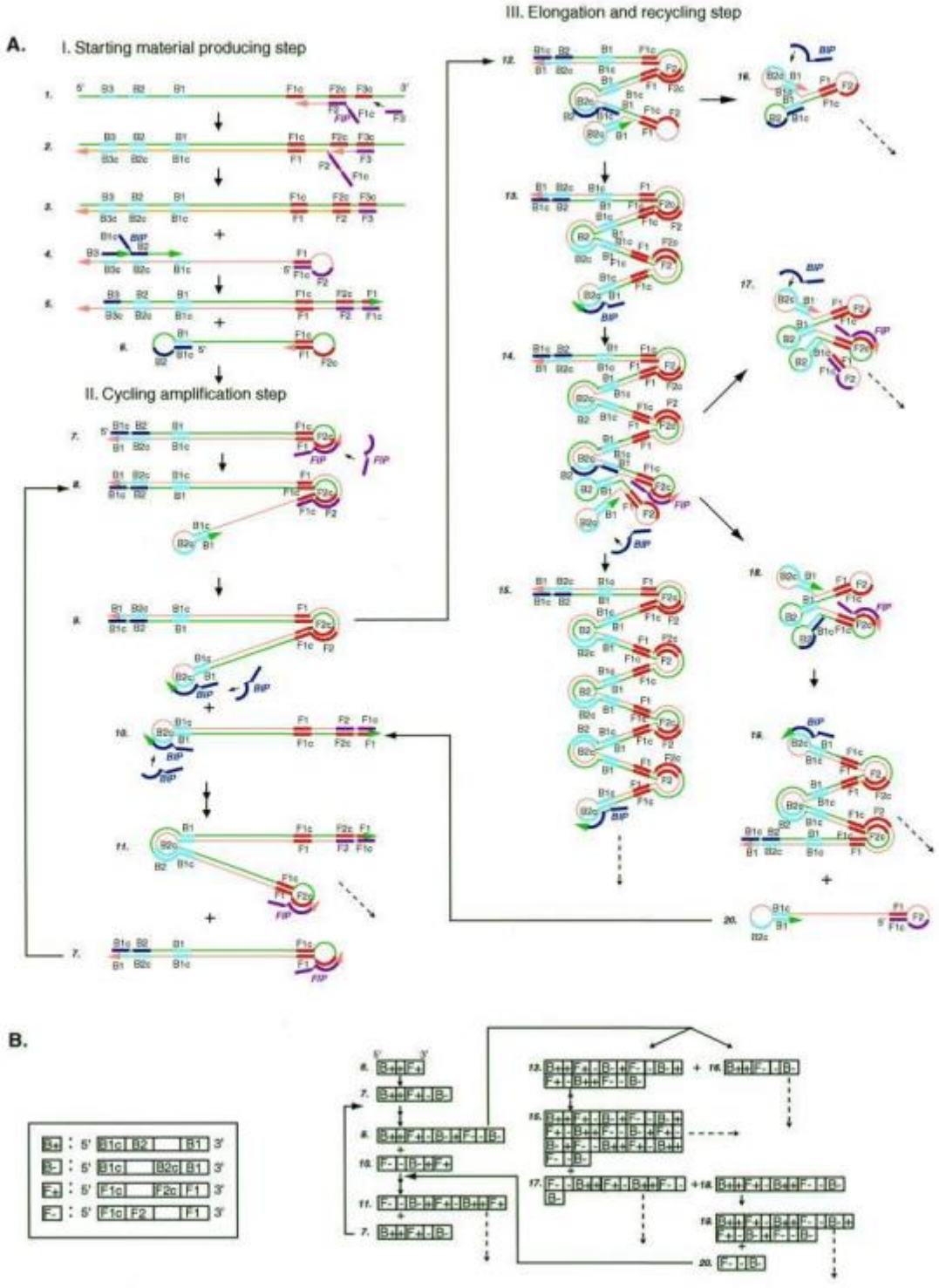
unlike other common propagation methods, it is possible to do without the need for temperature cycles and thermo cycler devices using inexpensive equipment such as a hot water bath or a thermal block.

To design a LAMP reaction, there need to be six primers that have the ability to completely connect to eight distinct areas of the target sequence. As a result of the LAMP reaction, a large amount of the product is produced without the need for a rigorous electrophoresis in the gel to be easily detected by simple methods such as observing turbidity or color changes caused by fluorescence dyes in the reaction mixture. Thus, the LAMP method can be used as a valuable tool for the rapid diagnosis of infectious diseases in clinical and hospital laboratories, especially in developing countries, due to its advantages such as sensitivity and proper characteristics, high efficiency, the need for expensive laboratory equipment and the simplicity of diagnosis.

LAMP reaction mechanism

Non-cyclic stage: Non-cyclic stage: When a two-string DNA is placed at a temperature of about 62-65 ° C, one of the internal primers (for example, the FIP primer) begins to react so that the TED F2 portion of the FIP primer is attached to the target sequence and from the end of the 3 That, the complementary strand begins with the Bst DNA polymerase enzyme.

Cycle Stage: In this step, external primers do not interfere, and only internal primers and, if necessary, loop primers are used. The free terminal 30 in the structure of the dumbbell (the arrow shown in structure 6) acts as a primer and initiates the new DNA thesis.


A.
I. Starting material producing step
II. Cycling amplification step
III. Elongation and recycling step
B.

LAMP Duplicate Product Detection Method

To confirm the products of the LAMP reaction, it can also be used as an ordinary PCR method by electrophoresis in an agarose gel. But due to the high amount of DNA produced by the LAMP reaction, it can be easily seen in the reaction mixture without the need for specific tools. In general, the following methods can be used to evaluate LAMP products:

1- Electrophoresis in agarose gel

2- Observational detection using fluorescent. The presence of LAMP propagation products can be observed by adding fluorescent dyes such as Cyber Green, Calcein, Picogreen, etc. directly into the tube containing the reaction mixture. Cyber Green's desire leaves a lot of double-stranded DNA, and emitting green light after excitement of the fluorescence color. Another way of adding calcein to the LAMP reaction mixture is after starting the proliferation. Calcium is a type of fluorescence that is silent before mixing with manganese ions before propagation begins. After

reproduction, peripheral products such as pyrophosphates are formed.

Methods based on immunology of ELISA method

ELISA is the most common method used to detect surface antigens or products of tested microorganisms. In the ELISA method, an antigen that is tested specifically for the microorganism is attached to an antibody that is fixed on a solid matrix. After the antigen-antibody complex is formed, the antigen concentration and, consequently, the microorganism present in the sample the color concentration device that is produced can be measured. It is commercially available on the market, which allows us to quickly, efficiently, inexpensively and easily identify the bacteria that we seeking.

Electrophoresis technique

It refers to the movement of particles in a liquid under an electric field. Because biological macromolecules such as DNA and proteins are pregnant, they can be separated by placing them in an electric field based on physical properties such as spatial shape,

molecular weight, and electrical charge. For this purpose is used a method called electrophoresis. Different methods of electrophoresis have been developed for the separation and study of biomolecules, including nucleic acids or proteins.

The concept of nanotechnology

While there are many definitions for nano technology, the NNI offers a definition of nanotechnology that includes three features.

1- The development of scientific and technological research at atomic, molecular or macromolecular levels at particle sizes of 1 to 100 nm.

2- Designing, creating, and using structures, tools and systems that, due to their small size (dimensions ranging from 1 to 100 nanometers), have new properties and functions. These structures are called nanostructures.

3- Ability to control or manipulate at the atomic level

In simple terms, nanoscale science represents the ability to produce new materials, tools and systems, by taking control of the molecular and atomic levels and using their properties on a

nanoscale. From the definitions mentioned, nanotechnology is not a field but it is a new approach in all fields.

For nanotechnology, applications have been made in a variety of areas, including food, medicine, medical diagnostics and biotechnology, including electronics, computers, communications, transportation, energy, the environment, aerospace, and national security.

Nano Materials Manufacturing Methods

In general, there are two methods for reaching the valley at the nanoscale: the top-down method and the bottom-up method. By using these methods, it is possible to produce nanoparticles of size, properties, morphology, and applications. The top-down method is in fact the same method used for centuries, and the scholars of the bronze age performed on wood and stone. The top-down approach was first introduced by Feynman as a method for making nano-sized devices. In this way, larger devices create smaller devices. This is the case, as long as nano-sized machines are obtained. In the top-down method, a bulk material is shaped and modified to

reach a nanometer size. In this method, the accuracy of the dimensions obtained depends on the accuracy of the tools. Lithographic, spinning, mechanical (like grinding) are methods that create nanostructures. In the bottom-up method, nano-materials are produced by combining constructive blocks such as atoms and molecules and placing them next to each other.

According to physics, the smaller the dimensions of a material, the ratio of the surface to volume increases, and the ratio of the atoms to the total atoms are increased. For example, in single-shell clusters, the ratio of surface atoms to total atoms is 92%, while this ratio is reduced to about 35% in clusters of seven shells. Hence, nanoparticles will have a very large surface-to-volume ratio. Since surface atoms determine chemical properties, including the reactivity of materials, it can be concluded that the effect of surface atoms on the determination of the properties of materials is much more and more evident in a material with smaller dimensions than materials with larger dimensions.

Applications of nanoparticles

If it is accepted that nanotechnology has the potential to produce new materials, tools and systems together with molecular, atomic and molecular-level controls, then it can be said that the applications of this technology are such that it is difficult to be a field which does not affect it. By using nanotechnology, you can make better and cheaper products available to humans than conventional ones. The first industrial production of nanoparticles in the twentieth century was produced by the production of soot, and subsequently by the production of foam silica in 1940.

The materials currently made in the form of nanoparticles only include compounds such as carbon nanotubes, cadmium selenide (CdSe) and quantum dots, but also a wide range of metal oxides and metal oxides, sulfides, fluorides, carbonates, silicates, and several categories of materials. Other is also included. The use of carbon nanotubes in tissues, and zinc or titanium oxide nanoparticles in solar panels, is part of the vast applications of nanoparticles.

Since the beginning of man-made artificial compounds, the addition of fine materials, but larger than nanoscale, has been one of the most commonly used methods to change the properties of materials. Therefore, the first application that can be made for nanoparticles is the use of these materials in the production of nano composites. By using nanoparticles in nano composites, many of its optical, electronic, magnetic, chemical and thermal properties will change.

The strength of a magnetic magnet increases with increasing cross-sectional area per unit volume. Studies have shown that magnetism based on etrium-samarium-cobalt nanoparticles, due to the high cross-sectional area, has very unusual magnetic properties. Other new applications of nanoparticles include the use of zinc oxide nanoparticles in the production of sunscreens.

Measure the LAMIP method specificity

In order to evaluate the specificity of the LAMP method for the proliferation of the Morganella bacterium from a nanodrop device, absorption at 550 nm was used. The results showed that the

absorption rate at 550 nm for bacillus, citrobacter, klebsiella, shigella, salmonella and pseudomonas is less than 0.093 for Morganella species than the basal area.

Genetic studies provide us with a quick overview of the data used to design molecular techniques for simultaneously identifying a large number of pathogens. Recent technologies accelerate the identification and differentiation of pathogens. In the next few years, a complete sequence of genomes will be available for many pathogenic strains, which can help design primers such as PCRs or probe probes for pathogenic strains.

Current detection methods, including direct diagnosis of microscopy and culture, have major and important disadvantages, such as the need for a long time to diagnose the infection and, consequently, a high cost of diagnosis and treatment.

This issue requires the alternative and suitable methods in terms of speed, accuracy and sensitivity, if the advantages of modern molecular and molecular research are remarkable and not comparable with conventional methods.

So today, researchers are trying to identify the best and most efficient and at the same time the easiest and cheapest molecular methods to identify clinical labs through various studies. The most critical task ahead is how to use new molecular-based classification systems to prevent and treat bacterial diseases and manage patients.

In this study, a combination of LAMP-nano-probes of gold was used to detect Morganela Morgani bacteria. In this method is used a DNA polymerase and a set of 4 specific primers, which identify 6 specific sequences of the target gene. Over the past 10 years,

LAMP has been widely used in the analysis of nucleic acids due to its simplicity, speed, efficiency and privilege.

Morganella is resistant to beta-lactam antibiotics, but is susceptible to antibiotics such as ciprofloxacin and the third generation of cephalosporins. Although the main method for detecting this bacterium in most laboratories is still traditional methods of culture and biochemical responses, however, time-consuming, high costs, and a lack of sensitivity and specificity have led to the development of molecular techniques to detect this bacterium.