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# CONVENTIONAL *VERSUS* MODERN TECHNIQUES USED FOR THE DETECTION OF PATHOGENS IN FOOD MATRICES: A REVIEW

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***Abstract***

*Microbial contamination is one of the most important obstacles in the food industry. In order to control microbial contamination, many methods have been developed over the years to reveal the behaviour and characteristics of microorganisms in order to control them and in order to understand the impact of microorganisms on foods. Increasing concerns about outbreaks of foodborne diseases require rapid on-site and sensitive methods for the detection of microorganisms in various food matrices. In the current review, a brief discussion is presented about the methods used for the detection of pathogenic microorganisms present in food matrices, especially the tools based on nucleic acids extraction.*

***Key words:*** *microbial contamination, food industry, food matrices, detection.*

## INTRODUCTION

Foodborne pathogens are considered a major public health risk, and diseases cause a significant burden on food workers, consumers, and governments.

Despite significant advances in diagnosis and awareness of food safety worldwide, many foodborne germs may be detected to the consumer, and many foods, such as meat and other animal products including dairy, are contaminated with potentially harmful microorganisms (Minarovikova et al., 2020). Also the impact of microorganisms such as bacteria, viruses and fungi on human life is significant. *Salmonella*, followed by *Escherichia coli*, are the two most common types of microorganisms responsible for outbreaks of food-borne illness and disease (Jayan et al., 2020).

Microorganisms can adapt to different environments and perform a variety of functions in diverse commodities. Therefore, in order to detect microorganisms, appropriate tools and techniques are needed.

In the recent years, the conventional detecting methods include, aside microscopy, cell culture, biochemical tests, tools which benefit of immunology (serotype, Elisa) or even molecular biology tools (classical PCR or DNA-DNA hybridisation).

In general, cell culture, colony counting, microscopic analysis, polymerase chain reaction (PCR), and immunoassay are often used to identify and quantify microbes. Sometimes the role of 'culture methods and colony counting for results' can be inappropriate. Especially in samples that contain different types and high concentrations of microorganisms.

Although PCR is a specific molecular method, this technique is time consuming, while the real-time PCR method can be more specific and faster (Persson et al., 2018). However, it is considered one of the most important modern methods for detecting microorganisms (Paniel & Noguer, 2019).

There are many methods for isolating bacteria from different food matrices. The traditional methods for isolating bacteria depend on a fresh medium, which requires a differential agar medium and colony counting (Paniel & Noguer, 2019) (Figure 1).

Some other disadvantages of conventional culture methods can be pointed out, like their low sensitivity, risk of bacterial contamination leading to inhibition in the growth of bacteria of interest, and presence of viable but non-culturable bacteria (VBNC).

Consequences of having VBNC in a food matrices include an underestimation of the number of viable cells or the impossibility of isolating pathogens from the sample.

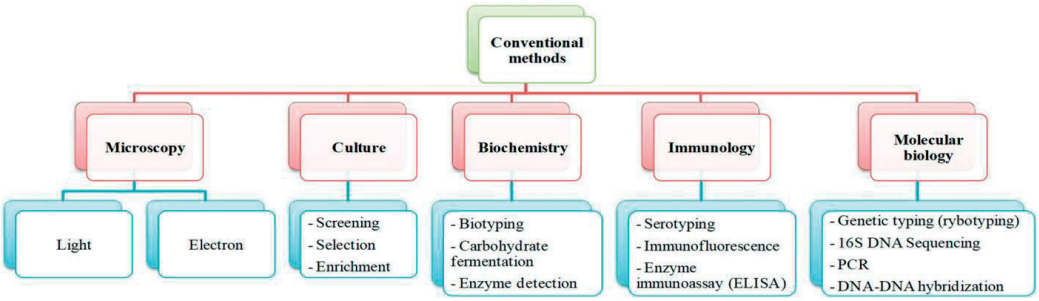


Figure 1. Conventional methods used for food borne pathogenic bacteria detection (Paniel & Noguer, 2019)

The VBNC condition is commonly found in environmental and food samples due to bacterial starvation and a large variety of stressful conditions, including growth inhibition temperature, hypoxia, suboptimal pH and salinity. In food, VBNC has been reported to occur, in some cases, while cannot be detected by bacterial culture, but can be detected with modern techniques. Therefore, these bacteria pose a growing threat in the food industry (Yang et al., 2021).

Some authors showed that VBNC cells can be found in the case of *Salmonella typhi* (*S. typhi*), as well as *Escherichia coli* (*E. coli*) and *Legionella pneumophila*. Therefore, in order to reach more accurate results, researchers often combine standard microbiological counting methods with other automated or semi-automated detection techniques that include DNA, antibody or biochemical methods (Cao et al., 2019). However, there are still many drawbacks to these traditional methods, and there is still a need to develop more rapid, sensitive and specific techniques for pathogen detection and quantification.

The development of new technologies with faster response time, better sensitivity and selectivity is very important to ensure the safety of consumers.

PCR-based methods have been applied to detect and identify bacteria in a large variety of samples. Compared with the old traditional methods, modern methods based on advanced qPCR techniques showed better specificity, higher sensitivity, shorter analysis time and better accuracy (Durand et al., 2020).

The PCR and qPCR methods can be applied to in situ and real-time monitoring for many applications, including the detection of many microorganisms.

These techniques are distinguished in the detection of bacterial populations even in the absence of selective culture medium and in the presence of other dominant groups.

To increase the accuracy of the analysis and reduce the time, some PCR methods have been developed that allow the identification of several pathogens in a single sample within a single reaction.

In the following, the classical pathogen detection methods will be described against the modern developed detection tools. This review is based mainly on the last five years scientific publications in the field of pathogen detection in different types of food. The study was structured based on different food matrices, respectively animal (Table 1), plant (Table 2) and beverages (Table 3), and for each were revealed the main pathogens to be detected and associated detection methods. Through this study it was emphasized that there is a difference between the old traditional methods and modern methods in detecting the existing germs in terms of work speed and results accuracy. Among the modern methods that are characterized by accuracy and speed in work, the most important are classical PCR and Real Time PCR, which is one of the most important modern methods for detecting microorganisms.

**Table 1. Different tools used for the pathogen detection in animal food matrices**

| Food matrices | Pathogen | Tools | Reference |
|---|---|---|---|
| Beef Burgers | *Escherichia coli* | Real-Time PCR | Rey et al., 2021 |
| Cheese | *Salmonella typhimurium, S. aureus, L. monocytogenes* | Real-Time PCR | Mendonça et al., 2019; Jana et al., 2020 |
| Chicken breast, turkey, Beef, Raw Pork, Sausage | *L. monocytogenes, E. coli, S. enterica, Campylobacter* spp., *C. coli, C. lari, C. upsaliensis, Salmonella* | PCR, Real-Time PCR | Kim et al., 2021; Vizzini et al., 2021; Hyeon et al., 2019 |
| Chicken carcasses | *Salmonella* spp., *Salmonella enteritidis* | Multiplex PCR | Ferone et al., 2020 |
| Dairy products | *Salmonella* spp., *Listeria monocytogenes*, *Cronobacter sakazakii* | Real-Time PCR, PMAxx-ddPCR | Ferone et al., 2020; Lv, 2021 |
| Fish | *Aeromonas* spp., *Streptococcus* spp., | Multiplex PCR, LAMP, Biosensors | Pires et al., 2021 |
| Fresh pork | *Staphylococcus aureus, Salmonella* and *Shigella* | Real-Time PCR | Ferone et al., 2020 |
| Meat | *Pseudomonas*, *Enterobacteriaceae*, *Brochothrix thermosphacta*, *Staphylococcus* | Multiplex Qpcr | Bahlinger et al., 2021 |
| Meat Products | *Listeria monocytogenes* | Real-Time PCR | Labrador et al., 2021 |
| Milk | *Salmonella, Listeria monocytogenes,* Pseudomonas, *Bacillus cereus, Staphylococcus aureus*, *Hafnia alvei, Serratia marcescens, Citrobacter freundii, E. coli* | Real-Time PCR, Multiplex Real Time PCR, qPCR, Biosensors | Wei et al., 2019; Zhou et al., 2019; Ferone et al., 2020; Jayan et al., 2020; Du, 2021; Huang et al., 2021; Lonczynski, and Cowin 2021; Maier et al., 2021 |
| Minced meat | *Listeria monocytogenes, Staphylococcus aureus, Bacteriophage* | Real-Time PCR, PCR | Ferone et al., 2020; Spilsberg et al., 2021 |
| Minced pork meat, Egg white, Egg yolk, Whole egg | *Salmonella enterica* | Direct PCR | Vinayaka et al., 2019 |
| Pork meat | *S. typhimurium* | Biosensors | Jayan et al., 2020 |
| Poultry meat, Red meat, Beef meat, Liver samples. | *Salmonella* spp. | Real-Time PCR | Siala et al., 2017 |
| Raw Poultry | *Salmonella* spp. | Real-Time PCR | O'Bryan et al., 2021 |
| Raw Seafood | *Vibrio vulnificus* | Real-Time PCR | Yang et al., 2021 |
| Sausages | *Latilactobacillus sakei* | ePCR | Iacumin, et al., 2020 |
| Shrimp, Mussels, Seafood | *Toxoplasma gondii, Vibrio parahaemolyticus, Vibrio cholerae, V. parahaemolyticus, V. vulnificus* | Real-Time PCR, LAMP Methods | Bonnin-Jusserand et al., 2019; Cao, 2019; Durand, 2020; Yang, 2020 |
| sliced turkey, raw cheese, chicken salad, shrimp | *Listeria* species *monocytogenes* | Multiplex Real Time PCR | Lonczynski et al., 2021 |
| Yogurt | *S. enterica*, *L. monocytogenes* | PCR | Kim et al., 2021 |

**Food born pathogen detection by cell culture**

The culture-based detection method has the advantages of being simple to use, detecting only live cells, and not requiring expensive experimental equipment. Sensitivity and selectivity are crucial features in choosing a selective/differential media, although certain culture-based methods lack the sensitivity and/or selectivity needed to isolate the target pathogen. When the media is insensitive, the chance of a food borne outbreak rises because the danger goes unrecognized. Target pathogens, on the other hand, cannot be separated from other microorganisms when selectivity is poor. Traditional media are constantly being improved, and new media are being developed to successfully isolate target microorganisms.

**Table 2. Different tools used for the pathogen detection in vegetable matrices**

| Food matrices | Pathogen | Tools | Reference |
|---|---|---|---|
| Cantaloupes, Watermelons, Pineapples, Radishes | *Salmonella enterica*, *Listeria monocytogenes* | Bacterial strains-Luria-Bertani (LB) media | Huang et al., 2019 |
| Chamomile, Mint | *Salmonella* | Multiplex Real-Time PCR | Koprinarova, 2021 |
| Cherry tomato | *Bacillus cereus, Listeria monocytogenes, Staphylococcus aureus* | Multiplex qPCR | Wei et al., 2019 |
| Chocolate bar | *Salmonella* spp., *Listeria monocytogenes* | Real-Time PCR | Ferone et al., 2020 |
| Fruits, Vegetables, | *Salmonella* spp. *and Listeria monocytogenes, Fungi* | Real-Time PCR | Ferone et al., 2020; Roumani et al., 2021 |
| Lettuce, Cabbage | *E. coli O157:H7, S. typhimurium, Salmonella* | Real-Time PCR | Kim et al., 2019; Kim et al., 2020; Huang et al., 2021 |
| olive fruit | *Colletotrichum acutatum* | Real-Time PCR | Azevedo-Nogueira, 2021 |
| Pineapple | *Aspergillu, Rhizopus, Geotrich , Neurospora, Candida, Fungi* | PCR, culture-based detection | Koffi et al., 2019; Koffi et al., 2021 |
| Soybean sprouts | *Listeria monocytogenes* | Real-Time PCR | Wei et al., 2019 |

**Table 3. Different tools used for the pathogen detection in beverages**

| *Food matrice* | Pathogen | Tools | Reference |
|---|---|---|---|
| Apple juice, grape juice. | *Salmonella paratyphi, S. typhimurium, Penicillium expansum, Paenibacillus* spp., *Alicyclobacillus* spp. | LAMP, PCR, qPCR, Biosensors | Frisch, L. M. et al., 2021; Li, H. et al., 2021; Elie, S. C. 2020; Jayan et al., 2020 |
| Beer, grape wine, other fruit wine, refined rice wine, traditional Korean turbid rice wines. | *Bacillus cereus* | Soy polymyxin broth | Kim et al., 2020 |
| Boza | *Enterococcus faecium* YT52 | PCR | Gök Charyyev, M. et al., 2019 |
| Coconut water | *Escherichia coli* | PCR | Wang et al., 2021 |
| Kombucha | *Starmerella davenportii Do18* | Potato dextrose agar (PDA) | Tu et al., 2020 |
| Orange juice | *E. coli, Salmonella typhimurium, Yersinia enterocolitis, Shigella boydii* | FTIR measurements, Biosensors | Paniel and Noguer 2019; Jayan et al., 2020 |
| Rice beer (makgeolli) | *Cronobacter, Enterobacter, Klebsiella* | qPCR | Jung et al., 2012 |

A variety of food samples were used, including leafy greens, seafood, beef, and pork items (Baek et al., 2021).

The researchers found many bacterial species in a viable but non-culturable state known as VBNC. These cells are characterized by their loss of ability to be cultured on routine agar, which impairs their detection by conventional techniques. Hence, failure to detect them poses a threat to public health. Therefore, the researchers had to find a way to detect all the germs present in the sample to be examined (Cao et al., 2019), and the methods take advantage of different molecular tools.

## Polymerase chain reaction (PCR)

Is a relatively newly used and widely method to quickly make millions of copies of a given DNA sample (complete copies or partial copies), in a series of cycles of temperature changes. It allows researchers to take very small samples of DNA and amplify them as much as possible

enough to study in detail. PCR was invented in 1983 by American scientist Cary Mullis at Cetus Corporation. It is fundamental to many procedures used in genetic testing and research, including analysis of ancient DNA samples and identification of infectious agents and diseases.
PCR is nowadays a common and often indispensable technique used in many medical laboratory research as well as for a variety of applications including biomedical research and forensics.
Applications of this technology include DNA cloning for sequencing, gene transcription and manipulation, and mutagenesis; DNA-based phylogenetic construction, or functional analysis of genes; diagnostics and monitoring of genetic disorders; ancient DNA amplification; Analysis of genetic fingerprints to determine DNA traits (for example, in pedigree and forensic testing); the detection of pathogens in DNA tests to diagnose infectious diseases. In food matrices, PCR was used for the detection of *Salmonella* or *E. coli* in different food products, as seen in Table 1.

**Real-time polymerase chain reaction (real-time PCR)**

It is one of the laboratory technique of molecular biology based on the PCR. It monitors the amplification of a targeted DNA molecule during the PCR, as in conventional PCR. Real-time PCR can be used quantitatively (quantitative real-time PCR) and semi-quantitatively (i.e., above/below a certain amount of DNA molecules) (semi-quantitative real-time PCR) (Minarovičová et al., 2020). Real-time PCR, sometimes referred to as quantitative or qPCR, determines the amount of PCR product present during a particular cycle. This approach allows you to quantify DNA creation in the qPCR experiment by employing a fluorescent report in the PCR reaction. SYBR Green and probe-based qPCR are the two types of qPCR. In a SYBR Green qPCR process, you start with a template that contains the target sequence you want to analyze. You'll also need primers, dNTPs, and your preferred DNA polymerase. The SYBR Green I dye is commonly included in the reaction mix that contains the DNA polymerase. In a probe-based qPCR process, you start with a template that includes the target sequence you want to analyze. You'll also need primers, dNTPs, and your preferred DNA polymerase. You'll also need a probe that's tagged with both a reporter and a quencher molecule. Probes are usually sold separately as custom items because they are unique to the target sequence. The first step in the PCR process is denaturation. The double-stranded DNA helix melts open into two single-stranded DNA templates as the thermocycler heats up to around 95 degrees Celsius. The temperature cools to 45-65 degrees Celsius during the annealing step, and the single-stranded primers connect to the appropriate ends of the target sequence. DNA polymerase connects to the prepared template and begins incorporating complimentary nucleotides during the cycle. Finally, the temperature rises to 65-75 degrees Celsius during extension. The sequence-specific primer is extended by DNA polymerase by adding complementary nucleotides to the DNA template.
Probe-based differs from SYBR Green in this stage because: in probe-based The fluorescence is caused when the DNA polymerase displaces the reporter molecule from the probe. The fluorescence builds up as the PCR cycle progresses, and it is measured at the end of each cycle. The number of freshly formed double-stranded DNA strands is quantified by measuring the intensity of the fluorescence created by the reporter molecule above background level (the Cq value). SYBR Green binds and fluoresces all newly produced double-stranded DNA complexes. The fluorescence builds up as the PCR cycle progresses, and it is measured at the end of each cycle. The amount of freshly produced double-stranded DNA is quantified by measuring the intensity of fluorescence emitted by SYBR Green I above background level (the Cq value). You're ready to start analyzing after repeating the denaturation, annealing, and extension processes 35-40 times. The Cq values can be used to quantify beginning DNA amounts, create a standard curve for gene expression research, and perform other analyses. Different examples of pathogens detection are provided in Table 1, like the detection of *Campylobacter* spp. in chicken samples by Real-Time PCR (Vizzini et al., 2021), or *Salmonella* in milk samples also by Real-Time PCR (Huang et al., 2021).

**End-point polymerase chain reaction (ePCR methods)**

The analysis performed after all PCR cycles have been finished is known as end point PCR. End point analysis is predicated on the plateau phase of amplification, unlike qPCR, which permits quantification as the template doubles (exponential phase).

Northern blot or P32 radioisotope (for RNA). Your RNA is run on an agarose gel, then transferred to a nucleic acid binding membrane, which is subsequently hybridized with a P32 labelled DNA probe that identifies your transcript. After exposure to an Xray film, the quantification is done using densitometry.

End-point polymerase chain reaction has been used in DNA amplification techniques since 1985, real-time polymerase chain reaction (RT-PCR) has been used since 1993, and reverse transcription real-time polymerase chain reaction (RT-qPCR) has been used in many research applications including cloning, analysis, gene expression genotyping, mutagenesis, sequencing, and many others. The cyclic amplification of a single DNA molecule into billions of copies of DNA molecules in a short period of time using DNA polymerase is the core premise of DNA amplification technologies (Figure 2). The DNA polymerases, on the other hand, stay with the result (DNA copies) in one system at the end of the enzymatic reaction and are normally discarded after usage. Separating the enzyme from the products, which is a difficult, expensive, and time-consuming process, is required to reuse DNA polymerases in a repeating reaction.

Furthermore, DNA polymerases are still quite expensive; therefore, to prevent wasting time and other resources, DNA-modifying enzyme immobilization method for nucleic acid detection is advised.

Research on the electrochemical real-time amplification technology, which is based on the solid-phase PCR methodology, have sparked a lot of curiosity. The electrochemical polymerase chain reaction (PCR), which is based on the hybridization of a target sequence and an oligonucleotide probe and immobilization on an electrode: graphite oxide, has been the focus of research for the past years.

The sample surface was passivated to prevent adsorption of additional PCR components, and the electrochemical response was accomplished in the requisite thermal conditions. The plots of the current signal against the number of ePCR cycles were recorded, and after 25-40 reaction cycles, a plateau was reached. However, this invention does not solve the problem because DNA polymerases remain with the product in one system and are typically discarded after usage. As a result, the use of electrochemical techniques as well as the immobilization of some extra enzymes makes a lot of sense (Dronina et al., 2021). Example for ePCR - detection of *Latilactobacillus sakei* in sausages samples (Iacumin et al., 2020).

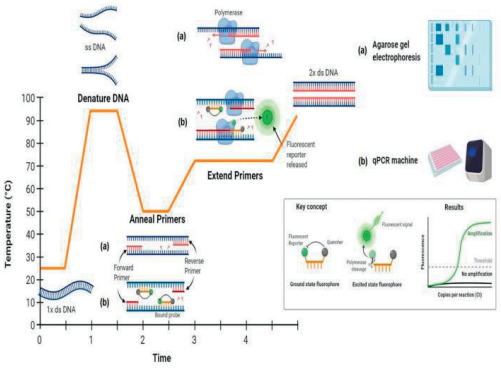


Figure 2. Schematic illustration of two DNA amplification techniques: (a) PCR; (b) real-time PCR (Dronina et al., 2021)

**Biosensor**

A biosensor is a three-part analytical instrument that includes: a bio-receptor or recognition element, a transducer, and a signal reading device (an enzyme, a receptor, an antibody fragment, a nucleic acid, a full microbial cell, plant or animal tissues, polysaccharides, and other biosensor recognition elements).

A transducer is a device that transfers a signal with high sensitivity from one form (physical, chemical, or biological) to another (electrical). Many transducer systems have been developed and are constantly being developed (Figure 3, after Rupak et al., 2021). An example is the detection of *Salmonella paratyphi, S. typhimurium* in Apple juice samples (Jayan et al., 2020).

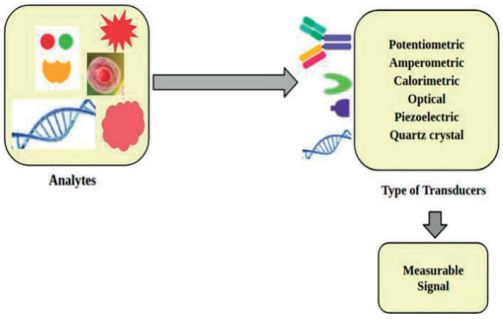


Figure 3. Detection of analytes by biosensor based methods (Rupak et al., 2021)

**FTIR Analysis**

FTIR Analysis, often known as FTIR Spectroscopy, is a technique for identifying organic, polymeric, and inorganic materials. Infrared light is used to scan test materials and examine chemical characteristics using the FTIR analysis method. The FTIR instrument passes infrared light in the range of 10,000 to 100 $cm^{-1}$ through a material, with part of it being absorbed and some passing through. The sample molecules transform the absorbed radiation into rotational and/or vibrational energy. The resulting signal at the detector appears as a spectrum, ranging from 4000 $cm^{-1}$ to 400 $cm^{-1}$, and represents the sample's molecular fingerprint. Because each molecule or chemical structure has its own spectral fingerprint, FTIR analysis is an excellent technique for chemical identification. When assessing industrially created material, FTIR spectroscopy is a well-established technique for quality control, and it is frequently used as the initial stage in the material analysis process. A change in the absorption bands' distinctive pattern suggests a change in the material's composition or the presence of contamination. If visual inspection reveals a problem with the product, FTIR micro-analysis is usually used to discover the source. This method is excellent for determining the chemical composition of tiny particles (10-50 microns) as well as larger areas on the surface.

FTIR analysis is used to: identify and characterize unknown materials (e.g., films, solids, powders, or liquids); identify contamination on or in a material (e.g., particles, fibers, powders, or liquids); identify additives after extraction from a polymer matrix; identify oxidation, decomposition, or uncured monomers in failure analysis investigations. An example is the detection of *E. coli, Salmonella typhimurium, Yersinia enterocolitis, Shigella boydii* in orange juice samples (Paniel and Noguer 2019).

**LAMP (loop-mediated isothermal amplification)**

Is a single-tube DNA amplification technology that offers a low-cost alternative for detecting certain diseases. To detect RNA, reverse transcription loop-mediated isothermal amplification (RT-LAMP) combines LAMP with a reverse transcription step.

LAMP stands for "isothermal nucleic acid amplification". In contrast to polymerase chain reaction (PCR) technology, which uses a number of alternating temperature steps or cycles to complete the reaction, isothermal amplification uses a constant temperature and does not require the use of a thermal cycler. An example is the detection of *Aeromonas* spp., *Streptococcus* spp. in fish samples (Pires et al., 2021).

## CONCLUSIONS

The purpose of this review is to point out the methods used recently in the food borne pathogens transmitted in different food matrices. Through the modern described methods (biochemical or molecular), it can be identified the best, most accurate and fastest the food borne pathogens. During the current period that the world is going through in the development in the production and transportation of food products in the same country or from one country to another.

As reported in the scientific publications, there are many methods used recently to detect bacterial contamination in different food matrices. Through PCR technology, we can know the characteristics and behaviour of the microorganisms present in the samples examined, even if they are few, simply by taking a small sample and multiplying it by copying it into millions of copies by PCR technology.

Different modern tools, like Real-Time PCR, or biosensors, were employed to identify the most common pathogenic bacteria, like *Salmonella, E. coli,* O157:H7, *L. monocytogenes*, *S. aureus*, as well as new emerging pathogens, like *Cronobacter* spp., *Hafnia* spp., *Shigella* spp. Such tests can be performed on a few samples by doubling the DNA to millions of copies, thus

obtaining more accurate results which cannot obtain it by the old traditional methods, nor by most modern methods**.**

It was found that one of the most employed of these methods is qPCR, which is one of the very successful and accurate modern methods, comparing to the old traditional methods, especially bacterial culture methods, which do not allow the detection of viable but non-cultivable bacteria (VBNC).

The ELISA approach has a benefit in that it may be used on-site, but it can only be used on a limited number of food samples, for example (ELISA) used for detection of foreign proteins in milk and other foods (Nagraik et al., 2021).

Few bacteriophage amplification-based detection techniques are now marginal, but they have a greater specificity than other methods.

The approaches based on gold nanoparticle aggregation are quick, and they can be used on-site, but they are challenging to employ in a liquid–solid matrix. Consumers and food companies may soon be able to conduct their own microbiological tests before purchasing or consuming food based on these efforts.

## REFERENCES


Azevedo-Nogueira, F., Gomes, S., Lino, A., Carvalho, T., & Martins-Lopes, P. (2021). Real-time PCR assay for Colletotrichum acutatum sensu stricto quantification in olive fruit samples. *Food Chemistry, 339*, 127858.

Baek, H.-W., Kim, S.-A., Min, W.-K., Dal Kang, S., Shim, S., Han, N. S., & Seo, J.-H. (2021). A species-specific qPCR method for enumeration of *Lactobacillus sanfranciscensis*, *Lactobacillus brevis*, and *Lactobacillus curvatus* during cocultivation in sourdough. *Food Analytical Methods, 14*(4), 750-760.

Bahlinger, E., Dorn-In, S., Beindorf, P.-M., Mang, S., Kaltner, F., Gottschalk, C., . . . Schwaiger, K. (2021). Development of two specific multiplex qPCRs to determine amounts of *Pseudomonas, Enterobacteriaceae, Brochothrix thermosphacta* and *Staphylococcus* in meat and heat-treated meat products. *International Journal of Food Microbiology, 337*, 108932.

Bonnin-Jusserand, M., Copin, S., Le Bris, C., Brauge, T., Gay, M., Brisabois, A., ... & Midelet-Bourdin, G. (2019). *Vibrio* species involved in seafood-borne outbreaks (*Vibrio cholerae, V. parahaemolyticus* and *V. vulnificus*): review of microbiological versus recent molecular detection methods in seafood products. *Critical reviews in food science and nutrition*, *59*(4), 597-610.

Cao, X., Zhao, L., Zhang, J., Chen, X., Shi, L., Fang, X., . . . Wang, L. (2019). Detection of viable but nonculturable *Vibrio parahaemolyticus* in shrimp samples using improved real-time PCR and real-time LAMP methods. *Food Control, 103*, 145-152.

Dronina, J., Bubniene, U. S., & Ramanavicius, A. (2021). The application of DNA polymerases and Cas9 as representative of DNA-modifying enzymes group in DNA sensor design. *Biosensors and Bioelectronics*, *175*, 112867.

Du, M., Li, J., Liu, Q., Wang, Y., Chen, E., Kang, F., & Tu, C. (2021). Rapid detection of trace *Salmonella* in milk using an effective pretreatment combined with droplet digital polymerase chain reaction. *Microbiological Research, 251*, 126838.

Durand, L., La Carbona, S., Geffard, A., Possenti, A., Dubey, J. P., & Lalle, M. (2020). Comparative evaluation of loop-mediated isothermal amplification (LAMP) vs qPCR for detection of *Toxoplasma gondii* oocysts DNA in mussels. *Experimental parasitology, 208*, 107809.

Elie, S. C. (2020). Heat resistance of *Paenibacillus* sp. spores isolated from carbonated juice beverage.

Ferone, M., Gowen, A., Fanning, S., & Scannell, A. G. (2020). Microbial detection and identification methods: bench top assays to omics approaches. *Comprehensive Reviews in Food Science and Food Safety, 19*(6), 3106-3129.

Frisch, L. M., Mann, M. A., Marek, D. N., & Niessen, L. (2021). Development and optimization of a loop-mediated isothermal amplification (LAMP) assay for the species-specific detection of *Penicillium expansum*. *Food Microbiology*, *95*, 103681.

Gök Charyyev, M., Özden Tuncer, B., Akpınar Kankaya, D., & Tuncer, Y. (2019). Bacteriocinogenic properties and safety evaluation of *Enterococcus faecium* YT52 isolated from boza, a traditional cereal based fermented beverage. *Journal of Consumer Protection and Food Safety*, *14*(1), 41-53.

Huang, C., Mahboubat, B. Y., Ding, Y., Yang, Q., Wang, J., Zhou, M., & Wang, X. (2021). Development of a rapid Salmonella detection method via phage-conjugated magnetic bead separation coupled with real-time PCR quantification. *LWT, 142*, 111075.

Huang, J., Luo, Y., Zhou, B., Zheng, J., & Nou, X. (2019). Growth and survival of Salmonella enterica and *Listeria monocytogenes* on fresh-cut produce and their juice extracts: Impacts and interactions of food matrices and temperature abuse conditions. *Food Control, 100*, 300-304.

Hyeon, J.-Y., Mann, D. A., Wang, J., Kim, W. K., & Deng, X. (2019). Rapid detection of Salmonella in poultry environmental samples using real-time PCR coupled with immunomagnetic separation and whole genome amplification. *Poultry science, 98*(12), 6973-6979.

Iacumin, L., Cecchini, F., Vendrame, M., & Comi, G. (2020). Emulsion pcr (Epcr) as a tool to improve the power of dgge analysis for microbial population studies. *Microorganisms, 8*(8), 1099.

Jayan, H., Pu, H., & Sun, D.-W. (2020). Recent development in rapid detection techniques for microorganism activities in food matrices using bio-recognition: A review. *Trends in Food Science & Technology, 95*, 233-246.

Jung, M.-J., Nam, Y.-D., Roh, S. W., & Bae, J.-W. (2012). Unexpected convergence of fungal and bacterial communities during fermentation of traditional Korean alcoholic beverages inoculated with various natural starters. *Food microbiology, 30*(1), 112-123.

Kim, E., Yang, S.-M., & Kim, H.-Y. (2021). Differentiation of lacticaseibacillus zeae using pan-genome analysis and real-time pcr method targeting a unique gene. *Foods, 10*(9), 2112.

Kim,[a] J. H., & Oh, S. W. (2019). Optimization of bacterial concentration by filtration for rapid detection of foodborne *Escherichia coli* O157: H7 using real-time PCR without microbial culture enrichment. *Journal of food science, 84*(11), 3241-3245.

Kim,[b] J.-H., & Oh, S.-W. (2020). Rapid and sensitive detection of E. coli O157: H7 and S. *Typhimurium* in iceberg lettuce and cabbage using filtration, DNA concentration, and qPCR without enrichment. *Food Chemistry, 327*, 127036.

Kim,[c] J.-H., & Oh, S.-W. (2021). Pretreatment methods for nucleic acid-based rapid detection of pathogens in food: a review. *Food Control, 121*, 107575.

Kim, S. A., Park, H. J., Cho, T. J., & Rhee, M. S. (2020). Toxic Potential of Bacillus cereus isolated from fermented alcoholic beverages. *Food Research International, 137*, 109361.

Koffi F. Y., Moumouny, T., Eric, Y. Y., Koffi, L. B., Diguta, C., Alloue-Boraud, M. W., & MATEI, F. (2021). Biocontrol of Post-harvest Fungal Diseases of Pineapple (*Ananas comosus* L.) Using Bacterial Biopesticides. *American Journal of Microbiological Research, 9*(2), 34-43.

Koffi, Y. F., Diguta, C., Alloue-Boraud, M., Koffi, L. B., Dje, M., Gherghina, E., & Matei, F. (2019). PCR-ITS-RFLP identification of pineapple spoilage fungi [J]. *Rom Biotechnol Lett, 24*, 418-424.

Koprinarova, M. (2021). Methods To Improve Molecular Detection of *Salmonella* in Complex Herbal Matrices Containing Inhibitors. *Journal of Food Protection, 84*(8), 1309-1314.

Labrador, M., Giménez-Rota, C., & Rota, C. (2021). Real-Time PCR Method Combined with a Matrix Lysis Procedure for the Quantification of Listeria monocytogenes in Meat Products. *Foods, 10*(4), 735.

Li, H., Chen, H., Liu, B., Cai, R., Jiang, N., Yue, T., & Wang, Z. (2021). Establishment of quantitative PCR assays for the rapid detection of *Alicyclobacillus* spp. that can produce guaiacol in apple juice. *International Journal of Food Microbiology*, *360*, 109329.

Lonczynski, T., & Cowin, L. (2021). Validation of the Applied Food Diagnostics, Inc. Simultaneous Multiplex Real Time PCR (SIMUL-qPCR) *Listeria* species and *L. monocytogenes* Assay in Selected Foods and Environmental Surfaces: AOAC Performance Tested MethodSM 062001. *Journal of AOAC International*.

Lv, X., Wang, L., Zhang, J., He, X., Shi, L., & Zhao, L. (2021). Quantitative detection of trace VBNC *Cronobacter sakazakii* by immunomagnetic separation in combination with PMAxx-ddPCR in dairy products. *Food Microbiology, 99*, 103831.

Maier, C., Hofmann, K., Huptas, C., Scherer, S., Wenning, M., & Lücking, G. (2021). Simultaneous quantification of the most common and proteolytic *Pseudomonas* species in raw milk by multiplex qPCR. *Applied microbiology and biotechnology, 105*(4), 1693-1708.

Mendonça, J. F. M. d., Vieira, F. d. O., Fonseca, I., Ribeiro, J. B., Arcuri, E. F., Borges, M. d. F., Martins, M. F. (2019). Detection of viable *Salmonella typhimurium* and *Staphylococcus aureus* in Coalho cheese by Real-Time PCR. *Food Science and Technology, 39*, 690-696.

Minarovičová J., Véghová A. and Kaclíková E. (2020). Evaluation of DNA extraction methods for culture-independent real-time PCR-based detection of *Listeria monocytogenes* in cheese. *Food Analytical Methods, 13*(3), 667-677.

Nagraik, R., Sharma, A., Kumar, D., Chawla, P., & Kumar, A. P. (2021). Milk adulterant detection: Conventional and biosensor based approaches: A review. Sensing and Bio-Sensing Research, 33, 100433.

O'Bryan, C. A., Ricke, S. C., & Marcy, J. A. (2022). Public health impact of *Salmonella* spp. on raw poultry: Current concepts and future prospects in the United States. *Food Control, 132*, 108539.

Paniel, N., & Noguer, T. (2019). Detection of *Salmonella* in food matrices, from conventional methods to recent aptamer-sensing technologies. *Foods, 8*(9), 371.

Persson, S., Eriksson, R., Lowther, J., Ellström, P., & Simonsson, M. (2018). Comparison between RT droplet digital PCR and RT real-time PCR for quantification of noroviruses in oysters. *International journal of food microbiology*, *284*, 73-83.

Pires, N. M., Dong, T., Yang, Z., & da Silva, L. F. (2021). Recent methods and biosensors for foodborne pathogen detection in fish: progress and future prospects to sustainable aquaculture systems. *Critical Reviews in Food Science and Nutrition, 61*(11), 1852-1876.

Rey, M. D. L. A., Cap, M., Favre, L. C., Rodríguez Racca, A., Dus Santos, M. J., Vaudagna, S. R., & Mozgovoj, M. (2021). Evaluation of PMA-qPCR methodology to detect and quantify viable Shiga toxin-producing *Escherichia coli* in beef burgers. *Journal of Food Processing and Preservation, 45*(4), e15338.

Roumani, F., Azinheiro, S., Rodrigues, C., Barros-Velázquez, J., Garrido-Maestu, A., & Prado, M. (2022). Development of a real-time PCR assay with an internal amplification control for the detection of spoilage fungi in fruit preparations. *Food Control*, *135*, 108783.

Siala, M., Barbana, A., Smaoui, S., Hachicha, S., Marouane, C., Kammoun, S., ... & Messadi-Akrout, F. (2017). Screening and detecting *Salmonella* in different food matrices in Southern Tunisia using a combined enrichment/real-time PCR method: correlation with conventional culture method. *Frontiers in microbiology*, *8*, 2416.

Spilsberg, B., Sekse, C., Urdahl, A. M., Nesse, L. L., & Johannessen, G. S. (2021). Persistence of a stx-encoding bacteriophage in minced meat investigated by Application of an improved DNA extraction Method and digital droplet PCR. *Frontiers in microbiology, 11*, 3305.

Tu, C., Hu, W., Tang, S., Meng, L., Huang, Z., Xu, X., Dong, M. (2020). Isolation and identification of *Starmerella davenportii* strain Do18 and its application in black tea beverage fermentation. *Food Science and Human Wellness, 9*(4), 355-362.

Vinayaka, A. C., Ngo, T. A., Kant, K., Engelsmann, P., Dave, V. P., Shahbazi, M.-A., Bang, D. D. (2019). Rapid detection of *Salmonella enterica* in food samples by a novel approach with combination of sample concentration and direct PCR. *Biosensors and Bioelectronics, 129*, 224-230.

Vizzini,[a] P., Manzano, M., Farre, C., Meylheuc, T., Chaix, , Ramarao, N., & Vidic, J. (2021). Highly sensitive detection of *Campylobacter* spp. In chicken meat using a silica nanoparticle enhanced dot blot DNA biosensor. *Biosensors and Bioelectronics, 171*, 112689.

Vizzini,[b] P., Vidic, J., & Manzano, M. (2021). Enrichment Free qPCR for Rapid Identification and Quantification of *Campylobacter jejuni*, *C. coli*, *C. lari*, and *C. upsaliensis* in Chicken Meat Samples by a New Couple of Primers. *Foods, 10*(10), 2341.

Wang, J., Kanach, A., Han, R., & Applegate, B. (2021). Application of bacteriophage in rapid detection of *Escherichia coli* in foods. *Current Opinion in Food Science, 39*, 43-50.

Wei,[a] S., Daliri, E. B. M., Chelliah, R., Park, B. J., Lim, J. S., Baek, M. A., . . . Oh, D. H. (2019). Development of a multiplex real-time PCR for simultaneous detection of *Bacillus cereus*, *Listeria monocytogenes*, and *Staphylococcus aureus* in food samples. *Journal of Food Safety, 39*(1), e12558.

Wei,[b] S., Park, B.-J., Kim, S.-H., Seo, K.-H., Jin, Y.-G., & Oh, D.-H. (2019). Detection of *Listeria monocytogenes* using Dynabeads® anti-Listeria combined with real-time PCR in soybean sprouts. *LWT, 99*, 533-539.

Yang, X., Zhang, X., Wang, Y., Shen, H., Jiang, G., Dong, J., Gao, S. (2020). A real-time recombinase polymerase amplification method for rapid detection of *Vibrio vulnificus* in seafood. *Frontiers in microbiology, 11*, 2751.

Yang, X., Zhao, P., Dong, Y., Chen, S., Shen, H., Jiang, G., Gao, S. (2021). An isothermal recombinase polymerase amplification and lateral flow strip combined method for rapid on-site detection of Vibrio vulnificus in raw seafood. *Food Microbiology, 98*, 103664.

Zhou, P., Xie, G., Liang, T., Yu, B., Aguilar, Z., & Xu, H. (2019). Rapid and quantitative detection of viable emetic *Bacillus cereus* by PMA-qPCR assay in milk. *Molecular and cellular probes, 47*, 101437.