DNA-based methods are becoming common techniques for the detection and identification of specific organisms. These methods rely on the ability to detect specific DNA sequences in the genome of target organisms. DNA-based methodologies have progressed impressively, being almost indispensable in most biology fields, including in disease diagnosis, forensic applications, and detection of several pathogenic microorganisms in clinical, food, and environmental samples (Jobling and Gill, 2004; Tang et al., 1997; Lockley and Bardsley, 2000). From all the methods available, amplification based methods, in particular polymerase chain reaction (PCR) and real time (rt)-PCR, are the most popular for diagnostics due to their simplicity, sensitivity, specificity, reproducibility, and ability to detect low amounts of the target.

Our knowledge about these methods has evolved rapidly. Many of the obstacles faced initially have been overcome, and many strategies have evolved that now allow us to increase the potential of these methodologies. In fact, there are several conditions that impact the result and the applicability of these techniques. One of the key steps to ensure a precise, consistent and successful result, based on these methods, is DNA extraction.

Many protocols have been published over recent years reporting different strategies for DNA extraction. These methods comprise enzymatic, chemical, thermal, or mechanic lysis, or even a combination of those (Rantakokko-Jalava and Jalava, 2002; Reischl et al., 2000; Tongeren et al., 2011). Thus, depending on the objective, different protocols can be appropriate for the same sample and/or for the same analysis method. The DNA quality and quantity directly influence the range of downstream methods that can be employed, as each method may be highly sensitive or not so sensitive and, therefore, may require different DNA amounts and purity before it can be considered adequate for its intended purpose. It is essential to select an appropriate DNA extraction method, in order to obtain a reliable result, and the option must be made considering both the objective and the downstream application.

DNA extraction methods follow some common steps: lysis, purification, and DNA recovery. These steps aim to effectively lyse cells, denature protein complexes, remove biological and chemical contaminants and, finally, recover the DNA. The simplest methods rely only on the lysis step. DNA extracts are used directly in the downstream applications, without any purification step. Indeed, lysis is the most
critical step to ensure a suitable and successful DNA recovery. This step is essential in all DNA extraction methods. The biggest differences between these methods are found in the purification step, since they apply different strategies to denature proteins and purify the DNA from the many biological and chemical compounds present in the sample.

It is also necessary to consider the origin of sample from which DNA will be extracted. There are a high number of different sources, such as human, animal, or vegetable tissues, hair, blood, stool, body fluid, and other clinical or veterinary samples, bacteria, yeast, fungi, spores, insects, soil, water, and food samples. These matrices will have different impacts on DNA quantity and purity, making some extraction protocols more appropriate for specific matrices (Di Pinto et al., 2007; Calvo et al., 2001).

Indeed, food safety and risk assessment of food contamination is of most importance nowadays. Thus, many organizations and governments are working together to implement a strict regulation, ensuring the public safety and confidence in the food chain and assessment of risks. These organizations include the World Health Organization (WHO), Food and Agriculture Organization of the United Nations (FAO), European Food Safety Authority (EFSA), and the European Commission.

Molecular analysis of food and water pathogens has developed rapidly in recent years. Although traditional culture methods are still an important tool for the detection of pathogens in food, they are laborious and time consuming, highlighting the need for the implementation of molecular methods that provide quick, sensitive, and robust results (López-Campos et al., 2012).

DNA-based analysis of food can be particularly difficult, since microorganisms (i.e., pathogens) are not uniformly distributed, the matrix may be very heterogeneous and complex, and there could be many inhibitors that need to be removed during DNA extraction (Di Pinto et al., 2007; Calvo et al., 2001; Arnal et al., 1999; Mandal et al., 2011; López-Campos et al., 2012).

In this chapter, several DNA extraction methods for the molecular detection of microorganisms in food and water will be reviewed. For each method, the advantages and disadvantages will be specified, as well as the strategies to overcome possible limitations. The most used methodologies – boiling method, column extraction, magnetic beads purification (both manual and automatic), and FTA cards – will be the focus of this chapter.

**BOILING METHOD**

In general, DNA extraction methods involve three main steps: cell disruption, DNA extraction, and DNA purification. The boiling procedure is one of the simplest protocols, and largely used for total DNA extraction from microorganisms (Reischl et al., 2000; Sepp et al., 1994; De Medici et al., 2003).

This simple method has evolved through the simplification of previous approaches that include the use of detergents as sodium dodecyl sulfate (SDS) or
cetyltrimethylammonium bromide (CTAB), enzymes like lysozyme and proteinase K, a phenol-chloroform purification and ethanol or isopropanol precipitation (Wilson et al., 1990; Neumann et al., 1992; Cheng and Jiang, 2006). The basis of these conventional extraction methods is that cells are lysed, cell components, such as lipids and proteins, are denatured in the presence of a detergent (proteins may also be hydrolyzed using a protease), and are removed by organic solvent extraction. These protocols have the advantages of having low cost, not requiring specific materials, and of being suitable for extracting DNA from many types of microorganisms. The disadvantages include laborious and time consuming manipulations, such as four to six changes of microcentrifuge tubes, incubations, precipitation, washing, drying, and resuspension steps. Also, depending on the protocol, they present highly variable DNA yields and/or do not remove some chemical compounds and cell debris that may inhibit downstream applications.

The boiling method is almost restricted to the lysis step, and the subsequent purification and precipitation steps described earlier are not performed, becoming much less laborious. Briefly, portions of individual bacterial colonies are suspended in a lysis buffer containing a detergent, (e.g., 1% Triton X-100 and/or 0.1% Tween 20), and a buffer solution (e.g., 10 mM Tris-HCl, 1 mM EDTA, pH 8). This cell suspension is incubated for 10–15 min at 95–100°C in a heat block or boiling water bath. Then, the tubes are briefly centrifuged to sediment the debris, and the supernatant is ready to be used (Figure 7.1). Nevertheless, it provides reasonable amounts of DNA that are usually enough to be used in amplification-based methodologies. It is also very easy and inexpensive. The major drawbacks are the low DNA yield and purity that are the result of the detergent usage, and the remaining cell debris that can contaminate

![Schematic Representation of the Boiling Method](image)

**FIGURE 7.1 Schematic Representation of the Boiling Method**

The sample is collected in a microtube and centrifuged to be concentrated. The supernatant is discarded and resuspended in the lysis buffer. This suspension is heated for cell lysis and DNA release. Finally, a brief centrifugation is performed to pellet cell debris and the supernatant containing the nucleic acid is transferred to a new microtube, and directly used in downstream applications.
the DNA samples and may inhibit the downstream applications. However, most amplification techniques are robust enough to be unaffected by inhibitors, and require very low amounts of target DNA. If inhibition occurs, most times it can be overcome just by diluting the sample, if the sensitivity of the assay is able to cope with this.

This method is also suitable for the DNA extraction of microorganisms that have been enriched from all kinds of foods, according to the international standards, or that have recovered from samples by filtration.

Microbial DNA extraction using the boiling method directly from food samples is also possible, although sometimes it may become challenging. This is due to the possible low numbers of microorganisms present in the sample and also due to PCR inhibitors present in food matrices (e.g., fats, proteins, polysaccharides, and calcium). In other words, the extraction might be affected by the physical, chemical, and biological characteristics of the food material (Wilson, 1997; Giacomazzi et al., 2005). Therefore, the method of choice is to perform an enrichment step to augment the bacterial population in the matrices, in particular the target microorganism. Still, as mentioned before, direct detection of DNA from the food matrix can be done, if the target microorganism is present in high enough numbers to be detectable by the assay. The low yield arising from the presence of small specific microbial populations is not easy to circumvent. However, when the objective is general microorganisms’ detection, or when the microbial contamination is high, the boiling method can be successfully applied to the sample. In this case, a good homogenization of the sample is crucial. In several food matrices, it is necessary to process the food prior to the boiling method, in order to promote the detachment of microorganisms from the food matrix, releasing them to form a suspension in the extraction medium. For this purpose, the use of a laboratory paddle shaker (e.g., Stomacher®) is recommended, together with specific bags. However, this homogenization can also be performed manually, using sealed plastic bags. For hard food materials, a mortar can be used prior to homogenization with a buffer. The choice is dependent on the sample characteristics, and sometimes the best strategy must be defined after testing different alternative methods to achieve optimum results. After processing, a small aliquot of the sample is centrifuged to concentrate the microbial population, the supernatant discarded, and the pellet resuspended in lysis buffer. As mentioned before, it is unavoidable that all the other sample components will also be centrifuged, and so they will impact upon the purity and quantity of DNA extracted.

In case of water or other liquids, the best strategy is usually to filter or centrifuge the sample, in order to concentrate the microbial population. For the filtration approach, it is essential to use a filter that allows the recovery of the cells that can subsequently be suspended in the lysis buffer, so that the remaining protocol can be applied. Most membrane filters retain microbial cells bound to the fibers. Such membranes comprise mixed cellulose esters, cellulose acetate, and nylon filters. These membranes are not appropriate for cell recovery. Polycarbonate filters are considered to be the most suitable option for this purpose, since they minimize the microorganisms’ entrapment within the filter, resulting in their retention on the surface (Smith et al., 1993).
The contaminants in DNA extracts, arising from food and water samples, are the major drawback of this method. As purification steps are not performed, the boiling method does not allow the recovery of high purity DNA, and inhibition of the enzymes used in the subsequent methods are a real possibility. Sample dilutions are often the best solution for this problem, as it reduces the interference of the inhibitor, and the target remains in an amount that is enough for target detection.

Another drawback might be the lysis efficiency of Gram-positive bacteria or fungi that have a more complex cell wall that might impose an obstacle to the DNA extraction. In those cases, glass or ceramic beads can be added to the sample, followed by a strong shaking in a vortex. This will allow a supplementary mechanic lysis prior to the boiling step, promoting the cell lysis and the DNA release. Additionally, incubation with lysozyme for bacteria, proteinase K (used for the destruction of proteins in cell lysates), or lyticase for fungi, might also be helpful. These enzymes have a lytic activity, helping the hydrolysis of the cell walls. Lysozyme hydrolyses the 1,4-β linkages between N-acetylmuramic acid and N-acetyl-d-glucosamine residues from the peptidoglycan in the bacterial cell wall. Proteinase K can digest proteins and aid in the removal of contamination from DNA preparations. In addition, it inactivates nucleases that can degrade nucleic acids during purification. Lyticase hydrolyzes poly-β(1,3)-glucose linkages in glutans, often present in the yeast cell wall. Consequently, these steps might provide increased lysis efficiency, since the combination of heat and enzymatic activity will enhance DNA recovery.

Like any other DNA extraction protocol, the boiling method presents advantages and disadvantages. However, due to its low yield and purity, its usage in subsequent analytical procedures must be validated, since it may not be suitable for every downstream application (Queipo-Ortuño et al., 2008; Ahmed et al., 2014). Due to the low yields of DNA recovery, this method is most suitable for PCR or other amplification based methods. For other techniques that require higher quantities of DNA or that are not so robust, the boiling method will not be appropriate. When appropriate, however, this method can be used on a routine basis being easy, rapid, and efficient.

**COLUMN EXTRACTION**

As stated earlier, DNA extraction using the boiling method is only suitable for applications that do not require high amounts of nucleic acids. Other methods that involve the use of detergents, organic solvents, and precipitation of the DNA are laborious, make use of several hazardous reagents, and are time consuming, especially when a high number of samples must be processed. Commercial kits take benefit of DNA-binding matrices to circumvent these disadvantages, allowing the recovery of high purity DNA, usually with good yields.

Generally, the basis of this system is the use of a column that has inside a chromatography affinity membrane (e.g., silica based) that adsorbs the DNA. This adsorption is achieved by the addition of reagents containing chaotropic salts (e.g., guanidine salts), at a particular pH, during or after the lysis step. These chaotropic salts
destabilize hydrogen bonds and hydrophobic interactions, denature proteins (including nucleases), and disrupt the association of nucleic acids with water, promoting the DNA binding to the silica membrane. Then, all impurities and contaminants are removed in the washing steps with a solution that contains a detergent and an alcohol (usually ethanol). The alcohol prevents the dissolution of the DNA that remains bound to the membrane while the other sample components are removed. Finally, DNA is eluted using a low salt buffer (e.g., TE or Tris buffer) (Figure 7.2). The various steps of eluting the reagents through the column can be achieved only by gravitational force or by centrifugation, depending on the column characteristics. These kits provide high quality, inhibitor-free DNA, suitable for use in several downstream applications, including those that require higher DNA amounts and purity.

As mentioned before, the sample matrix from which DNA extraction is intended impacts the final extraction product, both in terms of quantity and quality. Although column extraction methods are all based on the same principles, the commercial kits available are classified accordingly to the source material. Each brand presents specific categories, but, overall, they usually offer kits for DNA extraction from microbial cells, animal tissues, plant tissue, blood, soil, water, and food (e.g., http://www.lifetechnologies.com/; http://www.mn-net.com/; http://www.mobio.com/). The main difference between them is the sample pretreatment and lysis steps that are adapted to each type of matrix, according to their specific characteristics, in order to get the best final result.

For DNA extraction from microbial cultures, many commercial options are available, as all manufacturers offer one or several kits for these types of sample. However, for the detection of microorganisms in food and water, the purpose is, most
of the times, an extraction method that allows an efficient recovery of microbial DNA from enrichments, or directly from different kinds of food and water samples. For these type of matrices, some suppliers have developed suitable kits such as Nucleospin Food Kit (Macherey-Nagel), PowerFood® Microbial DNA Isolation Kit (MO BIO laboratories), or DNeasy mericon Food Kit (Qiagen), for food products; and PowerWater® DNA Isolation Kit (MO BIO laboratories), RapidWater® DNA Isolation Kit (MO BIO laboratories), or SurePrep™ Water RNA/DNA Purification Kit (Fisher Scientific), for water.

The choice for the most suitable kit should be made taking into account the sample characteristics. As mentioned previously, most of the times, a sample pretreatment, preconcentration, or processing must be performed, for reliable results. Microorganisms associated with food and environmental samples are often highly associated with the sample matrix, making it difficult for the lysis reagents to access microbial cells. In some other situations, microbial populations are very low, and it is necessary to process large sample volumes. Thus, it is usual that pretreatments include homogenization, enrichment, centrifugation, and filtration. These pretreatments must ensure that microbial cells become accessible, and in a sufficient number for an efficient DNA extraction. The best way to proceed may be evaluated in the laboratory, but commercial kits often provide standardized sample treatments and troubleshooting guidance to enhance the results.

In general, column-based technology is simple enough to apply to a large number of samples simultaneously, usually ensures high yield, concentration, and purity of the DNA extracted, enables the standardization of samples’ treatment, and the avoidance of possible contaminations as a result of intermediate processes.

The major disadvantages are related to the costs that can become more significant when a large number of samples have to be analyzed. It can also be time-consuming and labor-intensive, depending on the treatment procedures that must be performed before the kit application. It is also necessary to be aware that, with this silica-based technology, it is not possible to separate the DNA from some other contaminants like phenolic compounds and humic compounds. These substances can oxidize and form covalent bonds with nucleic acids, or mimic DNA in their chemical structure. Consequently, they bind to silica membranes in the presence of high salt concentrations, and elute with low salt solutions, as per the DNA. Hence, an additional step must be performed to remove these compounds, prior to DNA binding to silica membranes.

However, even considering these drawbacks, several authors have considered that this type of DNA extraction is very acceptable, and appropriate to routine usage in food and environmental analysis (Quigley et al., 2012; Lee et al., 2010; Giacomazzi et al., 2005; Rantakokko-Jalava and Jalava, 2002).

**MAGNETIC BEADS**

Magnetic beads purification of DNA is a technology that uses magnetic properties to separate the nucleic acids from chemical and/or biological contaminants present in solution or in suspension. For that purpose, the magnetic beads must reversibly bind
CHAPTER 7 DNA extraction: finding the most suitable method

DNA extraction becomes possible if the bead is prepared with a biopolymer with affinity to DNA, coated with a DNA binding antibody, or with a functional ligand presenting affinity to the DNA.

Nowadays, there are many commercially available beads for DNA purification, prepared with different materials, including porous glass (MGP), cellulose (MagaZorb®), silica (GenoPrep™ DNA), polystyrene (Dynabeads® DNA), inorganic magnetic materials like surface-modified iron oxide (MagneSil, SiMAG), etc.

The first steps of this method are similar to other methodologies, and rely on cell lysis with detergents and/or lytic enzymes, promoting the release of DNA from cells. After the addition of magnetic beads to the lysate, the released DNA binds to the beads, while the undesired chemical and biological compounds are maintained in solution, allowing a series of washing steps to be performed that result in highly purified DNA (Figure 7.3). This is achieved by applying a magnetic field that concentrates the beads on one side of the tube, enabling the removal of the contaminants in suspension by aspiration. Then, a washing reagent (commonly an ethanol based solution) is added and removed, also by aspiration. After the washing steps, the magnetic field is removed, and the beads with the bound DNA can be resuspended.

The beads may not interfere with the downstream application. In this case, the DNA remains bound and may be directly used, not requiring an elution step (Rudi et al. 1997; Berensmeier, 2006). However, if the retention of the magnetic beads does interfere with the downstream application (e.g., decreases the PCR sensitivity, or leads to false negative PCR results) an elution step is then required. Depending on the material composition of the beads, DNA elution can be accomplished by promoting a shift in the ionic strength of the solution, by heating, or by pH changes (Berensmeier, 2006). For silica coated beads, for example, the same principle of DNA binding

---

**FIGURE 7.3 Schematic Representation of the Magnetic Bead Purification Method**

After cell lysis, magnetic beads are added and DNA bound to them. The application of a magnetic field allows the beads with the bound DNA to concentrate in a specific site of the microtube, and to allow the supernatant to be discarded by aspiration. The same procedure enables the removal of undesired compounds with a washing reagent. Finally, a buffer is added to recover the purified DNA. The beads can be used directly in downstream applications, or the DNA can be eluted and beads removed.
in the presence of chaotropic salts, and elution with low salt buffer and pH changes, referred to before for silica columns, is typically applied.

As mentioned, to perform DNA extraction using this method, the lysis step has to occur previous to the addition of magnetic beads. Many of these commercially available kits describe their own protocol for DNA extraction from different samples, and provide the reagents needed to do so (MagJET Genomic DNA Kit, 2013; Axygen® AxyPrep Magnetic Bead Purification Kits, 2014; Magnetic Beads gDNA Kit, in press).

However, this purification method can be used with any customized pretreatment, processing, or lysis methods that can be chosen according to the objectives and downstream applications. If only a low amount of purified DNA is required, then a simple and rapid lysis, based on the boiling method, can be performed as an alternative to a more complex protocol. The direct usage of a preenriched or homogenized food mixture can also be used to detect microbial DNA, without performing a previous lysis. In such cases, the manufacturer supplies a detergent-based reagent that performs the lysis step, at the same time that the DNA binds to the beads (Caldarelli-Stefano et al., 1999; Rudi et al., 1997; Quigley et al., 2012; Moroney et al., 2012).

Magnetic bead DNA purification technology is also available in a fully automated extraction option, using specific equipment. Laboratory automation is increasingly important in molecular biology laboratories, as it allows the analysis of a large number of samples, saving handling time, and minimizing possible operator errors. Thus, this automated technology is less laborious, and reduces the training requirements of specialized personnel to carry out the DNA extraction (Oldham et al., 2012; Berensmeier, 2006).

Presently, there are various commercial automated systems available based on magnetic beads technology. Examples of these automated systems are MagNA Pure 96 System (www.roche-applied-science.com), AutoMate Express™ Instrument (http://www.lifetechologies.com), Liferiver™ EX2400/EX4800 Automated Nucleic Acid Extraction System (http://www.biosb.com/automated-nucleic-acid-extraction-system), and Maxwell® 16 Instrument (https://worldwide.promega.com), among others.

As described, for manual extraction using magnetic beads, the samples will either be placed directly into the instrument, or require a pretreatment procedure, depending on their characteristics. Similarly, in general, the manufacturer provides a protocol specific for several sample types, and the reagents required to carry it on.

This DNA extraction methodology is fast and simple to handle. It results in a very good yield and quality of the extracted DNA. It is highly reproducible, and less labor intensive than column extraction or classical protocols, since it avoids multiple centrifugation steps. With the automated system, the standardization and reproducibility are enhanced, and it allows the simultaneous extraction of several samples without much labor or time increase (Tan and Yiap, 2009; Smith et al., 2003; Caldarelli-Stefano et al., 1999; Chacon-Cortes and Griffiths, 2014).

The major disadvantage is the high cost, especially if automated systems are chosen due to the need of specific equipment and consumables. These automated
systems are also a less controllable technique, since they limit the possibility of optimization experiments, such as changing reagents, or making changes in the process because the programmed protocol cannot be modified or stopped once started.

**FTA™ CARDS**

FTA paper is an easy and fast technology, based on a cellulose-paper card that is impregnated with chemicals that lyse cells, denature proteins, inactivate bacteria and viruses, and protect DNA from nucleases, oxidation, and UV damage. It allows the collection and storage of biological material at room temperature simultaneously with DNA extraction.

FTA papers are available in two main formats: (1) sample DNA remains bound to the paper during the analysis – FTA “classical” card and (2) sample DNA is eluted from the card before further analysis – FTA elute card. This variation is due to the different chemistry on the basis of these two types of FTA cards. The FTA “classical” cards contain chemical denaturants and free radical scavengers that promote the DNA binding to the paper, while FTA elute cards are impregnated with a chaotropic salt that allows DNA elution from the card to a solution (www.gelifesciences.com). This is the principal characteristic that impacts directly the downstream application of the cards. Other features were developed by the manufacturers, in order to allow the use of the cards with different types of samples, or to make the use of the cards easier. As examples, there are cards with laminated flaps that allow the user to vigorously pound the samples (e.g., plant tissues), without damaging the card, and cards with an operator-help feature, such as an impregnated dye that changes from colored to white after sample application. There are also cards with different spot sizes and numbers, so as to be suitable for many applications.

Generally, sample preservation faces two major challenges: transportation and storage. During transportation, the samples are exposed to temperature variations and atmospheric changes that can lead to spoilage and DNA degradation. The FTA paper can prevent these problems, as their composition enables DNA protection from environment aggressions, such as the content of acid gases and free-radical-generating pollutants, ultraviolet irradiation, temperature, and humidity (GE Healthcare Life Sciences, 2012). Genomic DNA stored in the cards is stable for years, at room temperature. This was reported in some studies that tested DNA from samples stored on a card library at room temperature, without any atmospheric control, for at least 4 years, showing that it remained intact (Smith and Burgoyne, 2004). Moreover, buccal and blood derived DNA samples have been stored on FTA cards for more than a decade, at room temperature, and remain suitable for amplification (GE Healthcare Life Sciences, 2011b).

The other problem faced by laboratories is the sample storage. During storage, extracted DNA or stored tissues can degrade, resulting in problems for sample re-analysis. The space needed to keep these samples might also represent a cost-space problem. The chemistry of FTA paper enables cost-effective storage of biological
material at room temperature, for extended periods. Thus, no refrigerators or freezers are required (GE Healthcare Life Sciences, 2011b), a fact that significantly reduces storage costs.

Using an FTA card is very simple. A low volume of sample (few microliters or a few milligrams) is just applied onto the card, and let to air dry. DNA extraction and preservation occurs during this drying process. The system can be applied to clinical, environmental, food sample, and bacterial cultures. These include bone marrow aspirates, cultured cells, plasma (Qiagen, 2010), blood, saliva, tissue samples from several animals (Smith and Burgoyne, 2004), plants (Lin et al., 2000), insects (Harvey, 2005), viruses (Li et al., 2004), microorganisms (Rajendram et al., 2006), soil, water, and processed food.

To analyze the microbial population in food and water samples with this method may require a pretreatment step, using the strategies already described earlier, such as enrichments, homogenization, or water concentration with filters. The pretreated samples can then be applied to the card, enabling DNA targeting more easily.

Bacterial cultures can be loaded on to the FTA card from a cell suspension, or from cultures grown in liquid media. Rajendram et al. (Rajendram et al., 2006) tested long-term storage of DNA from bacterial cell suspensions, and the microbial inactivation properties of FTA cards. They reported that amplification of DNA from FTA cards was possible from low cell density suspensions (i.e., 10 cfu/mL), showing that a high recovery of bacterial DNA is possible using FTA paper. Also, all 100 bacterial samples stored on FTA cards were successfully amplified after 3 years storage. With respect to microbial inactivation, the FTA card showed that no viable cells were retained on the card, even at high cell densities, ranging from 10⁷ cfu/mL to 10⁸ cfu/mL for Gram-negative species. However, for the most robust species, such as spore-forming bacteria, complete inactivation was only achieved at cell densities ranging between 10⁴ cfu/mL and 10⁵ cfu/mL (Rajendram et al., 2006). Extraction of DNA from resistant bacterial forms, like bacillus spores, using FTA cards has also been successfully described (Lampel et al., 2004).

To load liquid samples into the card, the speed of application is very important. This needs to be rapid because the cells lyse soon after contact with the paper. Non-uniform spread of DNA often occurs when the sample delivery is too slow, and the aim is to prevent localized lysis and nonhomogenous sample distribution (Smith and Burgoyne, 2004).

After that, the released nucleic acids remain immobilized in the fibers of the matrix, and are preserved for transport, immediate processing, or long-term room temperature storage (www.gelifesciences.com). To use the sample applied in the card, a paper disk is punched with a small device (a puncher) provided by the manufacturer, and used in downstream applications. According to these applications, different disk sizes (between 1.2 mm and 6 mm) can be punched. For example, for DNA amplification, a 1.2 mm diameter disk will be enough, whereas for a more DNA demanding application, like RFLP, a larger disk should be punched. After punching, the disk is washed with purification buffers or/and TE buffer. The washing steps are necessary because, in addition to the target DNA bound to the card fibers, all the preserving
agents and sample contaminants are also in the cards. They are possible inhibitors of downstream reactions, and can be removed in the washing steps. The washing buffers vary between FTA paper brands, but the objective is always to remove all the contaminants, with just the DNA remaining (Figure 7.4).

After washing steps, the disks from the FTA “classical” cards can be used directly for carrying out the amplification reaction or other downstream application. For amplification methods, the disk can be reused in multiple reactions, since genomic DNA remains bound to the disk, while the amplification product remains in solution (Miles and Saul, in press). However, other methods, such as rt-PCR cannot be performed directly from the disk, as it will interfere with fluorescent acquisition. Thus, the option is to use the FTA elute system, in which DNA is eluted from the disks after the washing step. Elution is performed by incubating the disk in water at high temperatures for some minutes (Figure 7.4) (GE Healthcare Life Sciences, 2011a; www.zygem.com). More recently, different methods were described to elute the DNA from FTA “classical” cards. A number of chemical treatments were evaluated. These include the use of alkaline conditions (www.whatman.com), organic extraction (GE Healthcare Life Sciences, 2010), modified methanol fixation method (Johanson et al., 2009), prepGEM protocols from ZyGEM (Miles and Saul, in press), or using DNA extraction kits, like QIAamp™ DNA Investigator Kit (Qiagen), illustra™ tissue and cells genomicPrep Mini Spin Kit (GE Healthcare), or DNA IQ™ Kit (Promega) (GE Healthcare Life Sciences, 2010).
DNA purified from samples stored on FTA paper is of high quality, and can be used in many downstream applications, including qualitative and quantitative PCR, short tandem repeats (STR) analysis, restriction fragment length polymorphisms (RFLP), amplified fragment length polymorphisms (AFLP) analysis, human leukocyte antigen (HLA) gene typing, and whole genome amplification (WGA) (www.qiagen.com).

In addition to the advantages already mentioned, there are some limitations associated with this DNA extraction method. The major limitation is the maximum volume that can be applied into the FTA card. Processed and mixed samples are more challenging, since different components have to be homogenized, but only a small portion of this volume can be applied to the card. This can lead to a poor representation of the initial proportions and components of the original (heterogeneous) sample. In the particular case of samples for microbial detection, the population is often low, and the small volume applied to the FTA cards may not allow the loading of enough sample to extract sufficient microbial DNA. Sample pre-enrichment is a possible option in order to increase the amount of microbial population. Also, samples with a hard matrix, like dry fruit, cannot be applied directly to the card, and must be crushed and homogenized prior to application to the card.

There are different suppliers that sell FTA™ paper, but the base technology is the same – Whatman technology. Qiagen (QIAcards FTA spots), GE Healthcare Life-sciences (FTA cards), and ZyGEM (ZyGEM storage card) are different suppliers of this type of paper. International distributors like VWR and Sigma Aldrich also retail Whatman FTA cards.

In summary, FTA cards are considered a reliable option for the storage and transport of DNA samples from many different matrices. Samples can also be collected in situ, sent by mail or carried in personal baggage. Storage is much less space and cost consuming. Although some authors reported that long-term storage may hinder the release of the entrapped DNA from the card matrix (Ahmed et al., 2011), it is generally accepted that this technology allows the preservation of samples for years, at room temperature, in noncontrolled atmospheric conditions. The major drawback is undoubtedly the small maximum sample volume limitation, and the cost associated with the use of this methodology.

RNA EXTRACTION METHODS

Although beyond the scope of the present review, it is important to make some comments about RNA extraction, especially considering that some important food- and waterborne pathogens are RNA viruses (e.g., norovirus GI and GII and hepatitis A and E virus) (Costafreda et al., 2006; da Silva et al., 2007; Jothikumar et al., 2006; Le Guyader et al., 2009). In fact, RNA extraction methods are very similar to those described for DNA. The previously mentioned strategies for DNA extraction, namely column-based, magnetic beads, and FTA™ cards, are also available for RNA extraction and purification. The main differences are related to some purification solutions
that promote a preferential recovery of RNA in detriment of DNA. Another important issue for a successful RNA recovery is related to the requirements for all material, reagents, and solutions. RNA is more sensitive to degradation than DNA, and RNases are not easily destroyed by heat. Thus, all material and solutions used for handling RNA must be RNase free. Most kit suppliers also have available this kind of material (including tubes, tips, labware, etc.) and decontamination solutions for benches and other surfaces. Alternatively, materials and solutions can be decontaminated in-house, using diethylpyrocarbonate (DEPC), a chemical that can be used to inactivate RNase enzymes in water, and on laboratory utensils. It does so by the covalent modification of histidine (most strongly), lysine, cysteine, and tyrosine residues (Chirgwin et al., 1979). However, as it is highly toxic, if possible, it is always better to use commercially available products.

**CONCLUSIONS**

Detection of food and water pathogens is currently a major concern both for consumers and suppliers. Data from WHO suggest that food- and waterborne pathogens contribute to the mortality from many diseases, such as diarrheal disease, for which it estimates 2.1 million deaths in 2000. Each year, it is estimated that 76 million people suffer from food-related diseases in the United States, and that about 2,366,000 cases occur in England and Wales (Adak et al., 2005; Mead et al., 1999). These numbers demand a quick and accurate response to control outbreaks, or specific contamination issues. Additionally, foodborne diseases are changing, due to changes in the food industry, improved surveillance, detection methods, and lifestyles, all of which intensify the need for high control of the food industry.

DNA-based methods have evolved remarkably over the last few years, being an indispensable tool for the rapid detection of pathogens in food and water.

In this chapter, some strategies that are appropriate to ensure an accurate detection of microorganisms in food and water have been highlighted. Four different DNA extraction methods, presenting different outputs and possible downstream applications, have been discussed: boiling method, column extraction, magnetic beads, and FTA cards.

DNA extraction protocols have three basic steps in common: lysis, purification, and DNA recovery. For the four methods described, the strategies used in each of these steps may differ, and may even be absent in some protocols (Figure 7.5). The lysis step, and its high efficiency, is the most important, since the success of the downstream procedures depends on the availability of the DNA obtained at this initial stage. The amount of DNA recovered is directly dependent on the efficiency of the cell lysis. Reviewing the discussed methodologies, the boiling method comprises only the lysis step, achieved by combining a detergent action with high temperature application. In this protocol, hydrolytic enzymes can also be used specifically to enhance the lysis of Gram-positive bacteria and fungi, but no further purification procedures are usually applied. In column and magnetic bead-based strategies, lysis
is achieved using a lysis buffer, hydrolytic enzymes combined with incubations to enable the reagents to act on cell membrane targets and wall. In FTA cards, this step occurs by hydrolytic reagents impregnated within the paper.

The purification step is a requisite to isolate DNA from possible contaminants, and to obtain a pure DNA extract. As referred previously, the boiling method does not include a purification step. Both the column and magnetic bead methods have specific purification steps, based on the use of specific reagents that remove the undesired compounds, while the DNA is bound to the column or to the magnetic beads. In FTA cards, removal of DNA contaminants is performed by direct washing of punched disks with specific buffers.

Finally, in all the cited methods that include a purification step, the recovery step is usually made by elution with a low ionic strength buffer. However, it can be absent, when using FTA “classical” cards (Figure 7.5).

Furthermore, each method has its own characteristics, including advantages and limitations that are summarized in Table 7.1. The boiling method is the least
expensive of the four, and does not involve multiple manipulations of the samples. However, it has the limitation of yielding low amounts of DNA with low purity. On the other hand, column and magnetic beads extractions allow for good DNA yields presenting a high purity, as they effectively remove more of the contaminants present in food and water samples. Their major drawbacks are the costs and the time-consuming protocols.

An additional problem that arises from analysis of food and water samples is the need for sample pretreatment to meet the objective of the analysis. In some cases, the heterogeneous distribution of the microbial population, the differences in the microbial cell wall structures, and the low number of cells present in the sample, require strategies that will overcome these obstacles. Such strategies include, as mentioned before, homogenization, pretreatment with enzymes or microbeads, and pre-enrichments.

An advantage of the column extraction and magnetic bead purification is that, generally, the manufacturer includes in their protocol instructions on how to process various types of samples. Also, they include the reagents to achieve this, and even give troubleshooting advice for possible problems that may arise. When considering the boiling method or the FTA cards, the optimum protocol to process specific types of sample relies on previous knowledge and experience. Nevertheless, the same strategies may be applied to all of these methods.

Other important aspects in DNA extraction for the detection of microorganisms in food and water, is the time and labor required to extract the DNA. This is particularly important when a high number of samples have to be analyzed. The boiling method and FTA cards are the least laborious methods. On the other hand, column extraction is the most laborious, and automatic alternatives are not easily available. The automation potential in magnetic bead purification and the use of FTA cards are options to reduce the staff requirement, time, and personal specialization. The major drawback is, as mentioned before, the high cost and the low optimization potential when automated systems are used.

**Table 7.1** Comparison of the Main Features of Four DNA Extraction Methods: Boiling, Column Extraction, Magnetic Bead Purification, and FTA Cards

<table>
<thead>
<tr>
<th></th>
<th>Boiling Method</th>
<th>Column Extraction</th>
<th>Magnetic Bead</th>
<th>Automated Magnetic Bead</th>
<th>FTA Cards</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cost</td>
<td>Low</td>
<td>Medium</td>
<td>Medium</td>
<td>High</td>
<td>Medium</td>
</tr>
<tr>
<td>Laboring</td>
<td>Low</td>
<td>High</td>
<td>Medium</td>
<td>Low</td>
<td>Low</td>
</tr>
<tr>
<td>DNA yield</td>
<td>Low</td>
<td>High/medium</td>
<td>High/medium</td>
<td>High/medium</td>
<td>Medium</td>
</tr>
<tr>
<td>DNA quality</td>
<td>Low</td>
<td>High</td>
<td>High</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td>DNA stability (storage)</td>
<td>Low</td>
<td>High (-20°C)</td>
<td>High (-20°C)</td>
<td>High (-20°C)</td>
<td>High (RT)</td>
</tr>
</tbody>
</table>

*RT, room temperature.*
The stability of DNA during storage also differs between these four methods: the stability of the DNA recovered by the boiling method is very low, since the remaining contaminants and debris promote degradation of the DNA. DNA extracted by column and magnetic bead is very stable, if stored at −20°C. However, the DNA stored in FTA cards is highly stable at room temperature, for very long time periods.

Studies have attempted to compare these extraction methods and others, in order to determine the most suitable method. Results from these studies differ with the sample source and the downstream application, fact that means that, so far, there is no single technique that can meet all objectives for all types of samples and sample analysis situations.

Thus, there is no perfect universal method. They all are suitable for the detection of DNA from microbial populations present in food and water. Also, they are suitable and easily available for any laboratory that wishes to implement DNA based analysis. The choice for a specific method or methods must be made based on criteria such as the type and number of samples to be processed, the downstream analysis, and cost/benefit ratios. More robust applications, or those that are based on DNA amplification, will allow the use of simpler and more inexpensive techniques, such as the boiling method, while techniques that demand high yields of pure DNA will favor the use of column or magnetic beads-based extraction.

Regardless of the method selected, the cell lysis stage is one of the most important steps for a reliable result and its optimization, considering the particular characteristics of the samples that may prevent several further downstream problems. Additionally, strategies to remove particular sample contaminants, knowledge to overcome possible hitches, and experience to optimize a particular step, in order to increase the microorganism detection efficiency, is essential for a reliable, robust, and effective result.

In summary, many efforts have been made in order to standardize microbial food and water detection. However, particular characteristics of the samples must always be considered, making the complete standardization an impossible task. Nevertheless, many strategies are now accessible. The increasing knowledge about sample requirements, and the available techniques, allow the implementation of new, safer, and simpler methodologies to ensure food and water safety.

REFERENCES


GE Healthcare Life Sciences, 2010. Reliable extraction of DNA from Whatman™ FTA™ cards. GE Healthcare Application note 28-9822-22 AA.


GE Healthcare Life Sciences, 2011b. STR amplification of DNA from buccal and blood samples stored long-term on Whatman™ FTA™ cards. GE Healthcare Application note 29-0082-33 AA.


MagJET Genomic DNA Kit. Thermo Scientific product information, 2013. (www.thermoscientific.com/onebio)
Magnetic Beads gDNA Kit (Bacteria) Manual Protocol, Geneaid. Ver. 06.05.14 (www.geneaid.com).
Miles, M., Saul, D. Improved elution of DNA from Whatman FTA® cards using prepGEM®/forensicGEM® Storage Card extraction kits, ZyGEM Application note 103.


