



PATHfinder

Bacteria and Parasites
Real-Time PCR detection
Pathogens in milk and dairy

Food and microbes

How can DNA help?

***Food is rarely sterile, but
spoilage microorganisms
must always be excluded***

Food is rarely sterile, instead, the presence of micro-organisms is often required to obtain specific organoleptic characteristics. However, pathogenic microorganisms must always be excluded as well as spoilage microorganisms which do not cause any disease but destroy the value of food products. Foods can become contaminated with undesired microbes through exposure to animal manure, inadequate processing controls, cross-contamination, and improper storage or cooking.

***The disruptive effects on industry and
markets caused by pathogens outbreaks
can be dramatic.***

The spoilage of food products have tremendous direct costs for industry: production delays, plant shut-downs, product recall and product destruction. Furthermore, the indirect costs connected to the lost of brand reputation and consumer's trust on food-industry can even be higher.

***Traditional culture-based
detection of bacteria is
often laborious and time
consuming***

PCR-based methodologies are generally **more specific, informative** immediate strain identification), **sensitive**, and **faster** than conventional microbiological assays. Although, a pre-enrichment step is still often needed, the simplicity and time saving feature of the PCR reaction has made it increasingly applicable for detection of bacterial pathogens, but also of spoilage microorganisms, in food and beverages

***PCR based ISO standard methods, or the
detection and identification of
foodborne pathogens are accumulating***

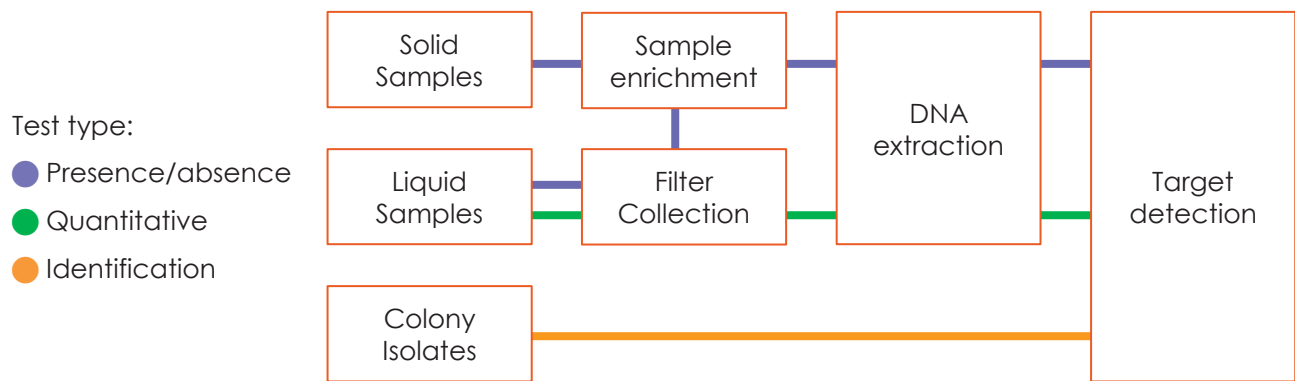
Real-Time PCR is often referred as an alternative method in food analysis. This is only partially true. More and more PCR based standard methods are appearing. For example, ISO 12869 rules how to detect *Legionella* and ISO 17919 specify how to detect *Clostridium botulinum* using PCR. Furthermore, since years, ISO 7218 endorsed the use of nucleic acid probes for the identification of colony isolates

PATHfinder portfolio

PATHfinder is the portfolio of products for Real-Time PCR detection of microbes relevant to food and water analysis

- **PATHfinder detection kits** developed on the basis of peer reviewed scientific literature and ISO technical specifications
- **PATHfinder SureXtra** heat inactivated bacterial pellets at known cell count for a punctual process control
- **PATHfinder DigiCount** innovative dPCR counted calibrators for best state-of-art accuracy in genomic unit quantification.

Possible workflow scheme for Real-Time PCR detection of microbes

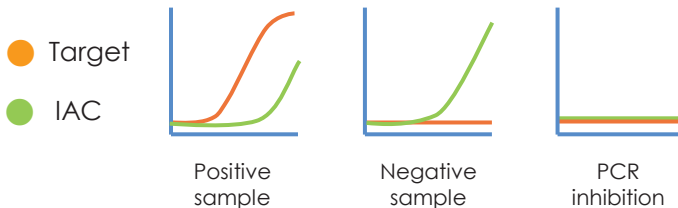


DNA extraction - Instructions for ordering

Kits	
EXD018K	ION Spin K DNA Extraction Kit (50 Ext.)
EXD009	FASTfood DNA Extraction Reagent (20 ml)
EXD199	Viral/Bacterial DNA/RNA Extraction kit (50 Ext.)
Ancillary reagents and equipment	
EXD010-P	DNA Extraction manifold
EXD011	Proteinase K (100 mg/vial)
EXD010	Proteinase K resuspension Buffer

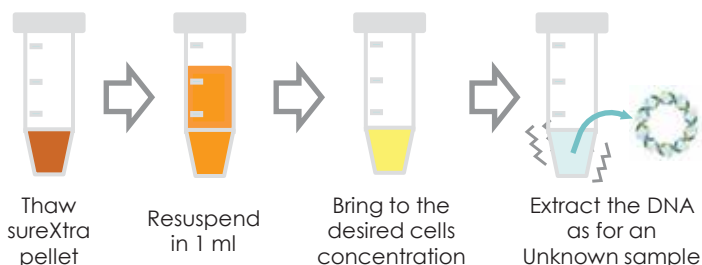
Controls to trust results

IAC demonstrates inhibitors or impurities, that interfere with DNA polymerase, are not lowering PCR reaction efficiency. IAC is co-amplified with the target sequence and detected on a separate optical channel.



PATHfinder kits include an Internal Amplification Control (IAC) respecting requirements of ISO 22119

IAC does not give any insight about the efficiency of extraction which can be deeply affected by the matrix. SureXtra can be dispersed in the enrichment broth containing a specific food/feed matrix to simulate the “real” extraction environment with all its interferences.



SureXtra address the indication of ISO 22174 to have a positive process control in a PCR session

Escherichia coli

Escherichia coli are facultatively anaerobic gram-negative bacteria that are naturally present in humans and animals as part of the intestinal microflora. Some strains can cause mild or lethal diseases and are referred as Shiga toxin-producing *E. coli* (VTEC or STEC). These strains are known to produce Shiga toxin 1 (Vtx1/Stx1) and Shiga toxin 2 (Vtx2/Stx2). In addition, another virulence-associated factors include the gene *eaeA*. Although serotype O157:H7 is the one that has been implicated most frequently in foodborne outbreaks worldwide, more than 100 STEC serotypes (e.g. members of the O26, O45, O103, O111, O121 and O145 serogroups) are known to cause human illnesses. Most human STEC infections have been traced to consumption of contaminated undercooked foods of bovine origin such as ground beef and **raw milk**. Other sources of infection include manure-contaminated vegetables, **raw milk**, **some dairy products**, **mayonnaise**, delicatessen food, lamb, venison, deer jerky, cured salami, contaminated water, cross-contamination, and direct contact.



Salmonella

Despite numerous legislative and educational initiatives to improve food hygiene, with a prevalence of over 90 million cases around the world per year. *Salmonella* is one of the most common foodborne pathogens. Because of its ubiquity in the environment and ability to colonize animals used in the human food chain, diseases caused by this bacterium are difficult to eradicate. Common contaminated foods associated to *Salmonella* infections in humans include poultry, poultry products, eggs and egg products, pork, beef, **milk and milk products**, seafood, fresh fruits, and vegetables.



Listeria

The genus *Listeria* contains six gram-positive species: *L. monocytogenes*, *L. innocua*, *L. seeligeri*, *L. welshimeri*, *L. ivanovii*, and *L. grayi*. Only the hemolytic species *L. monocytogenes*, *L. ivanovii*, and *L. seeligeri* are associated with human pathogenicity, although *L. monocytogenes* is the only species that has been involved in known foodborne outbreaks of listeriosis. *Listeria* can grow over a wide range of temperatures (-1.5 to 45-50°C) and pH ranges (4.3 to 9.6), survives freezing, and is relatively resistant to heat and acidic conditions. The primary source of food product contamination before release to consumers appears to be the processing environment including filling and packing equipment, conveyors, chill solutions, slicers, dicers, shredders and blenders. Furthermore, *Listeria* can form biofilms which allow the cells to survive stressing and sanitizing agents. Listeriosis has been associated with contaminated vegetables, **milk**, meat, poultry, fish and seafood products.



Clostridium botulinum

Foodborne botulism is a severe neurological disease affecting both humans and animals and is characterized by paralysis caused by a neurotoxin (BoNT). Seven serotypes (A to G) of *C. botulinum* have been classified however, the serotypes A, B, E, and F, account for almost all cases of human botulism. BoNT are produced also by strains of *C. baratii* (type E) and *C. butyricum* (type F). BoNTs, are produced during anaerobic growth of *C. botulinum* (but also by other species of *Clostridium*) and have an estimated ingested human toxic dose of 1 ng/kg body mass. A food may contain viable spores but not yet contain BoNT, because growth is required for toxin production. BoNT are heat labile and are rapidly inactivated by heating at 85°C or higher for at least 5 min). Most foods are likely to contain spores of *C. botulinum*; for example, it has been isolated from fish, meat, vegetables, fruits, honey.

mushrooms, **cheese, and nuts**. The heat-resistant spores are capable of surviving for up to 2 hrs at 100°C and can survive in foods that are incorrectly or minimally processed under anaerobic conditions. Hence, the most common cause of botulism is the consumption of home-canned foods prepared under inappropriate conditions. PATHfinder *C. botulinum* A, B, E, F qPCR kit described in ISO 17919 and validated in a comparative and collaborative trial.



Clostridium perfringens

Clostridium perfringens is a gram-positive, anaerobic, spore-forming bacterium classified into five toxinotypes (A, B, C, D, and E). Only a small fraction (1 to 5%) of all *C. perfringens* isolates (primarily type A), are capable of producing food poisoning. Bacterium exhibits growth at a temperature range of 15 to 50°C, with an optimal of 37 to 45°C, and with growth reported at temperatures as low as 6°C. The ability to form heat-resistant spores and the wide temperature range in which *C. perfringens* can grow are features that allow the bacteria to multiply and survive in different food.

This organism is commonly found in spices, and on the surfaces of vegetable products, as well as in other raw and processed foods. *C. perfringens* is also frequently found in meat and poultry products, generally through fecal contamination of carcasses, contamination from other ingredients, and/or post-processing contamination.



Campylobacter

Campylobacter spp. are gram-negative, non-spore-forming bacteria. Thermotolerant *Campylobacters* especially *C. jejuni* and *C. coli* are recognized worldwide as a leading cause of human infections. Humans can be infected by eating insufficiently cooked meat products (poultry, pork, and beef), by **drinking raw milk** and polluted water. *C. jejuni* has been isolated from a range of food sources, including poultry, red meat, and milk. The PATHfinder Thermotolerant *Campylobacter* Detection kit reflects the qPCR assay described in ISO 10272-1:2017/AMD and validated in a comparative and collaborative trial. The kit detects all food-borne thermotolerant *Campylobacters* (*C. jejuni*, *C. coli*, and *C. lari*).



Vibrio

The *Vibrio* genus encompasses Gram-negative bacteria species indigenous of marine and estuarine waters. Three species are considered important human foodborne pathogens - *V. cholerae* (Vc), *V. parahaemolyticus* (Vp) and *V. vulnificus* (Vv). *V. cholerae* is still a major health problem in parts of the developing world, contamination of fruits, vegetables and other foods usually occurs due to irrigation with polluted water or via an infected food handler. *V. parahaemolyticus* and *V. vulnificus* are adapted to salt or brackish water habitats and are halophilic to some degree, being unable to grow in the absence of sodium chloride, therefore they are most often associated with the consumption of raw, or undercooked,



Cronobacter

Cronobacter sakazakii is a gram-negative rod that causes severe illness in human infants, including necrotizing enterocolitis, septicemia, and meningitis. Most cases occur in infants less than 28 days old, and premature or low birth-weight infants are especially susceptible. Contaminated commercial infant formula powders have been implicated in several outbreaks and are suspected to be the main vehicle for *C. sakazakii* infections. The ability to adhere to surfaces, may explain the persistence of *C. sakazakii* on infant formula preparation equipment and in food-manufacturing environments. **Milk pasteurization** standards are more than sufficient to inactivate *C. sakazakii*, although it is remarkably resistant to osmotic stress and drying. PATHfinder *Cronobacter* Detection kit reflects the qPCR assay validated and described in FDA BAM Chapter 27.



Bacillus cereus

Bacillus cereus is a gram-positive spore-former often associated with two forms of human food poisoning, characterized by either diarrhea and abdominal distress or nausea and vomiting. As an important attribute, *B. cereus* can survive and grow in harsh conditions. This resistance could enable the bacterium to survive commercial food pasteurization and cooking at ambient pressure. *B. cereus* has been detected in heat-processed or cooked foods such as baking chocolate, baked bread, cooked rice, pasta, meats, **milk, and dairy products**, and its presence in spices, raw vegetables, salad dressing, and seafood has also been reported. Furthermore, an association between farinaceous foods and cereulide related food borne poisonings has been established. Standard cultural method ISO 7932 detects the so-called *B. cereus* group which includes also *B. anthracis*, *B. thuringiensis*, *B. weihenstephanensis*, *B. mycoides*. In its most recent release ISO 7932 includes also a qPCR test to identify colonies producing cereulide.



Staphylococcus aureus

S. aureus is a common cause of food borne poisoning worldwide which results from the ingestion of heat-stable enterotoxins produced in foods by enterotoxigenic *S. aureus*. Foods that are incriminated in staphylococcal food poisoning include beef, ham, pork, cooked sausage, chicken, turkey, egg products, tuna, canned lobster bisque, potato salad, canned mushrooms, bakery products such as cream-filled pastries, cream pies, and chocolate eclairs, sandwich fillings, spray-dried milk and other dairy products. These items may become contaminated during preparation, and toxin will form if these foods are subsequently mishandled prior to consumption.



Yersinia

The Yersiniaceae are gram-negative Enterobacteriaceae. *Y. enterocolitica* is a foodborne pathogen that can grow at refrigeration temperatures and survive repeated freezing and thawing. Although pork and pork products are the primary vehicles of *Y. enterocolitica* infection, drinking water and a variety of other foods, including milk, dairy products, beef, lamb, seafood, cheese, tofu, raw vegetables, fresh produce, and seafood, have also been implicated. PATHfinder Yersinia detection kit reflects the qPCR assays described in ISO 18867:2015 and validated in a comparative and collaborative trial.



Anisakis and Pseudoterranova

The human nematode infection most associated with seafood-borne disease is the anisakiasis. The species most implicated is *Anisakis simplex*, followed by *Pseudoterranova decipiens*. Humans can be infected by eating raw or undercooked fish or seafood that contains the third-stage larvae of *A. simplex*; cooking or freezing of all products from fish that are to be eaten raw are useful to reduce the transmission of the nematode. For freezing, a core temperature $\leq -20^{\circ}\text{C}$ for at least 24 h must be obtained prior to consumption. The PATHfinder Anisakis/Pseudoterranova is the first Real-Time PCR kit in the market for the detection of these nematodes. The kit was validated at the University La Sapienza in Rome (Dept. of Public Health and Infective Diseases) and accredited according to ISO 17025 at the Italian National Reference Centre for Anisakis (C.Re.N.A.).



Alicyclobacillus

Alicyclobacillus heat-resistant spores represent a severe quality risk for fruit juice industry. The bacteria mainly inhabit soil but can also infest fruit and crops; some species can cause deterioration of beverages by the production of guaiacol (*A. acidoterrestris*) or 2-methyltetrahydrothiophen-3-one (*A. acidocaldarius*) which have a peculiar off-flavor.

Importantly this fermentation occurs without CO₂ production (therefore, cans and briks shows no swelling) nor the bacteria cause discoloration of the product. Alicyclobacillus growth in beverages and tomato sauces can result in large-scale recalls with serious consequences for manufacturers. Screening of ingredients, especially fruit juices, for viable Alicyclobacillus spores has therefore become one of the key tools for preventing spoilage.



Brettanomyces

Dekkera bruxellensis (anamorph *Brettanomyces bruxellensis*) is a spoilage yeast especially important to the wine industry, where it has been shown to produce phenolic taints and to contribute to the production of biogenic amines in red wine. Traditional methods to identify spoilage yeasts in wine rely on culturing, in the case of *Dekkera* or *Brettanomyces* species, culturing usually involves selective media containing cycloheximide and typically takes 1 to 2 weeks to perform. Real-Time PCR offers significant advantages over traditional methods in terms of the speed by which assays are performed facilitating a better understanding of its origin in wineries and quickly assess the spoilage potential in wines during vinification.



Shigella

Dysentery caused by *Shigella* species is one of the common infectious diseases in developing countries and in travelers to tropical countries. Several food borne shigellosis outbreaks have been associated with the consumption of contaminated vegetable products, unpasteurized orange juice, salads, and dips. Furthermore, *Shigella* can contaminate, **milk**, poultry, and some dairy products.



Pseudomonas aeruginosa

Pseudomonas aeruginosa is a Gram negative commonly found in soil and water in a biofilm, attached to some surface or substrate. *P. aeruginosa* has very simple nutritional requirements. It is often observed "growing in distilled water", which is evidence of its minimal nutritional needs. *P. aeruginosa* is often found in potable water and in mineral water being the bacterium able to use IPA, PCB and PVC as a carbon source. *P. aeruginosa* is an opportunistic pathogen that can cause disease to skin, eye, ear, kidney and gastric tract especially in hospitalized patients and infants.



Giardia and Cryptosporidium

Pseudomonas aeruginosa is a Gram negative commonly found in soil and water in a biofilm, attached to some surface or substrate. *P. aeruginosa* has very simple nutritional requirements. It is often observed "growing in distilled water", which is evidence of its minimal nutritional needs. *P. aeruginosa* is often found in potable water and in mineral water being the bacterium able to use IPA, PCB and PVC as a carbon source. *P. aeruginosa* is an opportunistic pathogen that can cause disease to skin, eye, ear, kidney and gastric tract especially in hospitalized patients and infants.



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