



PATHfinder

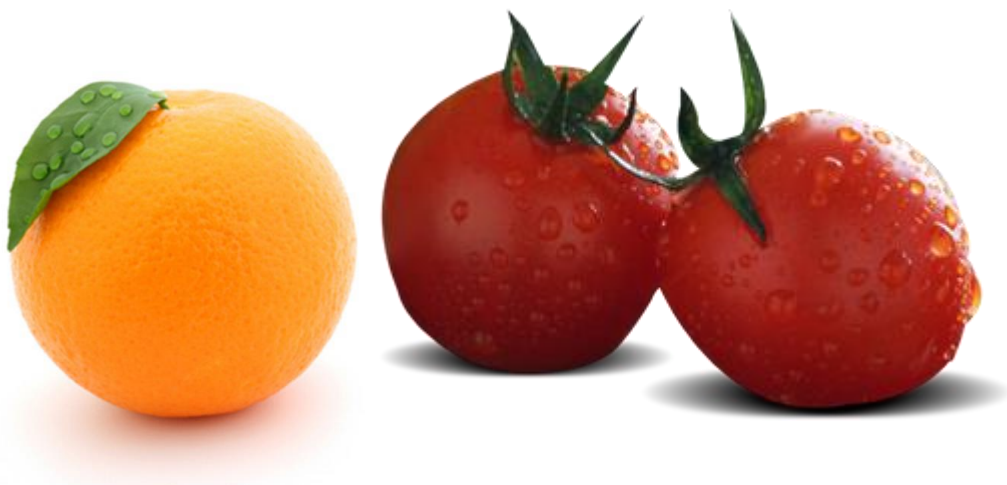
Bacteria and Parasites

Real-Time PCR detection and quantification

On a global scale, the food sector remains a major player in the lives and well being of the general human population, and considerable trust and confidence is invested in it by consumers. When food-borne related illnesses or epidemics hit the headlines, the public is understandably disturbed and claim for tighter regulations and more effective surveillance of food products.

The traditional culture-based detection of the bacteria is often laborious and time consuming and does not account for the rapid produce turnaround time of the modern food industry as very often the farm-to-fork time is 24 hrs.

A major advantage in the application of PCR-based methodologies lies in the fact that such assays are generally more specific, informative (e.g. immediate strain identification), sensitive, and faster than conventional microbiological assays. Although, a pre-enrichment step is 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.



Nucleic acids based analysis methods and their standardization are the core of **Generon** R&D and sales activities as we aim to be the most reliable partner in this field for food and beverage industry.

PATHfinder is the portfolio of products developed by **Generon** to exploit at best Real-Time PCR, and offers to the customer optimized and validated protocols to verify the presence of a wide range of pathogenic or spoilage micro-organisms by searching their DNA. The collection includes:

- **PATHfinder Assays** primers and probes developed on the basis of peer reviewed scientific articles after an in depth in-silico and in-vitro confirmation of the published data, blended with selected polymerases to ensure best-in-class detection.
- **PATHfinder SureXtra** heat inactivated bacterial pellets at known cell count ensuring a tight control of DNA extraction process efficacy;
- **PATHfinder DigiCount** innovative ddPCR counted calibrators for best state-of-art accuracy in genomic unit quantification.



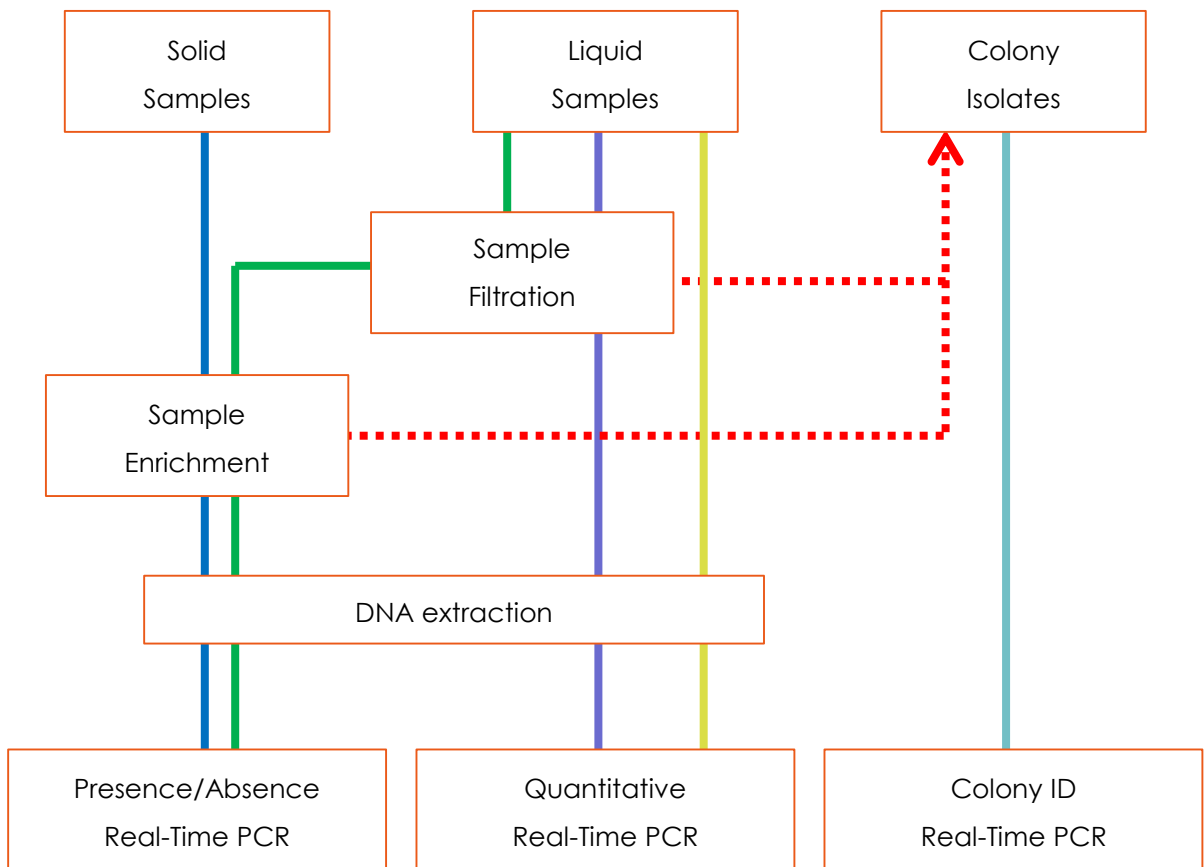
PATHfinder portfolio is complemented by kits for the extraction of nucleic acids from samples, products either developed by **Generon** or from selected technological partners.

ION-Liquid Force the quick extraction kit developed by **Generon** R&D team for the extraction of bacterial/fungal DNA from enrichment broth.

Ion Spin DNA Prep One-for-All developed by **Generon** R&D is specifically indicated when extracting from "difficult" matrices (e.g. chocolate samples). It is also the best solution when extracting Gram positive bacteria, spore-formers and parasites which need harsher extraction conditions to break cell walls.

Bio-Rad Aquadien™ kit specifically selected for the extraction of bacteria DNA from water samples of any kind.

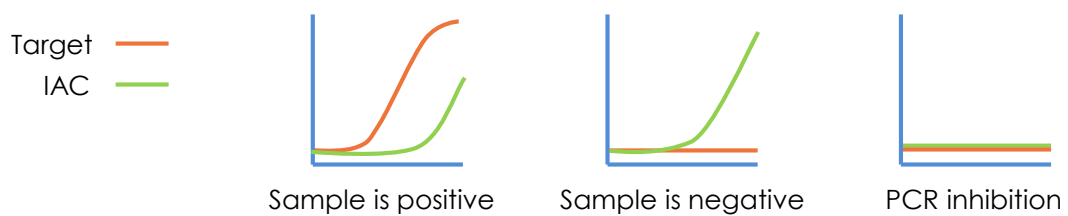
Figure 1 – Application of Real-Time PCR to microbiology



The most appropriate experimental approach to answer the analytical questions shall take into consideration the matrices tested, the characteristic of the bacteria, the expected concentration, and the technological limits. **Generon** assists you in finding the most convenient solution right from the start.

Generon kits include in each Real-Time PCR reaction an inhibition control, the so called **Internal Amplification Control (IAC)**, to demonstrate that PCR inhibitors or impurities that interfere with DNA polymerase are not lowering PCR reaction efficiency. IAC is a non-target DNA sequence present in the very same sample tube (as it is introduced through the assay mastermix), which is co-amplified simultaneously with the target sequence. In a PCR with an IAC, a control signal should always be produced even though there is no target sequence present.

Figure 2 – Internal Amplification Control



Although the IAC can reveal failure of a PCR reaction due to the presence of inhibitors it does not give any insight about the efficiency of the extraction reagents used. It has been demonstrated in many peer reviewed scientific magazines that not all the extraction kits/methods are equal in terms of DNA recovery and their performances are deeply affected by both the matrix and microbial target tested (for example there are huge differences for gram positive and negative bacteria). Some companies addressed this issue by providing exogenous DNA to spike the samples then being detected as if it was an IPC. The limit of this solution is that it does not account nor for the influence of the kind of bacteria tested nor for the effect of the matrix in bacterial lysis efficacy.

PATHfinder sureXtra is the answer developed by **Generon** to solve the problem of monitoring the efficiency of DNA extraction from microbial enrichment broths. **PATHfinder sureXtra** are heat inactivated bacterial pellets at known cells count, that can be dispersed in the enrichment broth containing a specific food/feed matrix to simulate the “real” extraction environment with all its interferences. This ensures a tight control of the whole Real-Time PCR experiment process from DNA extraction to target sequence detection.



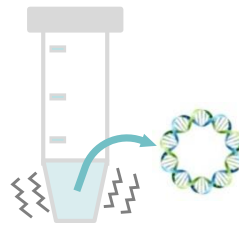
Thaw
sureXtra
pellet



Resuspend
in 1 ml



Bring to the
desired cells
concentration



Extract the DNA
as for an
Unknown sample



Amplify your
sureXtra DNA

PATHfinder sureXtra are manufactured under a strict quality control including: cell counting, sterility check (no colony growth after heat inactivation); DNA amplificability; in-lot reproducibility.

Salmonella

The genus *Salmonella* is divided into two species, *S. enterica* and *S. bongori*. To date, more than 2500 serovars of *S. enterica* have been identified, and most serovars have the potential to infect a wide variety of animal species and humans. *Salmonella* is a gram-negative mesophilic bacterium that can grow at refrigeration temperatures (4 to 10°C), with rapid growth between 25 and 43°C, although it is usually sensitive to temperatures above 55°C. *Salmonella* grows actively in the pH range 3.6 to 9.5 and optimally at nearly neutral pH values. Infections with *S. enterica* continue to be an important public health problem worldwide despite numerous legislative and educational initiatives to improve food hygiene. 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 with *Salmonella* infections in humans include poultry, poultry products, eggs and egg products, pork, beef, milk and milk products, seafood, fresh fruits, and vegetables.

PMB01A-50	PATHfinder <i>Salmonella</i> spp Assay	50 Reactions kit
PMB01X	PATHfinder <i>Salmonella</i> sureXtra	50 Vials



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* has the ability to 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.

PMB02A-50	PATHfinder <i>Listeria monocytogenes</i> Assay	50 Reactions kit
PMB03A-50	PATHfinder <i>Listeria</i> spp Assay	50 Reactions kit
PMB23A-50	PATHfinder <i>Listeria</i> Dual Triplex Assay	50 Reactions kit
PMB02X	PATHfinder <i>Listeria monocytogenes</i> sureXtra	50 Vials

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 are, however, able to cause disease ranging from mild to cholera-like diarrhea and may lead to potentially fatal complications such as hemolytic uremic syndrome (HUS).

On the basis of pathogenic features, the most important diarrheagenic *E. coli* are classified into at least six distinct groups: enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enterohemorrhagic *E. coli* (EHEC), enteroinvasive *E. coli* (EIEC), diffuse-adhering *E. coli* (DAEC), and enteroaggregative *E. coli* (EAEC).

EHEC, also referred to as Shiga toxin-producing *E. coli* (VTEC or STEC), are responsible for serious human infections such as uncomplicated diarrhea, hemorrhagic colitis, and HUS. These strains are known to produce Shiga toxin 1 (Vtx1/Stx1) and Shiga toxin 2 (Vtx2/Stx2). In addition, other virulence-associated factors include a pO157 plasmid, which encodes hemolysin and the enterocyte effacement locus containing the intimin 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, including HUS. 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.

PMB14A-50	PATHfinder <i>E.coli</i> (+ <i>Shigella</i> spp.) Assay	50 Reactions kit
PMB10A-50	PATHfinder <i>E.coli</i> VTEC stx1-stx2 & <i>eae</i> -IAC Duplex assays	50 Reactions kit
PMB10X-M	PATHfinder <i>E.coli</i> VTEC (stx1+stx2+ <i>eae</i>) sureXtra	50 Vial
PMB10A-V1-50	PATHfinder <i>E.coli</i> VTEC (stx1) Assay	50 Reactions kit
PMB10X-V1	PATHfinder <i>E.coli</i> VTEC (stx1) sureXtra	50 Vial
PMB10A-V2-50	PATHfinder <i>E.coli</i> VTEC (stx2) Assay	50 Reactions kit
PMB10X-V2	PATHfinder <i>E.coli</i> VTEC (stx2) sureXtra	50 Vial
PMB10A-VE-50	PATHfinder <i>E.coli</i> VTEC (<i>eae</i>) Assay	50 Reactions kit
PMB10X-VE	PATHfinder <i>E.coli</i> VTEC (<i>eae</i>) sureXtra	50 Vial
PMB10A-P103	PATHfinder <i>E.coli</i> O:103 wzx Assay	50 Reactions kit
PMB10X-P103	PATHfinder <i>E.coli</i> O:103 sureXtra	50 Vial
PMB10A-P104	PATHfinder <i>E.coli</i> O:104 wzx Assay	50 Reactions kit
PMB10X-P104	PATHfinder <i>E.coli</i> O:104 sureXtra	50 Vial
PMB10A-P111	PATHfinder <i>E.coli</i> O:111 wbdL Assay	50 Reactions kit
PMB10X-P111	PATHfinder <i>E.coli</i> O:111 sureXtra	50 Vial
PMB10A-P121	PATHfinder <i>E.coli</i> O:121 wzx Assay	50 Reactions kit
PMB10X-P121	PATHfinder <i>E.coli</i> O:121 sureXtra	50 Vial
PMB10A-P145	PATHfinder <i>E.coli</i> O:145 ihp1 Assay	50 Reactions kit
PMB10X-P145	PATHfinder <i>E.coli</i> O:145 sureXtra	50 Vial
PMB10A-P157	PATHfinder <i>E.coli</i> O:157 rfbE Assay	50 Reactions kit
PMB10X-P157	PATHfinder <i>E.coli</i> O:157 sureXtra	50 Vial
PMB10A-P26	PATHfinder <i>E.coli</i> O:26 wzx Assay	50 Reactions kit
PMB10X-P26	PATHfinder <i>E.coli</i> O:26 sureXtra	50 Vial
PMB10A-P45	PATHfinder <i>E.coli</i> O:45 wzx Assay	50 Reactions kit
PMB10X-P45	PATHfinder <i>E.coli</i> O:45 sureXtra	50 Vial
PMB10A-H4	PATHfinder <i>E.coli</i> H:4 fliC Assay	50 Reactions kit
PMB10A-H7	PATHfinder <i>E.coli</i> H:7 fliC Assay	50 Reactions kit

Campylobacter

Campylobacter spp. are gram-negative, non-spore-forming bacteria. Thermotolerant *Campylobacters* especially *Campylobacter jejuni* and *C. coli* are recognized worldwide as a leading cause of human infections. *Campylobacters* are unable to grow below 30°C, below pH 4.9, or in a 2% concentration of sodium chloride. Furthermore, these bacteria are very sensitive to desiccation and do not survive well on dry surfaces. 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. Almost all parts of poultry carcasses, whether fresh, chilled, or frozen, are frequently contaminated with *C. jejuni*. The primary reservoir for *C. coli* is pig, whereas *C. coli* constitute only a minimal percentage of the *Campylobacter* isolates from chicken and cattle.

The PATHfinder Thermotolerant *Campylobacter* Detection and Quantification Assay was developed following a public Real-Time PCR protocol (Hoorfar et al. 2006) validated in comparative and collaborative trials, based on recommendations from the Nordic system for validation of alternative microbiological methods (NordVal). The assay detects all food-borne thermotolerant *Campylobacters* (*C. jejuni*, *C. coli*, and *C. lari*).

PMB04A-50

PATHfinder Thermotolerant *Campylobacters* (*jejunii/coli/lari*) Assay

50 Reactions kit



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.

PMB06A-50

PATHfinder *Clostridium perfringens* Assay

50 Reactions kit

Vibrio

The *Vibrio* genus encompasses Gram-negative bacteria species indigenous of marine and estuarine waters. Three species are considered to be important human foodborne pathogens - *V. cholerae*, *V. parahaemolyticus* and *V. vulnificus*. *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, shellfish.

PMB13A-C-50	PATHfinder <i>Vibrio cholerae</i> Assay	50 Reactions kit
PMB13A-P-50	PATHfinder <i>Vibrio parahaemolyticus</i> Assay	50 Reactions kit
PMB17A-50	PATHfinder <i>Vibrio parahaemolyticus</i> /tdh/trh Triplex Assay	50 Reactions kit
PMB13A-V-50	PATHfinder <i>Vibrio vulnificus</i> Assay	50 Reactions kit
PMB13A-M-50	PATHfinder <i>Vibrio</i> (Vp - Vv - Vc) Multiplex Assay	50 Reactions kit

Quadruplex reactions kit for the detection of *Vibrio parahaemolyticus* (FAM), *Vibrio vulnificus* (Texas Red), *Vibrio cholerae* (Cy5) and Internal Amplification Control (HEX).



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 at low temperatures. The organism has also been reported to survive and grow in the pH range 4.3 to 9.3. The endospores allow the bacterium to enhance its resistance to wet heat, dry heat, radiation, desiccation, extreme pH, chemicals, enzymes, and high pressure. 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 foodborne poisonings has been established.

PMB09A-50	PATHfinder <i>Bacillus cereus</i> Assay	50 Reactions kit
PMB09X	PATHfinder <i>Bacillus cereus</i> sureXtra	50 Vials

Cronobacter sakazakii

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, probably due to impaired immune response compared to full-term infants and adults. 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, including rubber, silicon, polycarbonate, and stainless steel, 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.

PMB08A-50	PATHfinder <i>Cronobacter sakazakii</i> Assay	50 Reactions kit
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Staphylococcus aureus

S. aureus is a common cause of foodborne poisoning worldwide which results from the ingestion of heat-stable enterotoxins produced in foods by enterotoxigenic *S. aureus*. It is a gram-positive, it grows from 7 to 47.8°C, and in a pH range 4.5-9.3; the bacterium is also highly salt tolerant, resistant to nitrites, and capable of growth at low a_w values.

S. aureus is a ubiquitous bacterium, being both a human and a zoonotic commensal. 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.

PMB11A-50	PATHfinder <i>Staphylococcus aureus</i> Assay	50 Reactions kit
PMB11X	PATHfinder <i>Staphylococcus aureus</i> sureXtra	50 Vials

Clostridium botulinum

Clostridium botulinum is an anaerobic, gram-positive, spore-forming rod that causes botulism. 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. BoNTs are produced also by strains of *Clostridium baratii* (type E) and *Clostridium 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).

Foodborne botulism is a rare disease that results from the consumption of food contaminated with preformed BoNT or after botulinum toxin producing clostridia (BTPC) colonization of the gastrointestinal tract and secretion of the neurotoxin. Symptoms generally appear 18 to 36 h following ingestion of contaminated food, and persons with these symptoms require immediate specialized treatment.

The majority of 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 as a consequence the most common cause of botulism is the consumption of home-canned foods prepared under inappropriate conditions.

PMB44A-50	PATHfinder <i>Clostridium botulinum</i> (BTPC) Assay	50 Reactions kit
PMB44X	PATHfinder <i>Clostridium botulinum</i> sureXtra	50 Vials
PMB67A-50	PATHfinder <i>Clostridium botulinum</i> Assay (according to ISO-17919)	50 Reactions kit
PMB67X	PATHfinder <i>Clostridium botulinum</i> sureXtra (amplicon according to ISO-17919)	50 Vials
PMB67A-ID	PATHfinder <i>Clostridium botulinum</i> ID Assay (according to ISO-17919)	4 x 50 Reactions kit



Shigella

Dysentery caused by *Shigella* species is one of the common infectious diseases in developing countries and in travelers to tropical countries. *Shigella* are gram-negative bacteria. The genus is divided into four species or serotypes: *S. dysenteriae*, *S. flexneri*, *S. boydii*, and *S. sonnei*, representing subgroups A, B, C, and D, respectively. *Shigella* spp. can survive at a low pH for several hours and in acidic foods for extended periods. Several foodborne 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.

PMB61A-50	PATHfinder <i>Shigella</i> spp Assay	50 Reactions kit
PMB58A-50	PATHfinder <i>Shigella</i> / <i>E.coli</i> Discrimination Assay	50 Reactions kit



Yersinia

The yersiniae are gram-negative bacteria that belong to the family Enterobacteriaceae. *Y. enterocolitica* is a foodborne pathogen that has the ability to grow at refrigeration temperatures and survive repeated freezing and thawing, which is a concern for food safety. This bacterium has a temperature range for growth usually between 4 and 42°C. Although pork and pork products are considered to be 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.

PMB07A-50	PATHfinder <i>Yersinia enterocolitica</i> Assay	50 Reactions kit
PMB60A-50	PATHfinder <i>Yersinia pseudotuberculosis</i> Assay	50 Reactions kit

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 also 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. It's noteworthy also the resistance of *P. aeruginosa* to many antibiotics and disinfectants.

PMB05A-50

PATHfinder *Pseudomonas aeruginosa* Assay

50 Reactions kit

Cryptosporidium

Cryptosporidium is a protozoan parasite that causes waterborne outbreaks. *C. parvum* and *C. hominis* are the most prevalent species causing disease in humans, infection with this species is also common in livestock animals, especially cattle and sheep, although pigs, goats, and horses. The transmissible stage of *C. parvum* is the oocyst, which when carried in the feces can contaminate surface water. This protozoan parasite is a serious issue for the water and fresh produce industry, since contamination via contaminated irrigation waters may occur. Outbreaks of cryptosporidiosis have been associated with different foods, including inadequately pasteurized milk and raw milk, apple cider, basil, green onions, cold chicken salad, raw sausages, and tripe.

PMB42A-50

PATHfinder *Cryptosporidium* Assay

50 Reactions kit

Giardia

The common protozoan *Giardia lamblia* is a flagellate protozoan with trophic (feeding) and cystic (resting) stages. *Giardia* is an important human pathogen, possibly the most common parasite infection of humans worldwide. Infection can result after ingestion of at least 10 to 25 cysts through contaminated water or food, or by person-to-person contact. Cysts can be found in sewage effluents, in surface waters, and in some potable water supplies. Foodborne transmission can occur with ingestion of raw or undercooked foods. Food-associated outbreak cases have been associated with consumption of cysts of *Giardia*-contaminated salmon and cream cheese dip, cold noodle salad, and fruit salad.

PMB41A-50

PATHfinder *Giardia* Assay

50 Reactions kit

Legionella

Legionella is a pathogenic Gram negative bacterium, including species that cause legionellosis or Legionnaires' disease. *Legionella* is common in many environments, with at least 50 species and 70 serogroups identified. *L. pneumophila* is the most common pathogenic species, accounting for more than 90% of legionellosis cases and serogroup 1 is the predominant serotype (84.2%).

Legionella transmission is via aerosols containing the bacteria. Common sources include cooling towers, swimming pools, domestic hot-water systems, fountains, and similar disseminators that tap into a public water supply. Under appropriate conditions, *L. pneumophila* can also survive for long periods as a free organism in low-nutrient environments. Regular monitoring of potentially contaminated water sources is essential to prevent legionellosis outbreaks.

PMB15A-50	PATHfinder <i>Legionella pneumophila</i> Assay	50 Reactions kit
PMB15D-SG1-50	PATHfinder <i>Legionella pneumophila</i> Serogroup 1 Assay	50 Reactions kit
PMB15D-SG-50	PATHfinder <i>Legionella pneumophila</i> ID Assay [#]	50 Reactions kit
PMB15R	PATHfinder <i>Legionella pneumophila</i> DigiCount	>120 000 GUs in 120 µl
PMB16A-50	PATHfinder <i>Legionella</i> Dual Assay [§]	50 Reactions kit
PMB16R	PATHfinder <i>Legionella pneumophila</i> SG1 DigiCount	>120 000 GUs in 120 µl
PMB18A-50	PATHfinder <i>Legionella</i> spp. Assay	50 Reactions kit
PMB18R	PATHfinder <i>Legionella</i> spp. DigiCount	>120 000 GUs in 120 µl

[#] Duplex reactions kit for the identification of *Legionella* colony isolates. *Legionella pneumophila* SG1 isolates will result (FAM+/HEX+) *Legionella pneumophila* SG2-16 (FAM-/HEX+)

[§] Duplex reactions kit for the detection/identification of *Legionella pneumophila* (FAM+/HEX+) and other *Legionella* spp. (FAM-/HEX+)



Anisakis

The human nematode infection most commonly associated with seafood-borne disease is the anisakiasis. The species most commonly implicated is *Anisakis simplex*, followed by *Pseudoterranova decipiens*. The life cycle of these nematodes involves larval stages with several intermediary hosts and the adult stage, during which the worm parasitizes the stomachs of marine animals. 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 $<-20^{\circ}\text{C}$ for at least 24 h has to be obtained prior to consumption.

The PATHfinder *Anisakis/Pseudoterranova* Assay is the first kit Real-Time PCR 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).

PMB66A-50

PATHfinder *Anisakis/Pseudoterranova* Assay

50 Reactions



Alicyclobacillus

In the current competitive environment, safety in the Food and Beverage industry is a question of company credibility and stability. The definition of food quality may differ for processors and consumers, but generally organoleptic characteristics, such as flavor, odor and appearance, as well as extended shelf-life are among the most important of its attributes. Spoilage of food by microbial contamination may occur at any point during production, altering any one or all of these characteristics renders the product unusable and results in financial damage for the food manufacturer.

The fruit juice industry faces significant product spoilage challenges. Pasteurization is an effective microbial control method for common yeast, mold and lactic acid bacteria that tolerate or thrive in acidic fruit juices (pH < 4) due to their low heat resistance. Additionally, spores are common contaminants. However, most bacterial spores fail to germinate at pH levels below 4.1, thus product spoilage is limited to a small number of organisms able to grow at pH 3.8. For the past few years consumers are asking and purchasing "natural" products over those containing chemically synthesised preservatives. Concurrently, the emergence of novel spoilage heat-resistant spores forming microbes such as *Alicyclobacillus spp.*, have further complicated product quality and safety challenges for the fruit juice industry.

Alicyclobacillus genus is characterized by gram positivity, moderately thermophilic, acidophilic, strictly aerobic, endospore-forming bacilli. The bacteria mainly inhabit soil, but can also infest fruit and crops and some species can cause deterioration of beverages by the production of guaiacol (*A. acidoterrestis*) or 2-methyltetrahydrothiophen-3-one (*A. acidocaldarius*) which have a peculiar off-flavor. Importantly this fermentation occurs without CO₂ production (therefore cans and Tetra-briks shows no swelling) nor the bacteria cause discoloration of the product. *Alicyclobacillus* growth in beverages and tomato sauces can result in large-scale spoilage incidents with serious consequences for manufacturers. This means that control of the organism in the manufacturing process is essential, but the heat resistance of the spores (92°C for 10 seconds) dictates that it is not easy to achieve. Screening of ingredients, especially fruit juices, for viable *Alicyclobacillus* spores has therefore become one of the key tools for preventing spoilage.

PMB48A-AC-50	PATHfinder <i>Alicyclobacillus acidocaldarius</i> Assay	50 Reactions
PMB48X-AC	PATHfinder <i>Alicyclobacillus acidocaldarius</i> sureXtra	50 Vial
PMB48A-AT-50	PATHfinder <i>Alicyclobacillus acidoterrestis</i> Assay	50 Reactions
PMB48X-AT	PATHfinder <i>Alicyclobacillus acidoterrestis</i> sureXtra	50 Vial
PMB48A-M-50	PATHfinder <i>Alicyclobacillus</i> Multiplex Assay*	50 Reactions
PMB48A-G-50	PATHfinder <i>Alicyclobacillus</i> _species guaiacol producing + <i>Alicyclobacillus acidocaldarius</i> Multiplex Assay	50 + 50 reactions

* Duplex reactions kit for the detection of *A. acidoterrestis* (FAM), *A. acidocaldarius* (HEX), Internal Amplification Control (Texas Red).



Brettanomyces

Dekkera bruxellensis (anamorph *Brettanomyces bruxellensis*) is a spoilage yeast in the beverage industry that is found in soft drinks and alcoholic beverages. It is especially important to the wine industry, where it has been shown to produce phenolic taints (4-ethylphenol [4EP] and 4-ethylguaiacol) 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 and the ability to quantify the target microbial population. At Generon we developed a kit for the detection and quantification of *D. bruxellensis* in wine. This method will enable a comprehensive determination of *D. bruxellensis* in wine, thereby facilitating a better understanding of its origin in wineries and quickly assess the spoilage potential of *D. bruxellensis* in various juices and wines during vinification.

PMB65A-50

PATHfinder *Brettanomyces bruxellensis/anomalus* Assay

50 Reactions kit





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