SureFast® Microbiology

Qualitative detection of common foodborne pathogens with qPCR

Simple and straightforward: 10 min lysis protocol

Flexible: open platform

Broad portfolio

More information:

https://r-b.io/mibiwater

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Relevant food pathogens

Various disease-causing organisms can contaminate foods – more than 250 foodborne diseases have been identified. Most of them are infections, caused by a diversity of bacteria, viruses and parasites. But also harmful toxins and chemicals can contaminate foods and cause foodborne illness[1].

The pathogens can be categorized into three groups[2]:

- **Infectious invasive pathogens**
  - Enter the body and invade or colonize host
  - Typically > 8 hr for onset of illness
  - E.g. *Salmonella, Listeria monocytogenes, Campylobacter* and enteroinvasive *Escherichia coli*
- **Toxigenic pathogens:**
  - Produce enterotoxins in the food
  - Illness is due to toxic contamination of the food
  - Onset of illness can be as little as 1 hr, as the toxin is pre-formed in the food and consumed
  - E.g. *Staphylococcus aureus*, *Bacillus cereus* and *Clostridium botulinum*
- **Toxico-infectious pathogens:**
  - Cause illness through a combination of colonizing the host (infection) and releasing potent toxins (intoxication) in the gut
  - E.g. enterotoxigenic and enterohemorrhagic *E. coli* and *Clostridium perfringens*

Salmonella spp.

High impact, wide distribution, zero tolerance

Listeria monocytogenes

Critical for ready-to-use foods

STEC (verocytoxin-producing *E. coli)*

Severe human illness risk

[1] Centers for Disease Control and Prevention (CDC), Food Safety Homepage FDA "Bad Bug Book" http://vm.cfsan.fda.gov

[2] Behlinhg J., Kornacki L. (ed.), Principles of Microbiological Troubleshooting in the Industrial 5 Food Processing Environment, Food Microbiology and Food Safety, Chapter 2: Selected Pathogens of Concern to Industrial Food Processors: Infectious, Toxigenic,Toxico-Infectious, Selected Emerging Pathogenic Bacteria DOI 10.1007/978-1-4419-5518-0_2

Microbiological criteria for foodstuffs

Commission Regulation (EC) No. 2073/2005

This regulation for food business operators establishes strict microbiological limits for specific microorganisms and their toxins across various food categories in the European Union. It requires Food Business Operators (FBOs) to perform routine testing, implement Hazard Analysis and Critical Control Point (HACCP) principles, and take corrective actions if safety limits are exceeded.

Two primary categories of microbiological criteria are described:

- **Food safety criteria:** Used to determine whether a food batch or product is safe to place on the market. If these limits are exceeded, the product must be withdrawn or recalled.
- **Process hygiene criteria:** Used to verify that the manufacturing processes are working effectively and that hygiene standards are maintained during production.

Key Requirements for Food Businesses – Compliance & Testing: FBOs must test their products against defined microbiological limits (such as *Salmonella*, *Listeria monocytogenes*, and *E. coli*) during validation and verification procedures.

- **Corrective actions:** If criteria are not met, operators must take predetermined corrective actions, including improving hygiene, adjusting processes, or removing the affected food from the market.
- **Shelf-life studies:** Businesses must conduct studies to ensure that ready-to-eat (RTE) foods comply with the regulation's limits (especially for Listeria monocytogenes) throughout the entire shelf-life of the product.
- **Labelling:** Specific rules apply to minced meat and meat preparations (excluding poultry) that require thorough cooking, which must be clearly labeled for the consumer.

Why test with real-time PCR

Fast results
Results in hours, not days

Early risk detection
Identify contamination before it becomes a problem

High sensitivity & specificity
Reliable detection

Improved efficiency
Optimized production flow

Cost savings
Reduce recalls, rework and downtime

Exemplary laboratory workflow of a typical real-time PCR pathogen detection assay

Time requirement: 16 - 28 hrs

1 Sample enrichment

- 25 g of sample added to 225 ml of enrichment broth
- Overnight incubation

Time requirement: 0.5 - 1 hrs

2 DNA extraction – manual or automated

- Thermal lysis
- If applicable – DNA purification step

Time requirement: ~ 20 min

3 Real-time PCR set-up

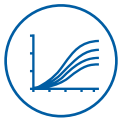
- Prepare master mix
- Add extracted DNA

Time requirement: ~ 1 hr

4 Real-time PCR analysis

All SureFast® kits can be used with common real-time PCR devices (FAM/HEX/ROX/Cy5)

- Standardized thermal profiles



Overview food source reference methods

Bacteria	Possible food source (examples)	Analytical reference method	Enrichment*
Gram-negative bacteria			
1 *Salmonella*	**Poultry & meat:** Raw or undercooked chicken, turkey, beef, and pork **Eggs:** Raw or lightly cooked eggs, or foods containing them **Dairy:** Unpasteurized (raw) milk and cheese. **Produce:** Contaminated fruits and vegetables (e.g., pre-packaged salads, sprouts, and cantaloupes) **Processed/dry foods:** Chocolate spreads, nut butters, and flour	ISO 10135: 2013-05 (PCR) ISO 6579-1: 2017-07-26	BPW 18 ± 2 h at 34 °C - 38 °C
2 *Campylobacter* spp.	Improperly handled or undercooked poultry products, unpasteurized ("raw") milk and cheeses made from unpasteurized milk, shellfish	ISO 10272-1: 2023-07	Bolton broth 4 - 6 h at 37 °C microaerobic, followed by 44 ± 4 h at 41.5 °C
Vibrio cholerae / parahaemolyticus	Raw or undercooked seafood/brackish water and seawater	ISO 21872-1: 2023-06	ASPW with 2 % NaCl **1. Step:** 6 ± 1 h at 41.5 ± 1 °C (fresh food) or 37 ± 1 °C (dried, frozen or salted food) **2. Step:** 10 ml from first step in 90 mL preheated ASPW, 18 ± 1 h at 41.5 ± 1 °C
Vibrio vulnificus	Particularly oysters/brackish water and seawater	ISO 21872-1: 2023-06	ASPW with 2 % NaCl **1. Step:** 6 ± 1 h at 37 ± 1 °C **2. Step:** 10 mL from 1. in 90 mL preheated ASPW, 18 ± 1 h at 37 ± 1 °C
Yersinia enterocolitica	Raw or undercooked seafood	ISO/TS 18867: 2016-01 (PCR) ISO 10273: 2017-08	Peptone-Sorbitol-Bile-Broth 48 h at 25 ± 1 °C
Cronobacter spp.	Particularly oysters, infant formula	ISO 22964: 2017-08	BPW 18 h ± 2 h at 34 °C to 38 °C
Pathogenic *Escherichia coli*			
E. coli	Raw or undercooked ground beef and beef products, raw milk, various water sources, fresh produce like lettuce, spinach, sprouts		BPW 16 - 24 h at 37 °C
E. coli – Enterohemorrhagic (*E. coli* O157:H7 and others)	Raw or undercooked ground beef and beef products, raw milk, various water, fresh produce like lettuce, spinach, sprouts	ISO/TS 13136: 2012 (PCR)	mTSB or BPW 18 - 24 h at 37 ± 1 °C
Gram-positive bacteria			
3 *Listeria monocytogenes/ Listeria* spp.	Raw milk, inadequately pasteurized milk, chocolate milk, cheeses, ice cream, raw vegetables, raw poultry and meats, fermented raw-meat sausages, deli meats, raw or smoked fish and other seafood	ISO 11290-1/2: 2017-09	**Step 1:** Half Fraser broth 24 - 26 h at 30 ± 1 °C **2. Step:** Transfer 0.1 mL of culture in 10 mL of Fraser Broth 24 ± 2 h at 37 ± 1 °C
4 *Staphylococcus aureus*	**Milk and dairy products:** Raw milk, soft cheese, cream, and ice cream **Meat and poultry:** Cooked ham, poultry, pâtés, and ready-to-eat meat dishes **Egg products:** Dishes containing raw or undercooked eggs **Deli meats and salads:** Potato or pasta salads with mayonnaise, as well as cream-based dishes and cakes	ISO 6888-3/AC: 2005-09-07	BPW 16 - 24 h at 37 °C
Bacillus cereus	A variety of foods, particularly (fried) rice and leftovers, as well as sauces, soups, and other prepared foods that have sat out too long at room temperature	ISO 7932: 2020-11 ISO 21871: 2007-04-18	TSB-P 30 °C to 32 °C for 24 to 28 h Glucose Tryptone Polymyxin (GTP) broth 48 h at 30 °C

Overview food source reference methods

Bacteria	Possible food source (examples)	Analytical reference method	Enrichment*
Gram-negative bacteria			
5 *Clostridium botulinum*	**Improperly canned and preserved foods:** Beets, corn, spinach, mushrooms and improperly preserved fruits or meats. Garlic in oil, fermented, salted, or smoked seafood **Honey:** Both raw and processed honey can contain spores	ISO/TS 17919: 2014-03	TPGY **1. Step:** 24 ± 2 h at 30 ± 1 °C (real-time PCR test, if result negative: Step 2) **2. Step:** 48 ± 2 h at 30 ± 1 °C
6 *Clostridium perfringens*	**Poultry:** Turkey and chicken, especially large roasts or stews **Meats:** Beef, pork, and rolled meats **Gravies and sauces:** Particularly those made from meat drippings **Soups and casseroles:** Thick, hearty dishes that take a long time to cool or heat evenly	ISO 15213-2: 2023	TPGY 48 h at 37 °C
Virus			
Hepatitis A	Raw or undercooked shellfish from contaminated waters, raw produce, contaminated drinking water, uncooked foods, and cooked foods that are not reheated after contact with an infected food handler	DIN EN ISO 15216-1: 2021-107	n.a.
Norovirus	Produce, shellfish, ready-to-eat foods touched by infected food workers (salads, sandwiches, ice, cookies, fruit), any other foods contaminated with particles of vomit or feces from an infected person	DIN EN ISO 15216-1: 2021-10	n.a.

1 *Salmonella*

If a hen's reproductive organs are infected, the yolk of an egg can be contaminated in the hen before it is even laid.

2 *Campylobacter* spp.

Foodborne *Campylobacter* infections have a characteristic seasonality with a distinct increase of cases in the summer and early autumn.

3 *Listeria monocytogenes*/*Listeria* spp.

Listeria have the ability to survive, multiply and persist under harsh conditions. They are for instance resistant to freezing, can grow in the presence of 10 % salt, survive in concentrated brine solutions, and are able to grow at 1 - 45 °C (optimum at 35 - 37 °C).

4 *Staphylococcus aureus*

S. aureus is a common bacterial pathogen causing staphylococcal food poisoning (SFP).
SFP is not caused by consumption of live bacterial cells but rather picked up from ingesting one or more heatstable pre-formed staphylococcal enterotoxins (SEs) in foods contaminated with e.g. *S. aureus*. This so called intoxication does not need the bacterial growth in the host. SEs are unique, because they survive heating including canning.

5 *Clostridium botulinum*

Botulism is categorized into following types:
- Foodborne
- Wound
- Infant
- Inhalation

There are 7 forms of botulinum toxin: types A - G. Types A, B, E and rarely F cause human botulism.

6 *Clostridium perfringens*

Spores of *C. perfringens* are able to survive normal cooking and pasteurization temperatures, after which they can then germinate and multiply during slow cooling, or storage at room temperatures and/or during inadequate re-warming. Sometimes it is referred to as the "food service germ", because foods served and left for long periods at room temperature have been linked with this illness.

* The enrichment conditions are only guidelines and may vary depending on the tested food matrices.
Please also consider national laws and regulations.

BPW – Buffered peptone water
ASPW – Alkaline Saline Peptone Water
mTSB – Modified Tryptone Soya Broth
TPGY – Tryptone Peptone Glucose Yeast Broth
TSB-P – Trypticase Soy-Polymyxin Broth
n.a. – not applicable

Kit overview

Available DNA extraction kits

Product	Pathogen type	Description	Steps	Hands on time/10 samples
SureFast® Speed PREP (Art. No. F1054)	Gram-negative bacteria & parasites	Fast & easy DNA isolation without purification For 100 preparations	2	~ 20 min
SureFast® PREP Bacteria (Art. No. F1021)	Bacteria	Complex matrices with strong inhibitors (enrichments, avulsions and swabs) For 100 preparations	7	~ 45 min
SureFast® PREP DNA/ RNA Virus (Art. No. F1051)	Viruses	Cell culture supernatants, foods (e.g. wash up fluids from fruits, salads etc.), filters from water samples For 100 preparations	7	~ 45 min
SureFast® Mag PREP Pathogens (Art. No. F1062)	Viruses & bacteria	Automated nucleic acid preparation in combination with TANBead Maelstrom™ Switch 8 (ZSWITCH) For 96 preparations	Walk away solution	~ 5 min

Bacteria

Product	Description*	No. of tests/amount	Art. No.
Salmonella	**Qualitative real-time PCR – food related pathogens**		
SureFast® Salmonella PLUS	FAM: *Salmonella* spp.	100 reactions	F5111
SureFast® Salmonella ONE AOAC PTM 081803; MicroVal 2014LR43	FAM: *Salmonella* spp.	100 DNA preparations & 100 reactions	F5211
SureFast® Salmonella species/ Enteritidis/Typhimurium 4plex	FAM: *Salmonella* spp. ROX: *Salmonella* Enteritidis Cy5: *Salmonella* Typhimurium	100 reactions	F5166
Escherichia coli			
SureFast® Escherichia coli PLUS	FAM: *Escherichia coli*	100 reactions	F5157
SureFast® EHEC/EPEC 4plex (stx1, stx 2, ipaH, E.coli/Shigella)	FAM: *stx1* (subtype a-d) & *stx2* (subtype a-g) ROX: *ipaH* (*E. coli* & *Shigella* spp.) Cy5: *eae*	100 reactions	F5128
SureFast® STEC Screening PLUS	FAM: *stx1/stx2*	100 reactions	F5105
SureFast® STEC 4plex ONE (0157, stx1, stx2, eae) AOAC PTM 052602	FAM: *E. coli stx1* (subtype a-d) & *stx2* (subtype a-g) ROX: *E. coli* O157 Cy5: *eae*	100 reactions	F5265
SureFast® Escherichia coli Serotype I 4plex AOAC PTM 052602**	FAM: O121 ROX: O103 Cy5: O26	100 reactions	F5167
SureFast® Escherichia coli Serotype II 4plex AOAC PTM 052602**	FAM: O45 ROX: O111 Cy5: O145	100 reactions	F5168
Listeria			
SureFast® Listeria Screening PLUS	FAM: *Listeria* spp.	100 reactions	F5117
SureFast® Listeria 3plex ONE OMA 2025.04 "First Action"; MicroVal 2023LR114	ROX: *Listeria* spp. Cy5: *L. monocytogenes*	100 DNA preparations & 100 reactions	F5217
SureFast® Listeria monocytogenes PLUS	FAM: prfA-gene of *L. monocytogenes*	100 reactions	F5113
Bacillus cereus			
SureFast® Bacillus cereus group PLUS	FAM: *Bacillus cereus* group (*B. anthracis, B. cereus, B. cytotoxis, B. mycoides, B. pseudomycoides, B. thuringiensis & B. weihenstephanensis*)	100 reactions	F5126
SureFast® Emetic Bacillus cereus PLUS	FAM: Specific cereulide synthetase DNA sequence of the emetic *Bacillus cereus*	100 reactions	F5127

Kit overview

Bacteria

Product	Description*	No. of tests/amount	Art. No.
Campylobacter			
SureFast® Campylobacter 4plex	FAM : *Campylobacter jejuni* ROX: *Campylobacter lari* Cy5: *Campylobacter coli*	100 reactions	F5170
Clostridium			
SureFast® Clostridium botulinum Screening PLUS	FAM: Botulinum neurotoxins (BoNT) A, B, E & F of *C. botulinum, C. baratii & C. butyricum*	100 reactions	F5110
SureFast® Clostridium estertheticum PLUS	FAM: *Clostridium estertheticum*	100 reactions	F5160
SureFast® Clostridium perfringens PLUS	FAM: Specific alpha-toxin DNA sequence of *Clostridium perfringens*	100 reactions	F5123
Cronobacter			
SureFast® Cronobacter sakazakii PLUS	FAM: *Cronobacter sakazakii*	100 reactions	F5115
Staphylococcus			
SureFast® Staphylococcus aureus PLUS	FAM: *Staphylococcus aureus*	100 reactions	F5116
MRSA			
SureFast® MRSA 4plex	FAM: *SCCmec/orfX* ROX: *Staphylococcus aureus* Cy5: *mecA/mecC*	100 reactions	F7117
Vibrio			
SureFast® Vibrio 4 plex	FAM: *Vibrio cholerae* ROX: *Vibrio parahaemolyticus* Cy5: *Vibrio vulnificus*	100 reactions	F5161
SureFast® Vibrio Virulence 4plex	FAM: *V. parahaemolyticus* tdh, toxR, trh ROX: *V. vulnificus* vvh Cy5: *V. cholerae* rtxA	100 reactions	F5181
Yersinia			
SureFast® Yersinia 3plex	FAM: *Y. pseudotuberculosis* Cy5: *Y. enterolitica*	100 reactions	F5132
Multiplex kits			
SureFast® Foodborne Pathogen 4plex	FAM: *E. coli stx1/2/2* ROX: *Listeria monocytogenes* Cy5: *Salmonella spp.*	100 reactions	F5175
SureFast® Enterobacteriaceae	FAM: *Enterobacteriacae* ROX: *Cronobacter* spp. Cy5: *Salmonella* spp.	100 reactions	F5180

* HEX: IAC
** Certified within a workflow of the SureFast® STEC 4plex ONE method.

Viruses

Product	Description*	No. of tests/amount	Art. No.
	Qualitative real-time PCR - food related viruses		
SureFast® Norovirus/Hepatitis A 3plex	FAM: Norovirus (genogroup I & II) Cy5: Hepatitis A	100 reactions	F7124
SureFast® Hepatitis A PLUS	FAM: Hepatitis A	100 reactions	F7125
SureFast® Hepatitis E PLUS	FAM: Hepatitis E	100 reactions	F7142