

SureFast® Microbiology

Qualitative detection of common foodborne pathogens with qPCR



Simple and straightforward: 10 min lysis protocol

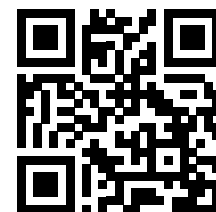


Flexible: open platform



Broad portfolio

More information:



<https://r-bio/mibiwater>

Causes of food poisoning

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 not depending on the organism traveling to the intestinal tract implanting and growing
 - 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:**
 - E.g. enterotoxigenic and enterohemorrhagic *E. coli* and *Clostridium perfringens*

Commission Regulation (EC) No. 2073/2005 on microbiological criteria for foodstuffs

This regulation sets harmonized microbiological criteria and how to perform the tests for certain microorganisms. The following pathogens are for example included:

- *Salmonella*
- *Listeria monocytogenes*
- *E. coli*
- *Enterobacter sakazakii*
- *Enterobacteriaceae*
- *Staphylococcal enterotoxins*

Moreover, it provides rules to be obeyed by food business operators when implementing general and specific hygiene measures referred to in (EC) No. 853/2004. Basically, two different types of criteria are established in Regulation 2073/2005:

- Food safety criteria: assess safety of a product/batch of foodstuff
- Process hygiene criteria: ensure production processes are operating properly

The main difference between them is the consequence: when a food safety criterion is not fulfilled, the batch of the affected food should be recalled or not placed on the market.

* References:

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

[2] Behlring J., Kornacki L. (ed.), Principles of Microbiological Troubleshooting in the Industrial

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

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



Time requirement: 16 - 26 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
- Eventually DNA purification step



Time requirement: ~ 20 min

3 Real-time PCR set-up

- Prepare master mix
- Add extracted DNA

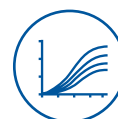


Time requirement: ~ 1 hr

4 Real-time PCR analysis

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

- Standardized thermal profiles



Available DNA extraction kits

DNA extraction kit	Pathogen type	Description	Steps	Hands on time/ 10 samples
SureFast® Speed PREP (F1054)*	GRAM-negative bacteria & parasites	Fast & easy DNA isolation without purification	2	~ 20 min
SureFast® PREP Bacteria (F1021)	Bacteria	Complex matrices with strong inhibitors	7	~ 45 min
SureFast® PREP DNA/ RNA Virus (F1051)	Viruses	Cell culture supernatants, foods (e.g. wash up fluids from fruits, salads etc.), filters from water samples	7	~ 45 min
SureFast® Mag PREP Pathogen	Viruses & bacteria	Automated nucleic acid preparation in combination with TANBead Maelstrom™ 4800 (ZMAL48)	Walk away solution	~ 5 min

* Same protocol as the „ONE“ kits.

Overview food source reference methods



Bacteria	Possible food source (examples)	Analytical reference method	Enrichment*
Gram-negative bacteria			
1 <i>Salmonella</i>	Meats, poultry, eggs, milk and dairy products, fish, shrimp, spices, coconut, sauces, cake mixes, dried foods and fruit, peanut butter, cocoa, produce (fruits and vegetables), chocolate	ISO 10135:2013-05 (PCR) ISO 6579-1:2017-07	BPW 18 ± 2 h at 37 ± 1 °C
2 <i>Campylobacter</i> spp.	Improperly handled or undercooked poultry products, unpasteurized ("raw") milk and cheeses made from unpasteurized milk, shellfish	ISO 10272-1:2017-09	Bolton broth 4 - 6 h at 37 °C microaerobic, followed by 44 ± 4 h at 41.5 °C
<i>Vibrio cholerae</i> / <i>parahaemolyticus</i>	Raw or undercooked seafood	ISO 21872-1:2017-10	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
<i>Vibrio vulnificus</i>	particularly oysters	ISO 21872-1:2017-10	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
3 <i>Yersinia enterocolitica</i>	Raw or undercooked seafood	ISO/TS 18867:2016-01 (PCR) ISO 10273:2017-08	Peptone-Sorbitol-Bile-Broth 48 h at 25 ± 1 °C
<i>Cronobacter</i> spp.	particularly oysters	ISO 22964:2017-08	BPW 18 h ± 2 h at 34 °C to 38 °C
Pathogenic <i>Escherichia coli</i>			
<i>E. coli</i>	Raw or undercooked ground beef and beef products, raw milk, various water sources, lettuce, spinach, sprouts		BPW 16 - 24 h at 37 °C
<i>E. coli</i> – Enterohemorrhagic (<i>E. coli</i> O157:H7 and others)	Raw or undercooked ground beef and beef products, raw milk, various water sources, lettuce, spinach, sprouts	ISO/TS 13136:2012 (PCR), DIN SPEC 10794 ISO 16654:2017-08	mTSB or BPW 18 - 24 h at 37 ± 1 °C
Gram-negative bacteria			
4 <i>Listeria monocytogenes</i> / <i>Listeria</i> spp.	Raw milk, inadequately pasteurized milk, chocolate milk, cheeses, ice cream, raw vegetables, raw poultry and meats, fermented raw-meat sausages, deli meats, and raw or smoked fish and other seafood	ISO 11290-1/2:2017-09	Half Fraser broth 25 ± 1 h at 30 ± 1 °C
5 <i>Staphylococcus aureus</i>	Meat and meat products; poultry and egg products, salads, bakery products, sandwich fillings, milk and dairy products	ISO 6888-1:2019-04	BPW 16 - 24 h at 37 °C
6 <i>Bacillus cereus</i>	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 17919:2013 / Messelh�usser et. al. 2014	TPGY 24 h ± 2 h at 30 ± 1 °C

* 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
n.a. – not applicable



Bacteria	Possible food source (examples)	Analytical reference method	Enrichment*
Gram-negative bacteria			
7 <i>Clostridium botulinum</i>	The types of foods involved in botulism vary according to food preservation and cooking practices	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
8 <i>Clostridium perfringens</i>	Meats (especially beef and poultry), meat-containing products, vegetable products, including spices and herbs, raw and processed foods, gravies - food left for long periods in steam tables or at room temperature for example	ISO 7937:2004	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	ISO 15216-1:2017-07	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	ISO 15216-1:2017-07	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 *Yersinia enterocolitica*

Up to date, there is a non-compulsory reporting on *Yersinia* and harmonized sampling and reporting rules do not exist yet.

4 *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).

5 *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.

6 *Bacillus cereus*

B. cereus intoxication has been linked to inappropriate food preparation and storage. A slow cooling process due to large containers is often a factor.

7 *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.

8 *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.








Kit overview

Bacteria



Product	Description*	No. of tests/amount	Art. No.
DNA preparation			
SureFast® PREP Bacteria	Preparation of bacteria DNA from enrichments	100 preparations	F1054
SureFast® Speed PREP	Speed preparation of bacteria- and parasites-DNA from enrichment cultures and tissue samples	100 preparations	F1021
SureFast® Mag PREP Pathogen	Automated viral and bacterial nucelic acid preparation in combination with TANBead Maelstrom 4800 (ZMAL48)	96 preparations	F1062
Salmonella			
Qualitative real-time PCR - food related pathogens			
SureFast® Salmonella PLUS	FAM: <i>Salmonella</i> spp.	100 reactions	F5111
SureFast® Salmonella ONE AOAC-RI 081803; MicroVal 2014LR43	FAM: <i>Salmonella</i> spp.	100 DNA preparations & 100 reactions	F5211
SureFast® Salmonella species/Enteritidis/Typhimurium 4plex	FAM: <i>Salmonella</i> spp. ROX: <i>Salmonella</i> Enteritidis Cy5: <i>Salmonella</i> Typhimurium	100 reactions	F5166
Escherichia coli			
SureFast® Escherichia coli PLUS	FAM: <i>Escherichia coli</i>	100 reactions	F5157
SureFast® EHEC/EPEC 4plex (stx1, stx 2, ipaH, E.coli/Shigella)	FAM: <i>stx1</i> (subtype a-d) & <i>stx2</i> (subtype a-g) Cy5: <i>eae</i> ROX: <i>ipaH</i> (<i>E. coli</i> & <i>Shigella</i> spp.)	100 reactions	F5128
SureFast® STEC Screening PLUS	FAM: <i>stx1/stx2</i>	100 reactions	F5105
SureFast® STEC 4plex ONE (O157, stx1, stx2, eae)	FAM: <i>E. coli stx1</i> (subtype a-d) & <i>stx2</i> (subtype a-g) Cy5: <i>eae</i> ROX: <i>E. coli</i> O157	100 reactions	F5265
SureFast® Escherichia coli Serotype I 4plex	FAM: O121 Cy5: O26 ROX: O103	100 reactions	F5167
SureFast® Escherichia coli Serotype II 4plex	FAM: O45 Cy5: O145 ROX: O111	100 reactions	F5168
Listeria			
SureFast® Listeria Screening PLUS	FAM: <i>Listeria</i> spp.	100 reactions	F5117
SureFast® Listeria 3plex ONE AOAC-RI 062501; MicroVal 2023LR114	ROX: <i>Listeria</i> spp. Cy5: <i>L. monocytogenes</i> Hex: IAC	100 DNA preparations & 100 reactions	F5217
SureFast® Listeria monocytogenes PLUS	FAM: <i>prfA</i> -gene of <i>L. monocytogenes</i>	100 reactions	F5113
Bacillus cereus			
SureFast® Bacillus cereus group PLUS	FAM: <i>Bacillus cereus</i> group (<i>B. anthracis</i> , <i>B. cereus</i> , <i>B. cytotoxicus</i> , <i>B. mycoides</i> , <i>B. pseudomycoides</i> , <i>B. thuringiensis</i> & <i>B. weihenstephanensis</i>)	100 reactions	F5126
SureFast® Emetic Bacillus cereus PLUS	FAM: Specific cereulide synthetase DNA sequence of the emetic <i>Bacillus cereus</i>	100 reactions	F5127
Campylobacter			
SureFast® Campylobacter 4plex	FAM : <i>Campylobacter jejuni</i> HEX: IAC ROX: <i>Campylobacter lari</i> Cy5: <i>Campylobacter coli</i>	100 reactions	F5170



Bacteria

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

Viruses

DNA preparation				
SureFast® Mag PREP Pathogen	Automated viral and bacterial nucleic acid preparation in combination with TANBead Maelstrom™ 4800 (ZMAL48)	96 preparations	F1062	
SureFast® DNA/RNA Virus	DNA preparation of viruses	100 preparations	F1051	
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	

Kit overview

Water analysis

Product	Description*	No. of tests/amount	Art. No.
DNA preparation			
SureFast® PREP Aqua	DNA preparation of bacterial cells from water samples	100 preparations	F1023
Qualitative real-time PCR - water related pathogens			
SureFast® Legionella Screen PLUS	FAM: <i>Legionella</i> spp.	100 reactions	F5502
SureFast® Legionella 3plex	FAM: <i>Legionella</i> spp. Cy5: <i>Legionella pneumophila</i>	100 reactions	F5505
SureFast® Pseudomonas aeruginosa PLUS	FAM: <i>Pseudomonas aeruginosa</i>	100 reactions	F5503
SureFast® Parasitic Water Panel 4plex	FAM: <i>Giardia intestinalis</i> ROX: <i>Entamoeba histolytica</i> Cy5: <i>Cryptosporidium</i> spp.	100 reactions	F5506
SureFast® Enterobacteriaceae Screening PLUS	FAM: <i>Enterobacteriaceae</i>	100 reactions	F5507
SureFast® Fecal Screen 4plex	FAM: <i>Enterobacteriaceae</i> ROX: <i>Enterokokken</i> Cy5: <i>E. coli</i>	100 reactions	F5504

* VIC/HEX: Internal Amplification control (IAC)

