Simultaneous detection of Salmonella spp., Escherichia coli O157:H7 and Listeria monocytogenes using multiplex PCR

Identification

Key words
multiplex real-time PCR, food-borne pathogen, simultaneous detection, salmonella, escherichia coli, listeria monocytogenes, multiple platform

Latest version
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Completed by
TTZ

How does it work?

Primary objective
analytical tool for simultaneous detection of the pathogenic microorganisms Salmonella spp., Escherichia coli O157:H7 and Listeria monocytogenes

Working principle
Samples are mixed with enrichment medium and incubated. After enrichment of the target organisms bacterial DNA is extracted and amplified with the multiplex PCR. The detection of PCR products can be done in real-time via fluorescent signals either via intercalating dyes within the bacterial DNA, followed by high resolution melting analysis for specification or with dual-labeled probes. Alternatively to real-time detection of PCR products, PCR can be followed by an electrophoresis with ethidium bromide staining and UV detection.

Images

Additional effects
time saving process (48 h instead of 7 days required in the standard culture method)
automatized high-throughput process higher sensitivity than conventional methods
allows quantification of pathogens reduced risk of cross-contamination during analysis

Important process parameters
suitable broth enabling simultaneous enrichment of the three bacteria (e.g. No17 enrichment broth according to Kawasaki et al. 2005)
enrichment time (15-20 h depending on food matrix) DNA extraction method (depending on food matrix)

Important product parameters

What can it be used for?

Products
relevant for all products exposed to contamination by the mentioned microorganisms

Operations
quality assurance, pathogen analytic

Solutions for short comings
The method enables inexpensive screening of several samples for more than one pathogen with low workload.
appropriate to support ISO culture methods with preliminary screening
What can it NOT be used for?

**Products**
no restricted products reported yet

**Operations**

**Other limitations**
The multiplex real-time PCR is not valid as an official reference method. Sensitivity: down to 1 cell of each species in 25 g sample enrichment media and PCR parameters have to be adapted for different pathogens

**Risks or hazards**
no risks

Implementation

**Maturity**
assays for single detection: commercially available
assays for multiple detection: lab scale, comparison with conventional methods showed reliability of the multiplex PCR

**Modularity**
PCR instrument and fluorescence or UV detector is needed

**/Implementation Consumer aspects**
Intoxications and infections caused by food-borne pathogens represent an increasing public health problem. The three discussed pathogens account for about 40% of pathogen intoxications in the EU. Suitable analytical methods are necessary for industry to combat this problem by detecting contaminations of their products at an early stage.

**Legal aspects**
limits for pathogens in food according to (EC) No 2073/2005 on microbiological criteria for foodstuffs

**Environmental aspects**
Rapid control and analysis of raw material is an important tool to ensure cleanability and to minimize the potential for cross-contamination and recontamination.

Facilities that might be interesting for you

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<tr>
<th>Title</th>
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<tr>
<td>Field Flow Fractionation INPT - EI Purpan</td>
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<td>Fruit &amp; vegetable analysis INRA</td>
<td>INRA - SQPOV</td>
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<td>Mastersizer FBR</td>
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<td>Microbiological analysis INRA</td>
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<td>Multi-user olfactometer INRA</td>
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<td>PlantLipPol-Green INRA</td>
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Further Information

**Institutes**
University of Parma, Urbino University, CTC

**Companies**
Barilla, Diatheva, Roche
References


