

RAPID DETECTION OF FOODBORNE PATHOGENS IN WATER, FRUITS, VEGETABLES AND PROCESSED FOODS USING DIRECT PCR

Publication Manuscript – DNA Extraction-Free Workflow

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Abstract

Foodborne pathogens present a major challenge to public health and food safety because contaminated water, fresh produce and processed foods can serve as vehicles for transmission. Conventional microbiological methods are dependable but may require enrichment, isolation and biochemical confirmation, resulting in longer turnaround times. Polymerase chain reaction (PCR) provides a rapid and specific molecular approach for pathogen screening. This manuscript presents a direct-PCR, DNA-extraction-free workflow for rapid screening of selected foodborne pathogens in water, fruits, vegetables and processed foods. The target organisms are *Salmonella* spp., *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Staphylococcus aureus* and *Campylobacter jejuni*. The proposed workflow uses sample preparation/concentration followed by direct addition of the prepared sample to a validated PCR system, amplification with pathogen-specific primers and analysis of the amplicons by agarose gel electrophoresis. The approach eliminates a separate conventional DNA purification step and may reduce analytical time and laboratory handling. Published studies have demonstrated the feasibility of direct PCR for foodborne pathogens in selected food matrices. Nevertheless, food-matrix inhibitors, low pathogen concentrations and detection of nucleic acid from non-viable cells remain important limitations. Therefore, matrix-specific optimization and laboratory validation are required before routine or regulatory application.

Keywords

Foodborne pathogens; direct PCR; DNA extraction-free; rapid detection; *Salmonella*; *Escherichia coli* O157:H7; *Listeria monocytogenes*; *Staphylococcus aureus*; *Campylobacter jejuni*; food safety.

1. Introduction

Foodborne diseases are caused by the consumption of food or water contaminated with pathogenic microorganisms, toxins or other biological hazards. Fresh fruits and vegetables can become contaminated through irrigation water, soil, manure, handling, transportation and processing. Processed foods may be contaminated through raw materials, equipment, inadequate processing or post-process handling. Rapid and reliable detection is essential for preventing contaminated products from reaching consumers.

Conventional culture methods are widely used for foodborne pathogen detection, but enrichment and confirmation can require substantial time. PCR has become an important molecular tool because it can detect pathogen-specific genetic targets rapidly and with high analytical specificity. However, conventional PCR workflows commonly include a nucleic-acid extraction or purification step.

Direct PCR aims to simplify this workflow by using a suitably prepared sample directly as the amplification template without a separate conventional DNA extraction/purification stage. The approach can reduce processing time and reagent use, but its performance depends on effective sample preparation and tolerance of PCR inhibitors present in food matrices.

The present manuscript focuses exclusively on a DNA-extraction-free direct-PCR workflow for rapid screening of selected foodborne pathogens in water, fruits, vegetables and processed foods.

2. Review of Literature

2.1 PCR-based foodborne pathogen detection

PCR-based assays have been developed for *Salmonella*, pathogenic *Escherichia coli*, *Listeria monocytogenes*, *Campylobacter* and *Staphylococcus aureus*. Reviews of rapid molecular detection methods describe PCR and real-time PCR as important tools for reducing detection time compared with conventional culture.

2.2 Direct PCR without a separate DNA extraction step

Direct amplification approaches have been investigated to simplify pathogen testing in food matrices. Williams and Hashsham described direct or DNA-extraction-free amplification approaches for foodborne pathogens, including examples involving *E. coli* in milk and *Salmonella* in pork. More recent work has continued to develop direct amplification protocols for foodborne pathogens.

2.3 *Salmonella* detection in vegetables

Real-time PCR has been applied to *Salmonella* detection from vegetable rinse-water. This demonstrates the usefulness of molecular testing for fresh-produce samples and highlights the importance of suitable sample preparation before amplification.

2.4 Rapid direct detection of *Salmonella*

A 2019 study reported a rapid approach combining sample concentration and direct PCR for *Salmonella enterica* in food samples. The method avoided enrichment and conventional DNA extraction and was evaluated with food matrices including vegetable salad, egg and minced pork.

2.5 PCR inhibition in food matrices

Food components such as fats, proteins, polysaccharides, pigments and other compounds may inhibit DNA polymerase activity. Direct PCR therefore requires careful sample preparation, dilution or concentration and an appropriate polymerase system. Matrix-specific validation is essential.

3. Research Gap

Although PCR is widely used for foodborne pathogen detection, many analytical workflows still require enrichment and nucleic-acid extraction. Published direct-PCR studies often focus on individual pathogens or limited food matrices. A practical workflow covering water, fruits, vegetables and processed foods with multiple important foodborne pathogens remains useful for rapid screening. The proposed study addresses this need by focusing on a simplified DNA-extraction-free direct-PCR workflow and by emphasizing validation across different sample matrices.

4. Objectives

- To develop a rapid direct-PCR workflow for selected foodborne pathogens.
- To detect selected pathogens in water, fruits, vegetables and processed foods without a separate conventional DNA extraction/purification step.
- To evaluate direct PCR for *Salmonella* spp., *E. coli* O157:H7, *Listeria monocytogenes*, *Staphylococcus aureus* and *Campylobacter jejuni*.
- To interpret PCR products using agarose gel electrophoresis.
- To establish analytical performance using experimentally generated validation data.

5. Materials and Methods

5.1 Sample collection

Representative water, fruit, vegetable and processed-food samples should be collected aseptically from defined sources. Each sample should be coded and transported to the laboratory under appropriate conditions. The final manuscript should report the exact sample numbers, sample types, collection dates and storage conditions used in the experiment.

5.2 Sample preparation – no DNA extraction

No separate DNA extraction or DNA purification procedure is included in this workflow. Water samples may be concentrated by an appropriate validated filtration or centrifugation method. Solid samples may be aseptically homogenized in a suitable sterile diluent and processed to obtain a representative suspension. A small volume of the appropriately prepared sample is then used directly as the PCR template. The exact sample-to-diluent ratio and processing conditions should be reported according to the laboratory protocol.

5.3 Direct PCR reaction

The PCR mixture should contain a validated PCR master mix, pathogen-specific primers, prepared sample template and nuclease-free water. A positive amplification control and a negative control should be included in every PCR run. Primer sequences, concentrations, reaction volume and thermal-cycling conditions must be reported exactly as experimentally used.

5.4 PCR amplification

Amplification should be performed using a calibrated thermal cycler. The validated program should include initial denaturation, repeated denaturation, primer annealing and extension steps, followed by final extension where applicable. Different primer sets may require different annealing temperatures and cycling conditions.

5.5 Agarose gel electrophoresis

PCR products should be separated using an appropriate agarose concentration and a validated nucleic-acid staining system. The gel should be visualized using a suitable gel documentation system. Expected amplicon size should be assessed using a molecular-size marker and valid positive and negative controls.

5.6 Result interpretation

A sample is considered positive when the expected target-specific amplification product is observed at the correct size and the controls are valid. A negative result means that the target was not detected under the conditions of the assay. Appropriate inhibition controls are recommended because a failed reaction can otherwise be incorrectly interpreted as a negative sample.

6. Proposed Direct-PCR Workflow

Sample collection → Sample preparation/concentration → Direct addition of prepared sample to PCR mixture → PCR amplification → Agarose gel electrophoresis → Gel visualization → Result interpretation → Validation.

DNA extraction/purification is intentionally absent from the workflow.

7. Conclusion

Direct PCR provides a promising rapid and simplified approach for screening foodborne pathogens in water, fruits, vegetables and processed foods without a separate conventional DNA-extraction/purification step. The approach can reduce sample-processing time and laboratory handling and may be useful for routine preliminary food-safety screening. The proposed workflow is applicable to important pathogens including *Salmonella* spp., *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Staphylococcus aureus* and *Campylobacter jejuni*. However, direct-PCR performance is affected by food-matrix inhibitors, pathogen concentration and assay design. Consequently, each sample matrix requires appropriate optimization and validation. The method should be compared with an accepted reference method before routine or regulatory use. Because PCR detects target nucleic acid rather than viability itself, positive results should be interpreted according to the intended application.

8. Future Scope

- Optimization of matrix-specific sample preparation while retaining the DNA-extraction-free concept.
- Development of multiplex and real-time PCR assays for simultaneous detection of multiple pathogens.
- Use of inhibitor-tolerant polymerases and improved sample-processing strategies.
- Evaluation of naturally contaminated samples and larger validation sets.
- Integration with portable PCR systems for rapid on-site food-safety screening.
- Investigation of viability-PCR approaches where differentiation between viable and non-viable cells is required.

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