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Sensitivity Molecular Detection for Salmonella *Enterica Serovars Typhi*

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ABSTRACT

Introduction: Salmonella infections contribute significantly to gastroenteritis cases, with the National Salmonella Reference Laboratory reporting 500 isolates in 2022. However, traditional culture-based methods for detecting Salmonella in samples can take 4 to 7 days to confirm a positive result, which poses health risks due to delayed detection. Given these health risks, swift and accurate detection methods are essential to minimize both false-positive and false-negative outcomes.
Methods: The qRT-PCR procedure involved the design of primers and probes targeting the same genes as the mPCR assay. These primer sets were reconfigured to generate smaller amplicons suitable for qRT-PCR systems
Results: qRT-PCR process, TaqMan probes were meticulously designed for specific target genes: FAM dye was employed to detect STM2745, Cy5 dye was used for STM4492, and Rox dye was utilized to detect. A standard curve was constructed using Typhimurium LT2 genomic DNA. Each sample underwent duplicate analysis, and Rotor-Gene software was employed to assign threshold values for each channel
Conclusion: The effectiveness of our qPCR assay for the detection of Salmonella across a diverse array of matrices. Notably, our results unveiled distinct limits of detection for Salmonella in various samples. Specifically, a parallel vein, the deployment of a PCR assay, leveraging an immunomagnetic separation technique for DNA extraction, was studied by another group. While subsequent analysis of Salmonella detected via our assay may necessitate the full ISO SMT method for live culture isolation, this supplementary step can be seamlessly conducted alongside qRT-PCR.

Keywords: Sensitive molecular; salmonella; *detection salmonella*

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Introduction

Salmonellosis stands as a prevalent infectious foodborne disease worldwide, affecting both animals and humans. *Salmonella enterica*, a bacterium within the Salmonella genus, is the primary culprit behind a broad spectrum of illnesses, ranging from gastroenteritis to severe, life-threatening enteric fever. Each year, the United States records an estimated 1 million cases of salmonellosis among humans. *S. enterica* ranked as the second most common cause of zoonotic infections among humans within the European Union in 2022, with 900 confirmed cases of salmonellosis reporter.[1]

Salmonella infections contribute significantly to gastroenteritis cases, with the National Salmonella Reference Laboratory reporting 500 isolates in 2022. However, traditional culture-based methods for detecting Salmonella in samples can take 4 to 7 days to confirm a positive result, which poses health risks due to delayed detection. Given these health risks, swift and accurate detection methods are essential to minimize both false-positive and false-negative outcomes. Salmonella, a gram-negative bacterium within the Enterobacteriaceae family, demonstrates remarkable hardiness, surviving for several weeks in dry environments and months in water.[2] Although most serotypes of Salmonella cause relatively mild gastroenteritis, some, particularly those transmitted from animals to humans, can lead to severe, life-threatening conditions. Salmonellosis outbreaks have been widely reported, highlighting the pressing need for improved prevention and control measures globally. Salmonellosis is characterized by fever, abdominal pain, diarrhea, nausea, and sometimes vomiting, after exposure to the bacterium. While most cases resolve without specific treatment, severe dehydration can occur, especially in children and the elderly. Additionally, the disease's transmission often goes unrecognized as a part of known outbreaks, with sporadic cases constituting a significant portion.[3]

Salmonella infections are zoonotic, meaning they can be transmitted between animals and humans. The bacterium is frequently found in the intestines of various animals, particularly poultry, pigs, and cattle. Contaminated foods like eggs and raw meat, as well as improper kitchen hygiene, can lead to human infection. Though many cases resolve on their own, individuals with weakened immune systems, infants, and older adults are at a higher risk of complications. Adequate hygiene, proper cooking, and safe handling of raw foods can help prevent the spread of this disease.[4]

Methods

A total of 150 bacterial isolates were utilized to validate the techniques formulated during this investigation. To assess the specificity of potential serovar regions identified through in silico genome comparisons, two reference collections were employed: Salmonella reference collection A, comprising 50 isolates from serovars within the *S. enterica* Typhimurium complex, and Salmonella reference collection

B, composed of 50 strains representing 50 serovars of *S. enterica.*[5] These collections encompass a diverse range of strains from various geographical origins. Additionally, 50 recent clinical and environmental isolates from Ireland were examined, covering 20 distinct serovars. As part of this study, three National Collection Type Culture (NCTC) strains, including *Escherichia coli* NCTC 9009, Listeria monocytogenes NCTC 11994, and *Staphylococcus aureus* NCTC 6571, were included as negative controls. DNA extraction from each strain was conducted using the Gnome DNA isolation kit (BIO 101, Inc., La Jolla, CA) and/or the Promega (Madison, WI) Wizard genomic DNA purification kit, following the manufacturer's instructions. The objective was to evaluate the developed techniques and investigate potential serovar-specific regions through comparisons of in silico genomes.[6]

The qRT-PCR procedure involved the design of primers and probes targeting the same genes as the mPCR assay. These primer sets were reconfigured to generate smaller amplicons suitable for qRT-PCR systems.[7]. The design of sequence-specific primers and TaqMan probes was facilitated by utilizing Primer3 software. The choice of dyes, including FAM, Cy5, and ROX, along with the quenchers BHQ1, BHQ2, and BHQ3, were tailored for compatibility with the Rotor-Gene 6000 system. For qRT-PCR execution, the QuantiTect multiplex PCR NoRox kit was employed, adhering to the manufacturer's guidelines from Qiagen. This approach aimed to ensure optimal primer and probe design for qRT-PCR analysis, facilitating the generation of meaningful results.[8]

Result

qRT-PCR process, TaqMan probes were meticulously designed for specific target genes: FAM dye was employed to detect STM2745, Cy5 dye was used for STM4492, and Rox dye was utilized to detect. A standard curve was constructed using Typhimurium LT2 genomic DNA. Each sample underwent duplicate analysis, and Rotor-Gene software were employed to assign threshold values for each channel. The study examined DNA samples from various common Salmonella serovars. The correlation coefficient was calculated to compare observed data points to the expected standard curve points. A higher R2 value approaching 1 indicated a better fit of observed data to the standard curve. The obtained R2 values from the mPCR reaction were all above 0.99, denoting a high level of accuracy. The slope of the standard curve, directly linked to the average amplification efficiency, allowed for calculation of PCR efficiency. Slope values for this qRT-PCR reaction ranged from 23.32 to 23.53. Additionally, the number of qRT-PCR cycles required to detect control strains was determined, yielding cycle threshold (Ct) values ranging from 13.41 to 18.12.

Both conventional mPCR and qRT-PCR assays were carried out at the preenrichment (BPW) and selective enrichment (RVS) stages. The validation of the mPCR assay was conducted using ground raw turkey samples containing S. enterica Heidelberg, Dublin, and Enteritidis cells, and various concentrations of Typhimurium were also utilized. The initial seeding levels for the meat samples were as follows: 6.1 x 10^8 S. enterica Typhimurium LT2/ml, 5.6 x 10^9 S. enterica Heidelberg (RL9)/ml, 7.5 x 10^8 S. enterica Dublin (CCC8)/ml, and 8.8 x 10^8 S. enterica Enteritidis (UCG1)/ml. This comprehensive approach allowed for effective assessment of the qRT-PCR reaction, highlighting its accuracy and applicability in detecting Salmonella strains across different stages of analysis.

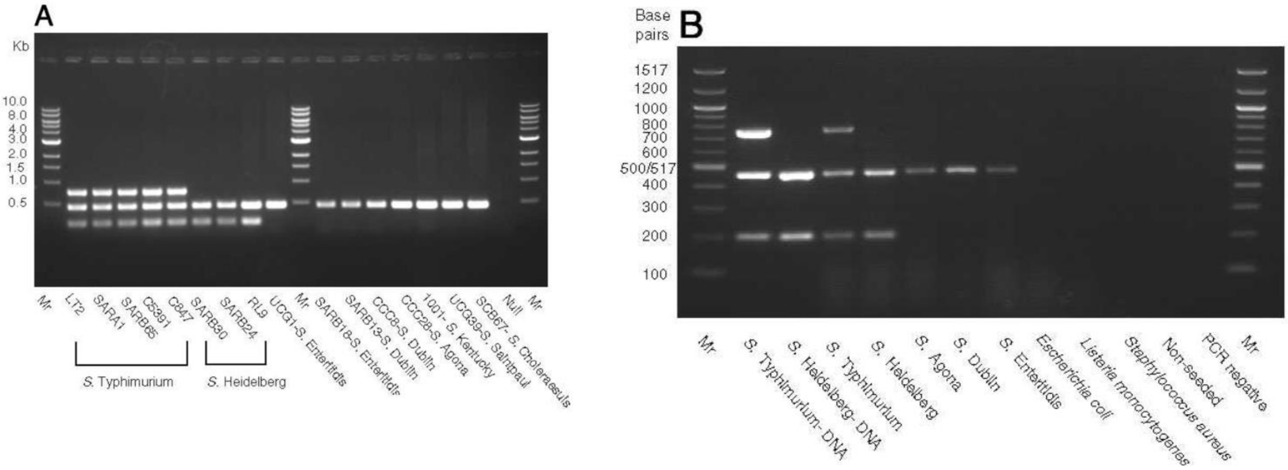

Figure 1 qPCR assays. (A) qPCR assay of a representative panel of Salmonella isolates. Typhimurium strains yielded three bands, Heidelberg isolates yielded the expected two bands, and all Salmonella serovars yielded the expected 461-bp band. (B) qPCR assay of food matrices seeded with Salmonella serovars Dublin, Agona, and Enteritidis, Escherichia coli, Listeria monocytogenes, and Staphylococcus aureus. DNA from Salmonella enterica Typhimurium LT2 and Heidelberg were used as positive controls yielded the expected three PCR bands and two PCR bands, respectively.

Discussion

We assessed the effectiveness of our qPCR assay in detecting Salmonella across various matrices. Our findings revealed distinct limits of detection for Salmonella in different samples: for cheese, raw turkey, and cooked turkey, the limits were 250 CFU/ml of selective enrichment, respectively. An PCR assay using an immunomagnetic separation technique for DNA extraction. Their investigation highlighted the inhibitory effect of food debris present in enrichment broths on PCR.[9]

Intriguingly, they discovered that high-fat-content samples like soft cheese displayed higher detection limits compared to lower-fat-containing foods such as eggs. For instance, the detection limits were 1.5 x 10^3 CFU/25 g of cheese sample and 1 to 5 CFU/25 g of egg sample. In contrast, our research demonstrated a higher sensitivity for detecting Salmonella in high-fat-content foods like grated cheese compared to raw ground turkey meat. The matrix that exhibited the most detrimental impact on sensitivity was raw turkey meat. This reduced detection limit could be attributed to the presence of inhibitory substances or competing microbiota in the matrix.[10] In the realm of PCR assays, TaqMan PCR assays have been created for various common gene targets to detect Salmonella, such as the stn gene, the ttrRSBCA locus, and the invA gene. Within our study, innovative qRT-PCR targets were devised for identifying Salmonella and differentiating between S. enterica Typhimurium and Heidelberg. Impressively, this differentiation could be achieved with a detection limit of 6.0 x 10^1 CFU/ml from RVS broth within 48 hours.[11]

This speed far surpasses the ISO SMT method, which typically takes 4 to 7 days to complete. While further analysis of Salmonella detected via this assay would necessitate the full ISO SMT method for live culture isolation, this additional step can be concurrently conducted with qRT-PCR–negative samples being disregarded. This streamlined process leads to significant time and labor savings in research.[12]

We employed a meticulous approach to designing a multifaceted mPCR assay for the detection and differentiation of Salmonella. Our assay hinged on three distinct primer pairs: the first targeting an inner membrane protein, the second focusing on another inner membrane protein identified, the third primer pair designed to pinpoint the oriC locus present across all Salmonella strains. By leveraging these primers, we were able to specifically identify and differentiate S. enterica Typhimurium and Heidelberg. Rigorous validation processes substantiated the sensitivity and specificity of our assay.[13]

Our investigation extended further to demonstrate the specificity of our mPCR assay across a range of sample types, including artificially spiked incurred samples, broth media, Cheddar cheese, and raw and cooked ground turkey meat. We conducted these tests on samples seeded with various Salmonella serovars (Dublin, Agona, and Enteritidis) as well as other foodborne pathogens like E. coli, L. monocytogenes, and S. aureus.[14] The outcomes of these tests were definitive, with the presence of Salmonella-specific amplicons observed in all screened Salmonella strains. In contrast, non-Salmonella strains yielded no such amplicons.[15]

Conclusion

The effectiveness of our qPCR assay for the detection of Salmonella across a diverse array of matrices. Notably, our results unveiled distinct limits of detection for Salmonella in various samples. Specifically, a parallel vein, the deployment of a PCR assay, leveraging an immune-magnetic separation technique for DNA extraction, was studied by another group. While subsequent analysis of Salmonella detected via our assay may necessitate the full ISO SMT method for live culture isolation, this supplementary step can be seamlessly conducted alongside qRT-PCR. This streamlined approach contributes to significant time and labor savings in research. Furthermore, our meticulous approach extended to the design of a multifaceted mPCR assay. This assay hinged on three distinct primer pairs targeting specific elements: an inner membrane protein, a different inner membrane protein, and the oriC locus present across all Salmonella strains. Our utilization of these primers enabled the specific identification and differentiation of S. *enterica Typhimurium* and Heidelberg. This differentiation was fortified by rigorous validation, underscoring the sensitivity and specificity of our assay.

Conflicts of Interest

There is no conflict of interest

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