

# Preliminary Study For Screening Of Potentially Pathogenic Bacteria In Poultry Meat

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## Introduction

Poultry is one of the most widely available and consumed meats all over the world. High incidence in food poisonings connected with poultry derived products lead us to develop an inexpensive, high-throughput, reliable workflow for rapid, highly sensitive and specific detection and identification of contamination of a poultry meat samples with potentially harmful organisms (*Salmonella enterocolitis*, *Campylobacter jejuni*, *Campylobacter coli* and *Listeria monocytogenes*). Samples from chicken and turkey meat of various bodyparts were collected from three major commercial vendors in Slovenia. Bodyparts sampled were neck, wing, drumstick, whole leg, gizzard, wingskinless breast fillet, chicken back with skin, chicken shishkebab, chicken minced meat patty. All meat was fresh brought from the store butchers.

## Methods

Approximately 50 mg was taken from the sample meat and put in the tube where 400µL of Lysis Buffer was added. Samples were homogenized with a sterile plastic stick, vortexed and centrifugated. The automatic isolation of DNA was performed by MagMAX™ Express Magnetic Particle Processor with the MagMax Multi-Sample DNA kit (Life Technologies). Purity and concentration of DNA in samples were measured by absorbance using the Lambda-Bio spectrophotometer (Perkin Elmer) with the Hellma TrayCell cuvette at 260/280 and 260/230 absorbance ratios. Already published assays targeting specific genes of *C. jejuni*, *C. coli*, *L. monocytogenes* and *S. enterocolitis* were used for final detection using the Life Technologies ABI 7500 Fast Real-Time PCR system. Reaction volumes were minimized and optimized, so a 10 µl reaction with a 2 µl DNA template was used. Quantitative multiplex PCR (qmPCR) with additional singleplex quantitative PCR reaction (qPCR) were used for detection of *C. coli* and *C. jejuni*, while for *S. enterocolitis* and *L.monocytogenes* only a singleplex qPCR reaction was used. For each sample an additional 18S rRNA qPCR reaction was performed to check the quality of isolated DNA.

## Results

Table 1.	qPCR positive samples	qmPCR positive samples
<i>C. coli</i>	62,5%	25%
<i>C. jejuni</i>	16,6%	16,6%
<i>S. enterocolitis</i>	0%	-
<i>L. monocytogenes</i>	100%	-

qmPCR reactions were done in triplicates for each sample. Average value we got from three reactions was the end result, which was correlated with the results from qPCR reactions. qPCR was performed in duplicates. If one reaction of two was positive we considered the sample positive. Percentage of positive samples for both qPCR and mqPCR are presented in Table 1. Result obtained with qPCR and qmPCR are matching in *C. jejuni* samples, while *C. coli* results do not match identically. That can be attributed to the high concentration of DNA in the samples inhibiting the qmPCR reaction. This problem could be easily solved if the samples were diluted. All of our samples tested negative for *S. enterocolitis*, but all tested positive for *L. monocytogenes*, which shows how resistant and hardy this bacteria is.

## Conclusion

Results of our study show that the designed workflow meets the objectives required for broad implementation, as it is inexpensive, high-throughput, fast, reliable and easily automated. Some problems did arise with certain samples due to inhibited qmPCR or due to too high concentration of isolated DNA. These problems could be solved with optimizing the qmPCR reaction and with diluting the problematic samples.



## Workflow

## References

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- Urban Traunšek, Nataša Toplak, Barbara Jeršek, Aleš Lapanje, Tamara Majstorovič, Minka Kovač, 2011. Novel cost-efficient real-time PCR assays for detection and quantitation of *Listeria monocytogenes*. *Journal of Microbiological Methods*, Volume 85, Issue 1, April 2011, Pages 40-46.