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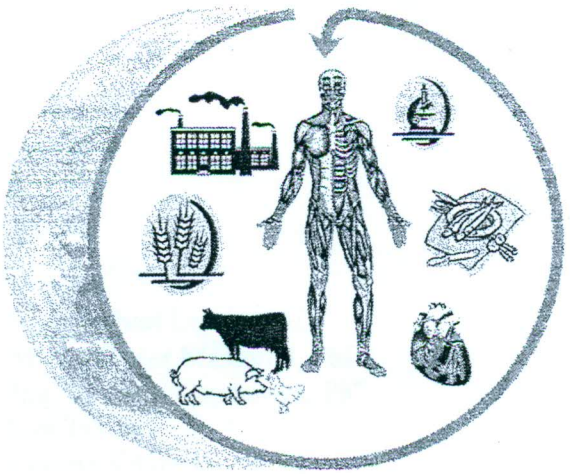

SEPTEMBER, 13th – 14th 2010,

NITRA, SLOVAKIA

Editors: doc. Ing. Norbert Lukáč, PhD.
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Authorized by the Rector of SUA in Nitra on 30.08.2010 as a Proceedings Book of Abstracts

ISBN 978-80-552-0437-6

THE STEP ONE REAL-TIME PCR FOR DETECTION OF *SALMONELLA SPP.*, *SALMONELLA ENTERICA SER. TYPHIMURIUM* AND *ENTERITIDIS* IN AXILLA OF CHICKENS

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Abstract

The aim of this study was follow the contamination of axilla of live chickens with *Salmonella* spp. by using the Step One real-time PCR. Detection of food-borne pathogens using conventional culture techniques takes up to 5 days to get a result. This includes primary and secondary enrichment and serological confirmation of colonies grown on agar plates. We used the PrepSEQ Rapid Spin Sample Preparation Kit for isolation of DNA and MicroSEQ® Salmonella spp. Detection Kit for pursuance the real-time PCR (Applied Biosystems). The samples were obtained by taking swabs from axilly of live chickens. In the investigated samples before incubation we could detect strain of *Salmonella* spp. in three out of five swabs, as well as internal positive control (IPC), which was positive in all samples. In the samples after 16 hours incubation we could detect strain of *Salmonella* spp. in samples, as well as internal positive control (IPC), which was positive in all samples. This Step One real-time PCR assay is extremely useful for any laboratory in possession of a real time PCR. It is a fast, reproducible, simple, specific and sensitive way to detect nucleic acids, which could be used in clinical diagnostic tests in the future.

Key words: *Salmonella* spp., real-time PCR, chicken axilla

Introduction

Salmonella is one of the most prevalent foodborne pathogens and infects over 160, 000 individuals in the EU annually, with an incidence rate of 35 cases per 100,000 (Anonymous, 2007). Salmonella testing in the slaughter environment is important as intestinal pathogens are carried into the abattoir in the bowels and on the skin of the animals (Wray, 2000).

Salmonellosis, caused by infection with bacteria from the genus *Salmonella*, is one of the most common foodborne illnesses and is manifested by diarrhea, mild fever, nausea, and abdominal pains, with the symptoms developing in 12–72 hours after consumption of contaminated food. In extreme cases it can also lead to death (Abubakar et al., 2007).

Meat, including poultry, is one of the major sources of human *Salmonella* infection (Denny et al., 2007; Foley et al., 2008), and therefore, efficient and rapid monitoring of *Salmonella* in the poultry production chain is necessary (Carrique-Mas and Davies, 2008).

Classical microbiological methods for detection of foodborne bacteria involve the use of pre-enrichment and/or specific enrichment, followed by the isolation of the bacteria in solid media and a final confirmation by biochemical and/or serological tests (Rodriguez-Lazaro et al., 2007).

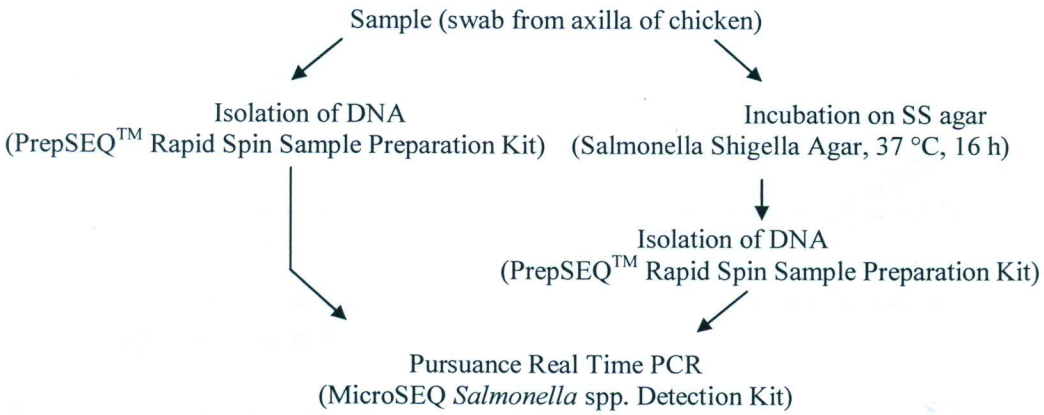
Determining bacterial viability is a key issue for the application of food risk management; thus a rational approach for detecting only viable bacterial cells by using molecular-based methods is necessary. However, the PCR does not distinguish among viable and dead bacterial cells. DNA

from bacterial dead cells can serve as a template for the PCR many days after cell viability has been lost (Allmann et al., 1995; Rodriguez-Lazaro et al., 2006a).

Material and Methods

The samples were obtained by taking swabs from axilla of live chickens. After the taking of the samples, we were advanced as shown in the Scheme 1.

Scheme 1 Progress after taking the samples



Bacterial strains and DNA extraction

As a pre-preparation step for the Step One real-time PCR, DNA extraction was performed using DNA extraction method: PrepSEQ Rapid Spin Sample Preparation Kit (Applied Biosystems).

General sample preparation protocol

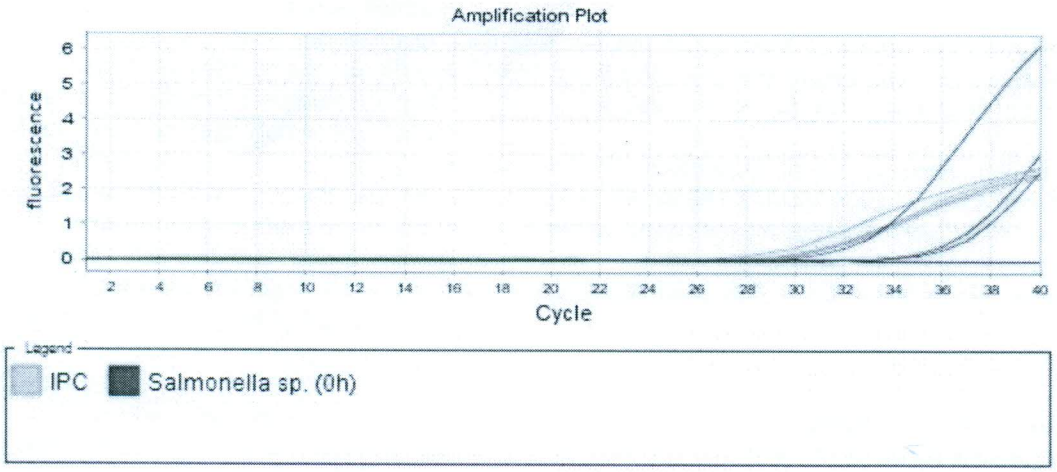
Load 750 µl of sample onto the spin column and cap the column. Microcentrifuge for 3 min at max speed. Aspirate, then discard the supernatant. Add 50 µl of Lysis Buffer to the pellet. Resuspend by pipetting up and down, or vortex until the pellet is resuspended. Cap the tube, then incubate at 95 °C for 10 min. Allow the sample to cool for 2 min at room temp. Microcentrifuge for 1 min at max speed. Add 250 µl of water. Microcentrifuge for 1 min at max speed. Proceed with PCR, or store the tube at –20 °C.

MicroSEQ® Salmonella spp. Detection Kit

We used 8-tube strips containing assay beads compatible with StepOne™ Systems. For 8-tube strips with seven or fewer reactions, add additional empty tubes as needed so that each strip contains a full set of 8 tubes. Add empty tubes as needed. Cap the tubes, sealing each tube with the transparent optical strip caps provided in the kit. Cap the tubes with the strip caps using the MicroAmp® 48-Well Base and the MicroAmp® Cap Installing Tool (Handle) to avoid collapsing, bending, or misaligning the tubes. Confirm that the strips are straight and that each tube is in line with the adjacent tube. Make sure reactions are thoroughly mixed. Make sure reagents are at the bottom of the tubes. If available, spin down the tube contents at 2000 × g for 20 seconds using a centrifuge with a plate adapter. Place the MicroAmp® Fast 48-Well Tray on the sample block of the StepOne System. Load the 8-tube strips horizontally. For example, in Row C, load an 8-tube strip across columns 1 through 8. A minimum of one 8-tube strip is recommended. It is not necessary to balance the tube strips on the tray.

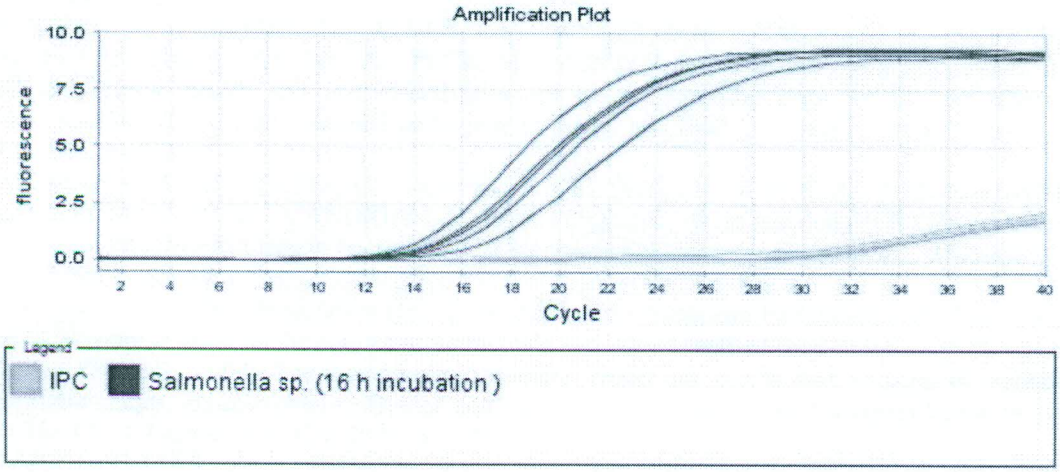
Results and Discussion

In the investigated samples before incubation we could detect strain of *Salmonella* spp. in three out of five samples (swabs), as well as internal positive control (IPC), which was positive in all samples, as shown in Graph 1. The real-time PCR performed for 40 cycles of 32 minutes.



Graph 1 Process Real Time PCR

In the samples after 16 hours incubation we could detect strain of *Salmonella* spp. in samples, as well as internal positive control (IPC), which was positive in all samples, as shown in Graph 2.



Graph 2 Process Real Time PCR

The multiplex real-time PCR developed in this study was the first to detected all *Salmonella* spp. possibly related with meats and to differentiate simultaneously *S.* Typhimurium from *S.*

Enteritidis in meats. Previously, real-time PCR assays had been applied for *Salmonella* spp. and other food-borne pathogens (Malorny et al., 2004; Rossmanith et al., 2006; Sails et al., 2003).

Although there has been a fall in the number of human *Salmonella* infections in Europe over the past number of years (Anonymous, 2007), foodborne infection from *Salmonella* continues to pose a great risk to public health. It is widely believed that pathogen reduction in animals, efficient Quality Control Systems (e.g. HACCP principles and GHP) and Quality Assurance (QA) at all stages of the food chain from ''farm to fork" is the most effective way to prevent the spread of infection via food (Anonymous, 2003).

Possible biological contaminants such as blood and fats present on animal carcasses which can cause PCR inhibition are also diluted. The bacterial cell pellet was washed in PBS prior to DNA isolation to reduce the concentration of enrichment medium which may also cause PCR inhibition (Rodriguez-Lazaro & Hernandez, 2006b).

Whereas most of the target genes used for PCR detection of foodborne pathogens encode proteins involved in virulence and were identified as the result of many years of work involving gene/protein structure–function studies, the progress in computational genomics has led the way to more efficient and customized mining of genomes for species-specific nucleotide sequences. Several software packages for such data mining have been developed based upon sequence alignment (Lu et al., 2006; Yao et al., 2007).

Conclusion

This Step One real-time PCR assay is extremely useful for any laboratory in possession of a real time PCR. It is a fast, reproducible, simple, specific and sensitive way to detect nucleic acids, which could be used in clinical diagnostic tests in the future.

Acknowledgement

This work was funded by Grant Agency KEGA 430-014SPU-4/2010, VEGA 1/0372/09 and APVV-VMSP-P-0057-09.

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