# *SALMONELLA*-PCR: SCREEN FOR MICROBIOLOGICAL SAFETY

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Keywords: *Salmonella*, rapid detection, implementation, validation

## Introduction

We present the development and application of (IMS-)PCR methods for routine detection of *Salmonella* in specific food matrices. Enrichme techniques, Polymerase Chain Reaction (PCR) and optionally Immunomagnetic Separation (IMS) were combined into fast screening metho adapted to the particular food matrix and the specific requirements of the PCR. The performance of these methods was evaluated i implementation studies with artificially contaminated and fresh samples from the meat production line, and in field trials at plant laboratories.

## *Salmonella* specific PCR

PCR primers were derived from *Salmonella*-specific probes selected from a library of randomly cloned DNA fragments (*Salmonella* subclass I An internal control, consisting of a genetically modified region of the *Salmonella*-specific probe, can be added to the samples before PC analysis. Amplification of this control DNA with the *Salmonella*-specific primers results in a PCR product which is larger than the *Salmonell* specific fragment (figure 1).

## Development of (IMS-)PCR methods

For implementation of our specific PCRs we focused on meat and meat products as well as on swab samples from pig carcasses an slaughterhouse equipment. Three (IMS-)PCR methods for routine detection of *Salmonella* in the meat production line were develope Detection levels were estimated at < 10 cells/swab (pigcarcasses) and 1 cell/25 grams (meat products, even after storage at -20°C for 15 days).

## Implementation

Large numbers (N=207) of fresh swabsamples from fresh pigcarcasses and slaughterhouse equipment were tested with both 44-hours and 2 hours PCR method and with the standard method (figure 2). A good correlation was found between the different methods (≥ 98%). With PC always a few additional *Salmonella* positive samples were found, but no false negatives. With IMS included (IMS-PCR method) results ar available after 25 hours and intenser PCR signals are found for artificially contaminated meat products.

Experimental field trials for the (IMS-)PCR method were conducted in 10 slaughterhouses and meat product factories with 526 samples (tabl 1). Experiments were carried out at the plant laboratories, if available. Special attention was paid to sensitivity, specificity and simplicity of th method for application in the routine laboratory, and to correlation with standard methods. All (IMS-)PCR methods were more sensitive tha the standard methods. *Salmonella* was detected in 63 samples by (IMS-)PCR and results could be confirmed by isolation of the microorganism 7 samples were found negative by the standard method but positive by (IMS-)PCR. However, after prolonged selective culturing an reinvestigation of these samples with standard techniques, *Salmonella* could also be isolated from these samples. Inclusion of IMS into the PC method improved the sensitivity of the assay only slightly for some matrices.

The performance of the developed methods will be statistically evaluated by comparison of the results with the reference method for th detection of *Salmonella* in food (ISO 6579/DIS 3565) for large numbers of fresh samples.

## Conclusions:

- reduction of the time for *Salmonella* detection to 24-25 hours with a PCR based screening method
- improved PCR signal and sensitivity when IMS is included into the PCR method
- ≥ 98% correlation between the standard detection method for *Salmonella* and the (IMS-)PCR methods
- all PCR results could be confirmed by isolation of the microorganism with standard techniques

## Acknowledgement

This work was supported by the Dutch Product Board for Livestock and Meat (PVV) and the Dutch Ministry for Welfare, Public Health an Culture (WVC).

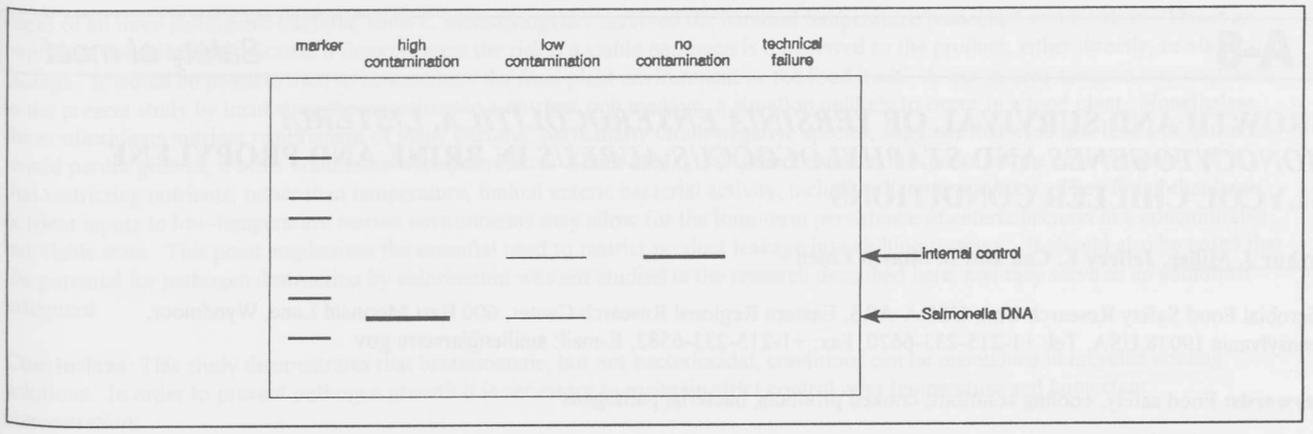


**Figure 1:** Interpretation of the PCR results. Results were visualized by agarose gel electrophoresis and ethidium bromide staining. Modification of the PCR will allow replacement by other visualization techniques in the near future, e.g. colorimetry.

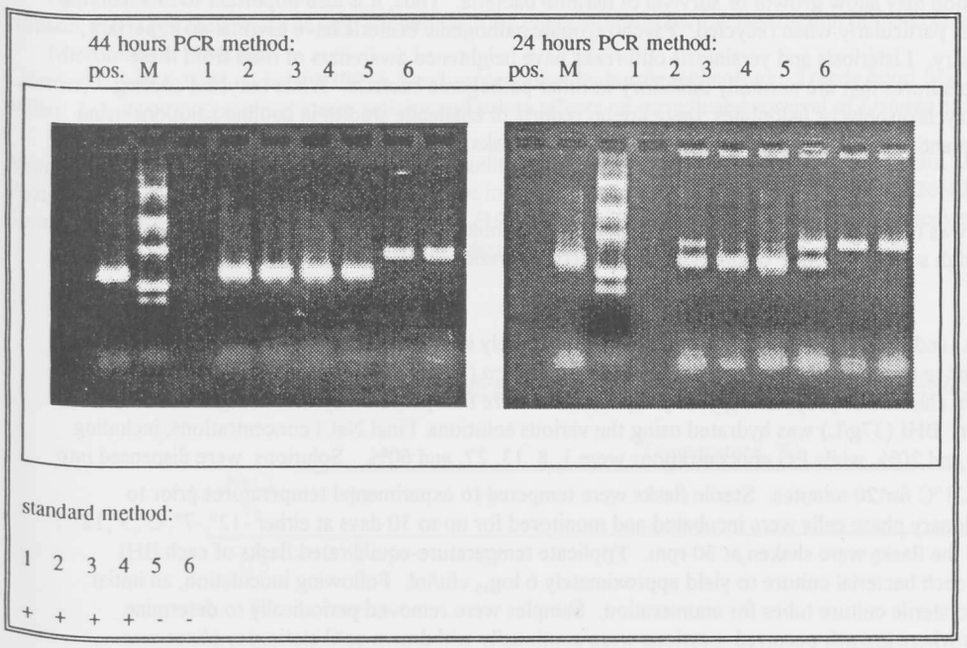


**Figure 2:** Comparison of PCR results for 44-hours PCR method, 24-hours PCR method and standard method for 6 fresh meat products.

| | |
|---|---|
| PCR positive, Standard method positive:<br>PCR positive, Standard method negative: | 63<br>7[1] |
| PCR negative, Standard method negative:<br>PCR negative, Standard method positive: | 456<br>0 |
| Number of investigated samples: | 526 |

**Table 1:** PCR results for 24-hours PCR method and standard method in field trial. 10 food microbiology laboratories were involved. In total 526 samples (meat products, swab samples from carcasses and slaughterhouse equipment) from the production line of pork and beef were investigated. [1] after prolonged selective culturing and reinvestigation of the samples with standard techniques, *Salmonella* could also be isolated from these samples.