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# MANUFACTURING A MODEL MEAT MATRIX TO INVESTIGATE THE SENSITVITY OF RAPID TESTING METHODS ON CAMPYLOBACTER SPP.

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## Introduction

For the examination of meat referring to the occurrence of Campylobacter spp. many different testing methods are developed. Rapid testing methods (e.g. PCR, ELISA) give faster results and are easy to handle compared to cultural methods. The food industry and the administration of food surveillance make use of these advantages. Therefore the validity of the results, got by rapid testing methods, has to be proved by comparing with the results of cultural methods. Validating new methods includes the examination of the sensitivity and specificity. The investigations have to be done with naturally contaminated material and additionally with artificial contaminated materials for receiving the detection limit.

## Objectives

There are no standardized instructions for the production of a reference material, which could be used for the validation of rapid testing methods on Campylobacter spp.. Seasonally poultry meat has a high contamination rate with Campylobacter spp.. To produce reference material poultry meat would be suitable as a model matrix.

## Methodology

For the testing procedure a mixture of poultry meat (turkey, chicken) was minced (3 mm). The absence of *Campylobacter spp.* was examined using cultural and rapid testing method.

Until the tests started the meat was stored at -18° C and was defrosted at 2° to 4° C 24 hours before use. Preliminary tests (without adding the test germ) were made in order to find out which machine is more suitable to get the best homogenisation. The meat was mixed/chopped in the universal mixing machine UM12 (Company Stephan, Germany) or in the 10L circuit-cutter (chopper) (Company Mueller, Germany). The selection of the machine which should be used for further procedure was made by comparing the cutting result and the rising of the temperature.

In further tests 1.8 kg meat was artificially contaminated with *Campylobacter jejuni ssp. jejuni* DSM 4688 (ATCC 33560) and chopped by using the 10L circuit-

cutter (chopper). Additionally 0.2 kg ice was added. A germ-suspension of 20 ml was inoculated, so that the matrix contained about 3.2 $x10^4$ to 5.0 $x10^6$ cfu/g *Campylobacter jejuni*. The matrix was chopped at knife-speed 1500 rpm. After 50, 100, 200 seconds 10 g samples were taken, diluted into 90 ml 0,1% buffered Peptone and mixed by stomacher (lab blender Seward Ltd., UK). The dillution was investigated quantitatively on Karmali-Agarplates by using the spiral-plater (Meintrup DWS, Germany). The Agarplates were incubated microaerophilic at 42° C for 48h. The evaluation of the characteristic colonies was done by analysis equipment ProtoCOL (Meintrup DWS, Germany).

In the main tests the meat matrix was handled the same way (1.8 kg meat + 0.2 kg ice). The inoculation level of *Campylobacter jejuni* was about 4.2 x $10^2$ to 4.1 x $10^{-1}$ cfu/g meat matrix. The chopping time at 1500 rpm took 150 seconds.

Afterwards samples of 25 g were diluted in 225 ml Preston-broth for qualitative determination. Additionally quantitative investigation of the enrichment broth was made by spiral-plating on Karmali Agarplates. The broth and the agarplates were incubated microaerophilic at 42° C for 48h.

## Results & Discussion

In the preliminary tests the temperature gradient in the meat matrix was determined during processing with the UM12 and the circuit cutter. The temperature of the meat was higher by using the UM12 machine (fig. 1). The circuit-cutter had a lower temperature increase and showed a better homogenity of the meat. The preliminary tests showed that circuit-cutter is assessed as the better system to produce the model meat matrix.

Further tests showed a good distribution of the microorganism after short chopping time. The germ concentration varied within tolerable ranges in the meat matrix. The determinated standard-deviation of log cfu/g were from s = 0.05 to 0.24. A longer chopping time showed, that the isolated colonie forming units (cfu) decreased (tab.1). A reason could be that the matrix was warming up and the intake of oxigen, which might transfere *Campylobacter* cells into VNBC-form.

In the main tests the qualitative determination in all samples was performed by a minimum inoculated concentration of *Campylobacter jejuni* of 4.0 x $10^0$ cfu/g. At the given conditions meat with a contamination rate lower than 4.0 x $10^0$ cfu/g showed culturally positive and negative results (tab.2).

The incubation of the enrichment broth for 24 hours determines presumtive positive results. If the enrichment broth was incubated for 48 hours, presumptive results would be significant positive with a contamination rate of *Campylobacter jejuni* over 1 x $10^4$ to 1 x $10^7$ cfu/ml. Therefore a 48 hour enrichment step is recommended according to the comparison of the sensitivity of detection methods.

If technological parameters are not adjusted, e. g. temperature of the meat below 0° C, stress might have an effect on the organisms and this could lead to negative results for meat with an even higher contamination rate. By optimisation of technological parameters the qualitative determination in artificial contaminated meat might possible be at a lower rate than 4.0 $x10^0$ cfu/g.

## Conclusions

The suitability of the circuit-cutter (chopper) for producing a meat matrix as a reference material for the determination of Campylobacter jejuni has been shown. The

samples could be used to investigate the sensitivity of rapid screening methods. The results of the cultural method are crucial for the determination of the sensitivity of rapid screening methods.

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## Tables and Figures

Table 1. quantitative determination after chopping

| Test No. | chopping time (1R =5 Sek.) | samples (n) | average cfu/g | standard-deviation log10 –transformed (s) |
|---|---|---|---|---|
| B | 10 | 6 | $3,6 \times 10^6$ | 0,23 |
| | 20 | 6 | $4,8 \times 10^6$ | 0,05 |
| | 40 | 6 | $3,4 \times 10^6$ | 0,06 |
| C | 10 | 6 | $2,8 \times 10^6$ | 0,06 |
| | 20 | 6 | $1,8 \times 10^6$ | 0,05 |
| | 40 | 6 | $1,4 \times 10^5$ | 0,24 |
| D | 10 | 6 | $1,0 \times 10^5$ | 0,05 |
| | 20 | 6 | $7,9 \times 10^4$ | 0,04 |
| | 40 | 6 | $5,0 \times 10^4$ | 0,08 |
| E | 10 | 6 | $1,6 \times 10^5$ | 0,04 |
| | 20 | 6 | $6,5 \times 10^4$ | 0,04 |
| | 40 | 6 | $2,6 \times 10^4$ | 0,20 |
| F | 10 | 10 | $1,2 \times 10^4$ | 0,09 |
| | 20 | 10 | $8,9 \times 10^3$ | 0,07 |
| | 40 | 10 | $5,7 \times 10^3$ | 0,07 |
| G | 10 | 10 | $1,0 \times 10^4$ | 0,14 |
| | 20 | 10 | $5,6 \times 10^3$ | 0,07 |
| | 40 | 10 | $3,1 \times 10^3$ | 0,10 |

Table 2. qualitative determination of *Campylobacter jejuni* in the main tests

| Test No. | conc. inoculation (cfu/ml) | calculated average conc. in meat matrix (cfu/g) | samples (n) | 24 h enrichment positive samples | 48 h enrichment positive samples |
|---|---|---|---|---|---|
| H | $4{,}2 \times 10^4$ | $4{,}2 \times 10^2$ | 6 | 6 | 6 |
| I | $3{,}1 \times 10^4$ | $3{,}1 \times 10^2$ | 6 | 6 | 6 |
| J | $5{,}6 \times 10^3$ | $5{,}6 \times 10^1$ | 6 | 5 | 6 |
| K | $3{,}0 \times 10^3$ | $3{,}0 \times 10^1$ | 6 | 5 | 5 |
| L | $4{,}5 \times 10^2$ | $4{,}5 \times 10^0$ | 6 | 1* | 2 |
| M | $4{,}0 \times 10^2$ | $4{,}0 \times 10^0$ | 6 | 6 | 6 |
| N | $8{,}5 \times 10^2$ | $8{,}5 \times 10^0$ | 12 | 12 | 12 |
| O | $5{,}2 \times 10^1$ | $5{,}2 \times 10^{-1}$ | 12 | 3 +3* | 6 |
| P | $4{,}0 \times 10^1$ | $4{,}0 \times 10^{-1}$ | 12 | 0 +12* | 5 +7* |
| Q | $5{,}7 \times 10^1$ | $5{,}7 \times 10^{-1}$ | 20 | 1 +3* | 8 +10* |

* presumptive

**Figure 1. Temperature gradient by mixing/chopping the meat matrix in preliminary tests**