Quantitative detection of *Campylobacter jejuni* on fresh chicken carcasses by real-time PCR

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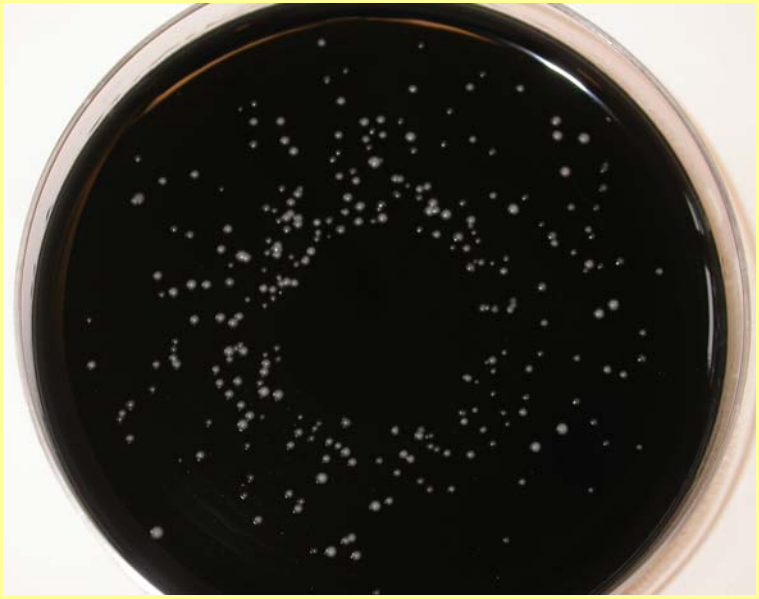
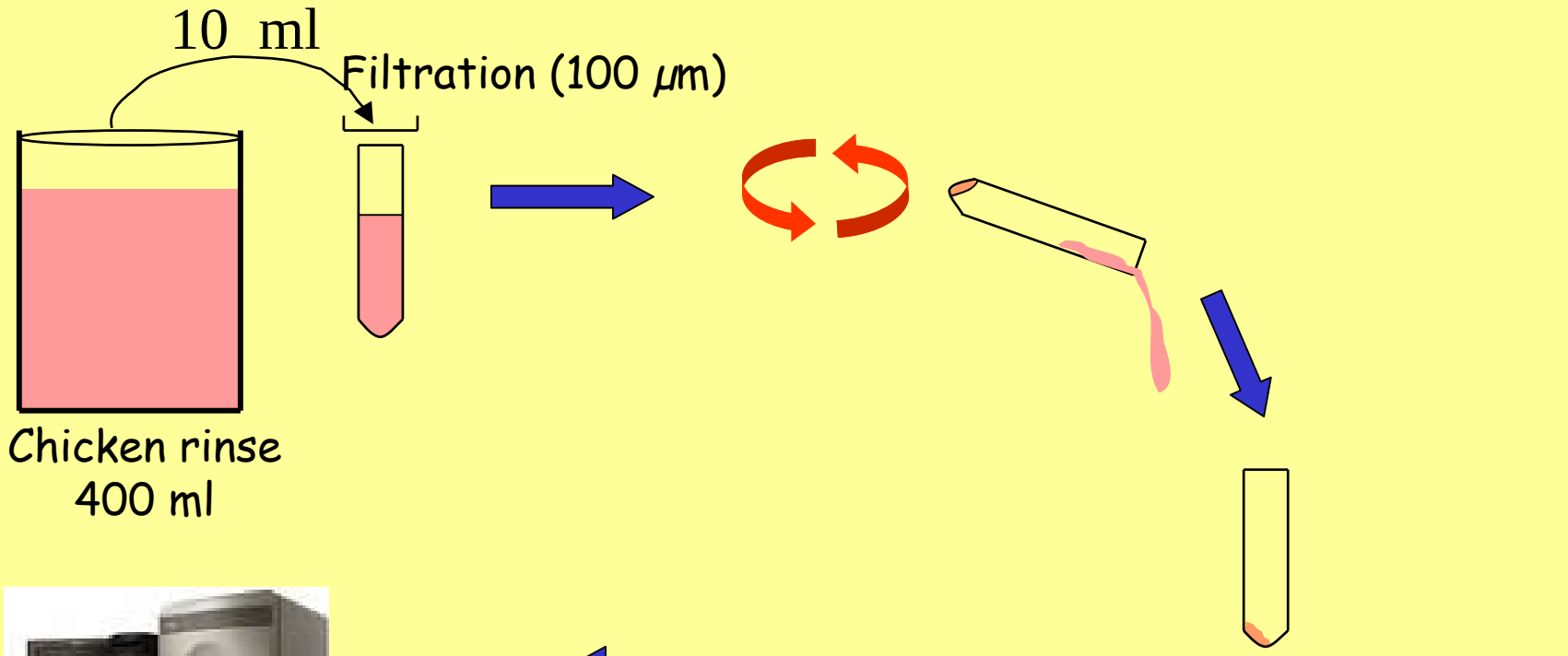
Problems with traditional direct plating on mCCDA

- Overgrowth by other species
- Growth of campylobacter-like colonies
- Swarming



Extraction of total DNA from chicken rinse



APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Sept. 2000, p. 4029–4036 Vol. 66, No. 9
0099-2240/00/$04.00+0

Application of the 5′-Nuclease PCR Assay in Evaluation and Development of Methods for Quantitative Detection of *Campylobacter jejuni*

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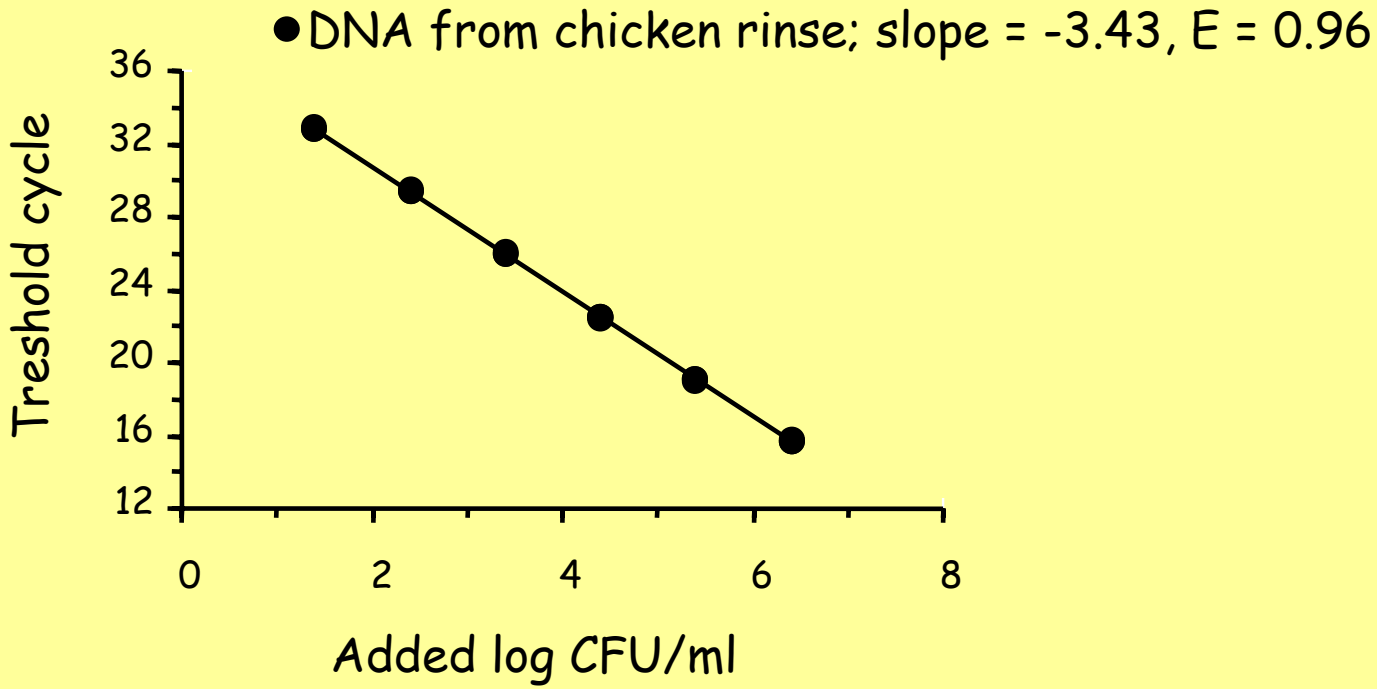
Received 22 February 2000/Accepted 11 July 2000

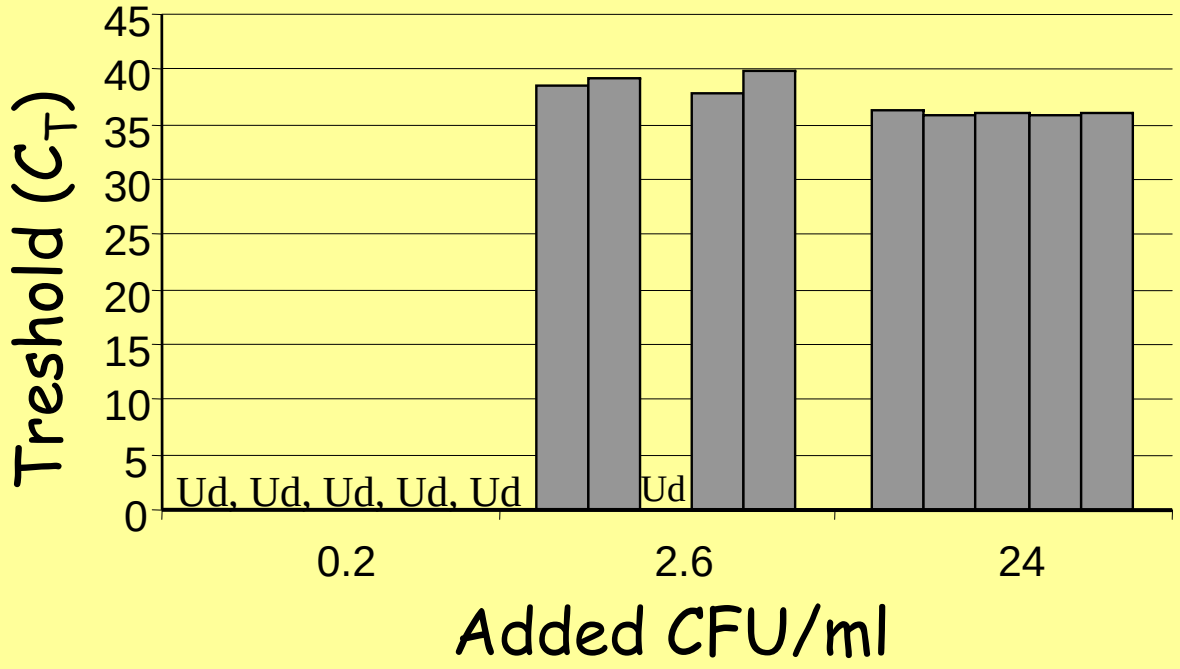
Campylobacter jejuni is recognized as a leading human food-borne pathogen. Traditional diagnostic testing for _C. jejuni_ is not reliable due to special growth requirements and the possibility that this bacterium can enter a viable but nonculturable state. Nucleic acid-based tests have emerged as a useful alternative to traditional enrichment testing. In this article, we present a 5′-nuclease PCR assay for quantitative detection of _C. jejuni_ and describe its evaluation. A probe including positions 381121 to 381206 of the published _C. jejuni_ strain NCTC 11168 genome sequence was identified. When this probe was applied, the assay was positive for all of the isolates of _C. jejuni_ tested (32 isolates, including the type strain) and negative for all other _Campylobacter_ spp. (11 species tested) and several other bacteria (41 species tested). The total assay could be completed in 3 h with a detection limit of approximately 1 CFU. Quantification was linear over at least 6 log units. Quantitative detection methods are important for both research purposes and further development of _C. jejuni_ detection methods. In this study, we used the assay to investigate to what extent the PCR signals generated by heat-killed bacteria interfere with the detection of viable _C. jejuni_ after exposure at elevated temperatures for up to 5 days. An approach to the reduction of the PCR signal generated by dead bacteria was also investigated by employing externally added DNases to selectively inactivate free DNA and exposed DNA in heat-killed bacteria. The results indicated relatively good discrimination between exposed DNA from dead _C. jejuni_ and protected DNA in living bacteria.

The efficiency of the PCR reaction

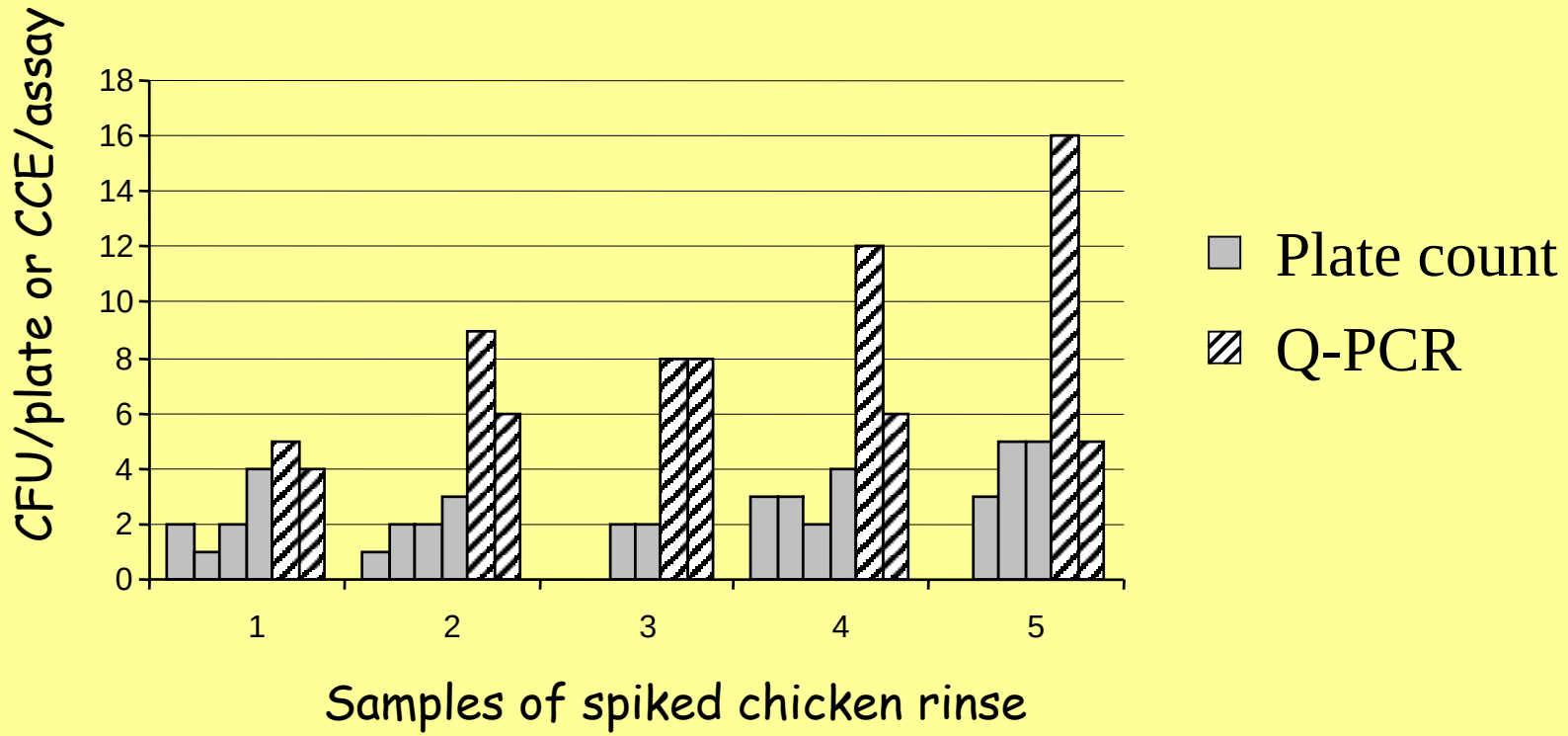


Detection limit

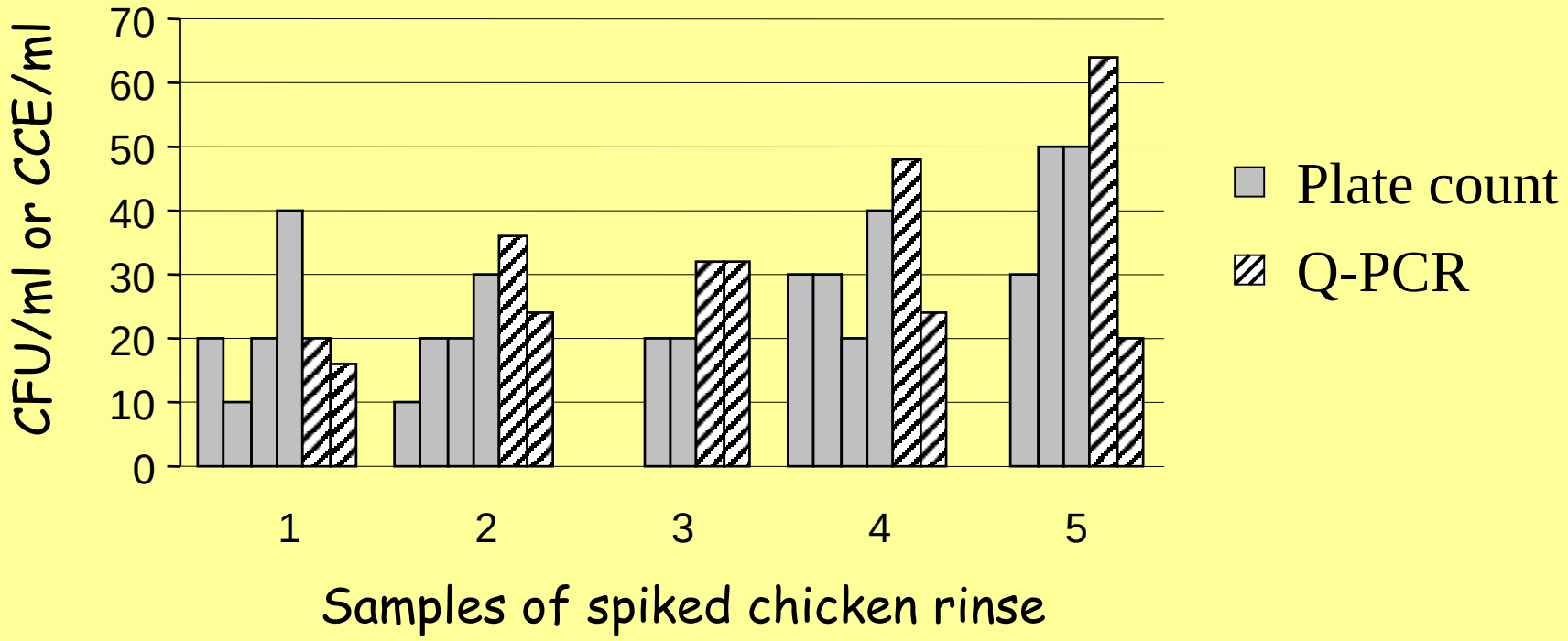
Theoretical limit = 4 bacteria per ml chicken rinse



Detection of a low number of *C. jejuni* by both direct plating and real-time PCR



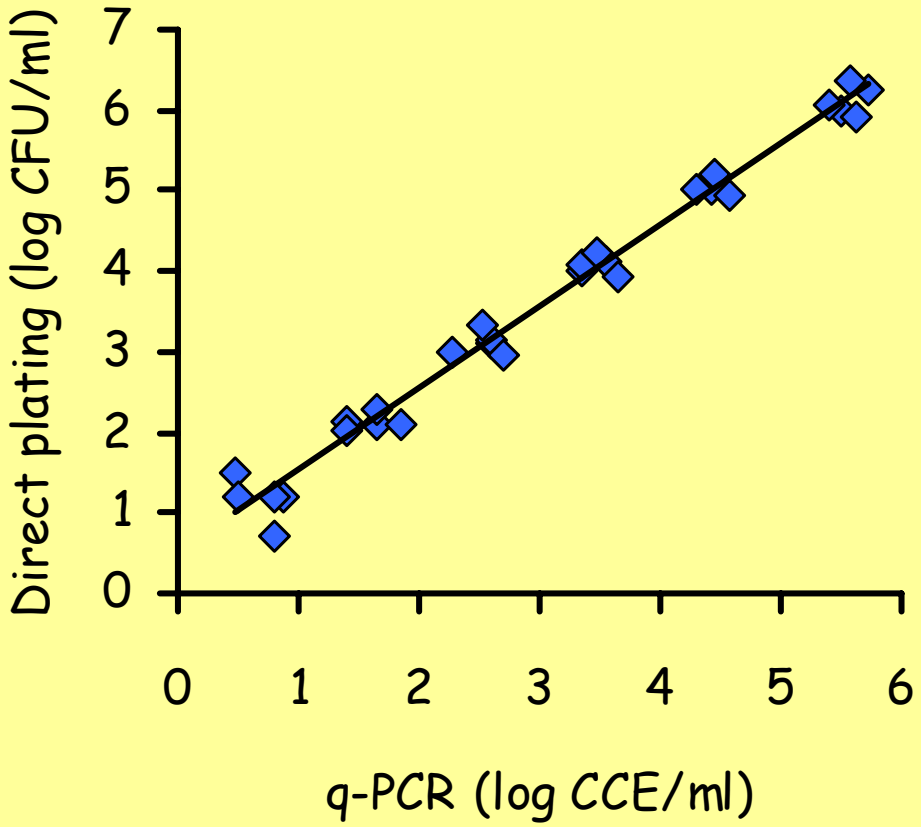
Detection of a low number of *C. jejuni* by both direct plate and real-time PCR



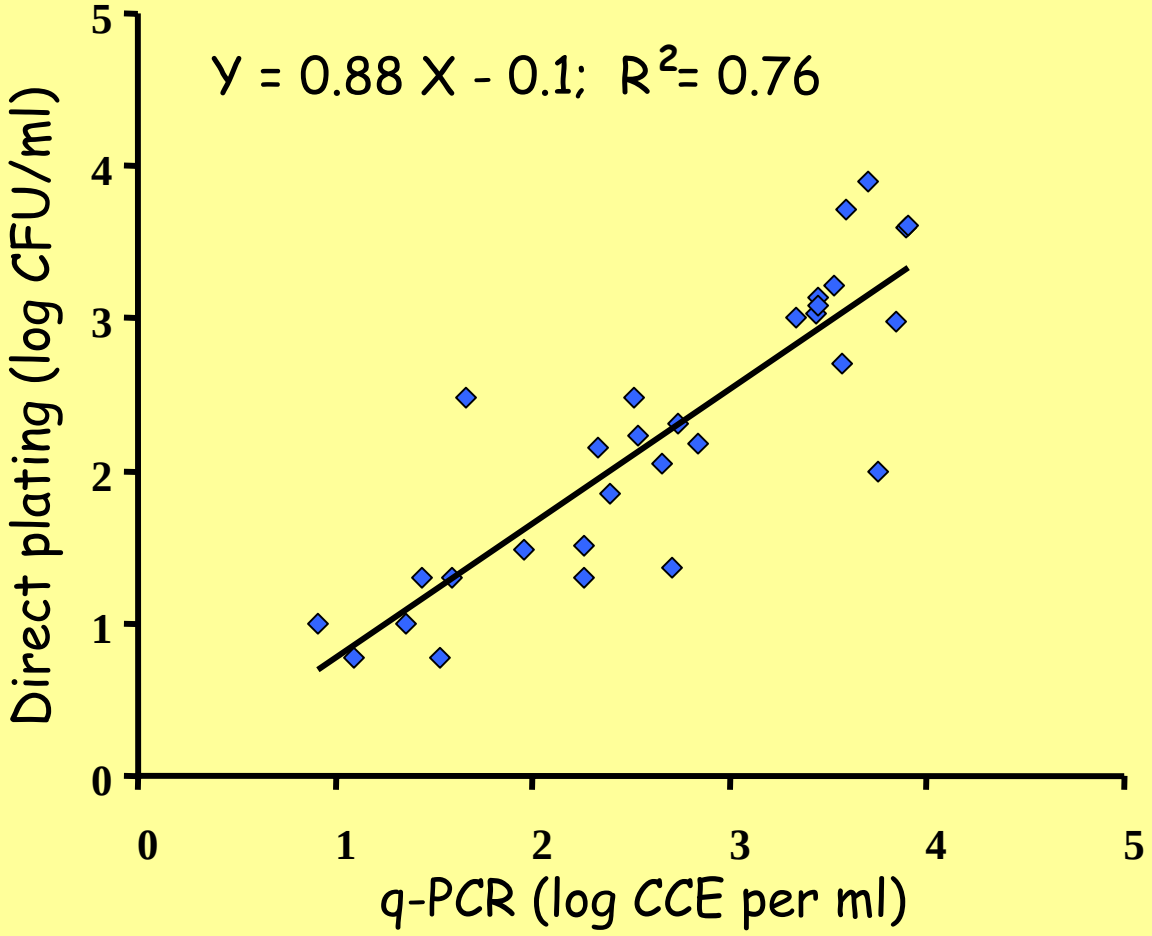
Correlation between direct plating and q-PCR

$R^2 = 0.98$

$Y = 0.51 + 1.0X$



30 positive carcasses sampled at slaughter

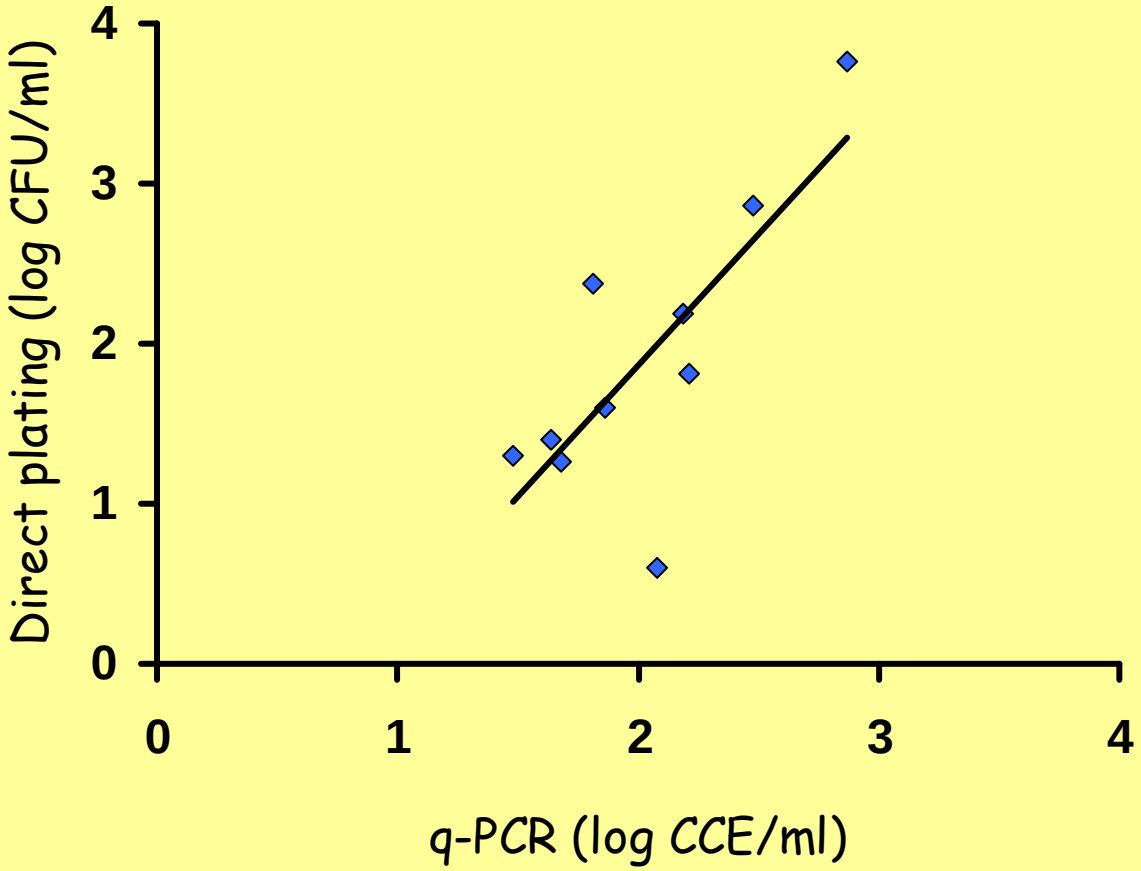


Analysis of 45 carcasses from retail

		Real-time PCR: Positive	Real-time PCR: Negative
Plate count	Positive	13	1
	Negative	6	25

Carcasses sampled at retail

$Y = 1.6X - 1.4$; $R^2 = 0.58$



Conclusions

- The sensitivity of the developed method is similar to direct plating.

- The correlation of the two methods is good when spiked or samples directly from slaughter are analysed.

- The correlation is less good with samples from retail, probably due to larger variation of dead and/or VNC *Campylobacter* between the samples.

- The purified DNA can be used for detection and quantification of other species, *e.g. Salmonella*.

Acknowledgments

The presented work was performed by:

- Anna-Clara Rönner, The Swedish institute for food and biotechnology
- Paula Ågren, National Food Administration
- Hans Lindmark, National Food Administration