Using 3D laser scanning microscopy for the absolute quantification of viable *Campylobacter* spp. in chicken rinse matrix

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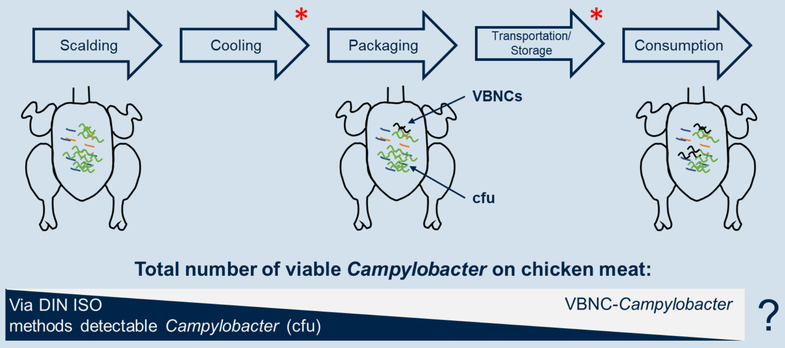
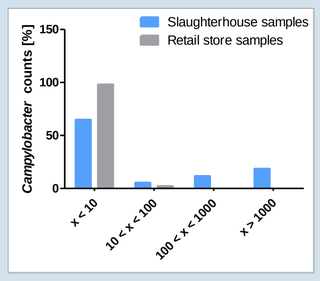




Distribution of *Campylobacter* spp. counts (x) in neck skin samples from broilers at the slaughterhouse or fresh chicken meat from the retail store (cfu/g)[1]

Campylobacter spp. on chicken meat

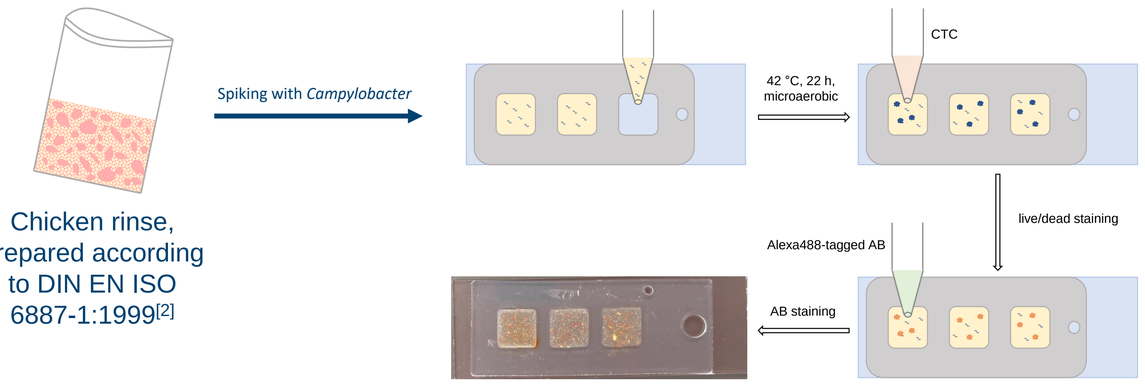
- Chicken meat is the main source for campylobacteriosis infection
- Discrepancy: High number of campylobacteriosis cases and few highly contaminated chicken meat samples (x > 1000 cfu/g)
- VBNC formation as a possible explanation: **V**iable **B**ut **N**on-**C**ulturable *Campylobacter* are not detectable by cultivation-dependent routine laboratory analysis

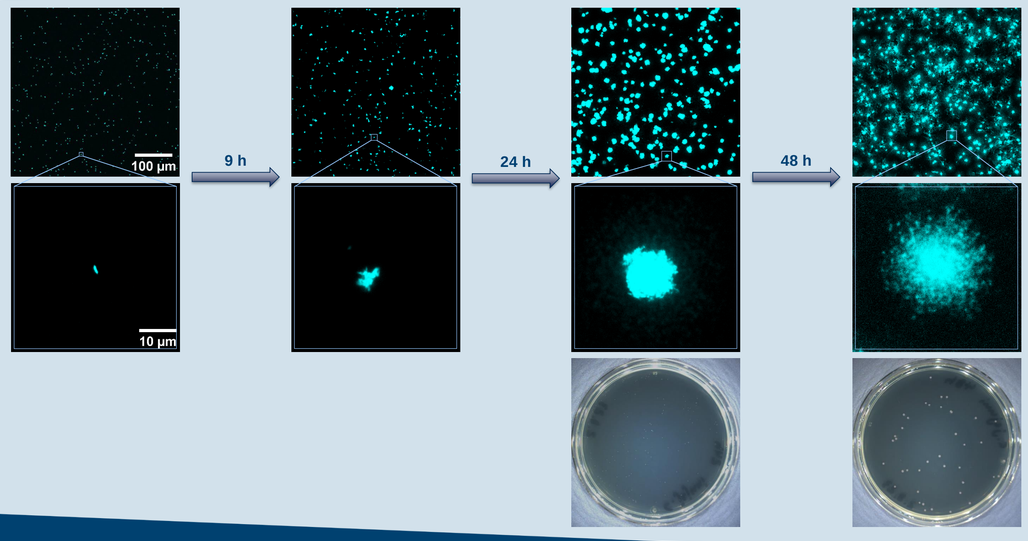
Objective:

Development of a quantification method for viable *Campylobacter* spp. in chicken meat samples with fluorescence microscopy, combining live-dead and genus-specific antibody staining.

Sample preparation workflow

- Preparation of chicken rinse according to DIN EN ISO method; spiking with a known number of living *Campylobacter* cells and the 10-fold amount of dead cells
- Microcolony growth in an agarose-based growth matrix under microaerobic conditions
- Staining of microcolonies (µCFU) and living single cells (VBNCs) with the tetrazolium salt CTC, an indicator for metabolic activity, for live-dead discrimination
- Staining of all *Campylobacter* cells and µCFU with a *Campylobacter*-specific, fluorophore-tagged antibody (AB)





Time-dependent µCFU growth

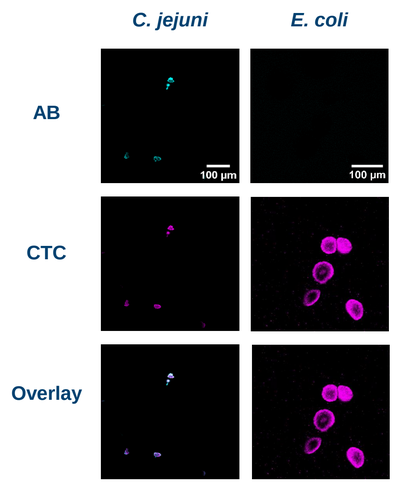
- Using 25x magnification, AB stained *Campylobacter* single cells (1-2 µm) as well as microcolonies (10-30 µm) can be visualized in 3D scans via confocal laser scanning microscopy (CLSM)
- First microcolony-like structures are visible after a few hours
- Best microcolony size for quantitative analysis is reached after 18-24 h, when colonies on Bolton agar plates are barely visible
- DIN EN ISO 10272-2:2017[3] plate counting method takes 48 h of incubation time, that's when microcolonies are already growing into each other and are no longer quantifiable

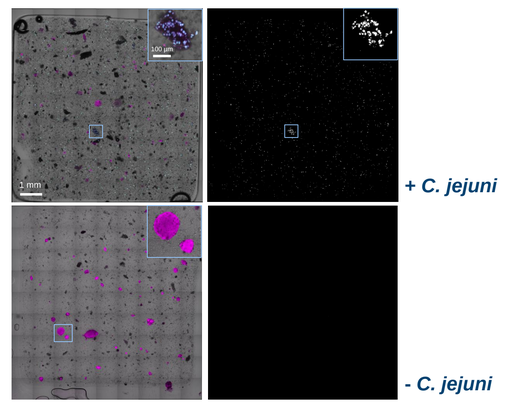
→ Time savings using the CLSM µCFU analysis method

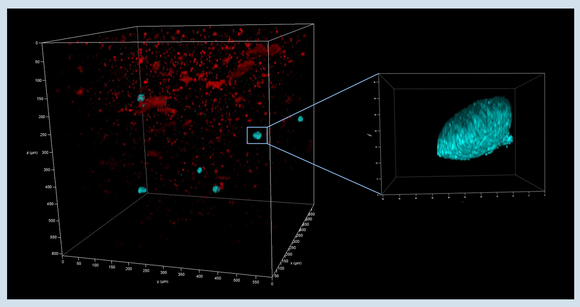
Specific detection of live *Campylobacter* spp. in complex 3D chicken rinse matrix

- Genus-specific antibody binding in Bolton broth and in complex chicken rinse matrix enables clear differentiation between *Campylobacter* cells/µCFU and other bacteria/components of the chicken rinse, that are stained with the viability marker CTC
- *Campylobacter* microcolonies grown on chicken rinse particles can be visualized and differentiated from the matrix and therefore quantified in 3D CLSM scans

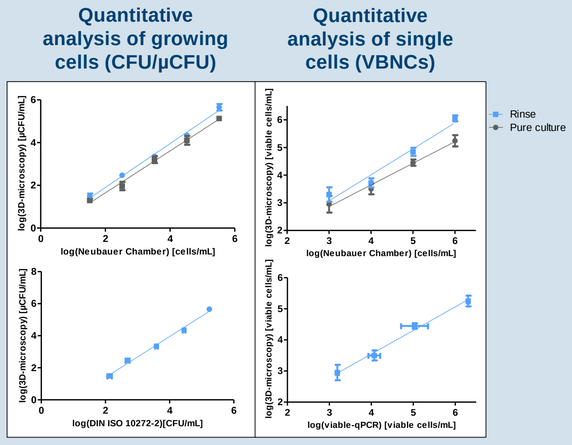
→ Possibility to detect µCFU and VBNCs simultaneously with enhanced resolution







3D CLSM scan of *C. jejuni* µCFU (cyan) in chicken rinse (red)



Quantification of viable *Campylobacter* cells (µCFU and VBNC)

- Live-dead discrimination could be achieved quantitatively in serial tenfold dilutions of living *C. jejuni* cells mixed with the 10-fold amount of dead cells.
- Absolute quantification was linear for µCFU formation within a range of ~10^1-10^5 µCFU/mL and for live single cells in a range of ~10^3-10^6 cells/mL, both in pure culture (Bolton broth) and chicken rinse.

[1] BVL (2023): Berichte zur Lebensmittelsicherheit –Zoonosen-Monitoring 2022. www.bvl.bund.de/ZoonosenMonitoring
[2] ISO 6887-1:1999, Microbiology of food and animal feeding stuffs - Preparation of test samples, initial suspension and decimal dilutions for microbiological examination - Part 1: General rules for the preparation of the initial suspension and decimal dilutions. International Organization for Standardization, Geneva, Switzerland.
[3] ISO 10272-2:2017, Microbiology of the food chain - Horizontal method for detection and enumeration of *Campylobacter* spp. - Part 2: Colony-count technique. International Organization for Standardization, Geneva, Switzerland.