Update on protocols

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NEW: Quantification of ESBL/AmpC-producing *E. coli* in caecal content and fresh meat samples

Commission Implementing Decision 2013/652/EU also includes voluntary assessment of the proportion of ESBL-/AmpC-producing *E. coli* within the whole *E. coli* population of a sample according to the most recent version of the protocol of the EURL-AR (point 4.3 of the Annex). Within-sample quantification of ESBL-/AmpC-producing *E. coli* is particularly relevant for MSs that detected a high prevalence of samples positive for ESBL-/AmpC-producing *E. coli*.


DTU Food
National Food Institute

LABORATORY PROTOCOL

Quantification of ESBL/AmpC-producing *Escherichia coli* in caecal content and fresh meat samples

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Version 1

Authored by the EURL-AR

NEW: Quantification of ESBL/AmpC-producing *E. coli* in caecal content and fresh meat samples

Material:

Caecal content: pig, cattle, chicken

Meat from: pig, cattle, chicken

Pre-test to assess presence of ESBL/AmpC-producing *E. coli*

Preparation of **spiking solutions**:

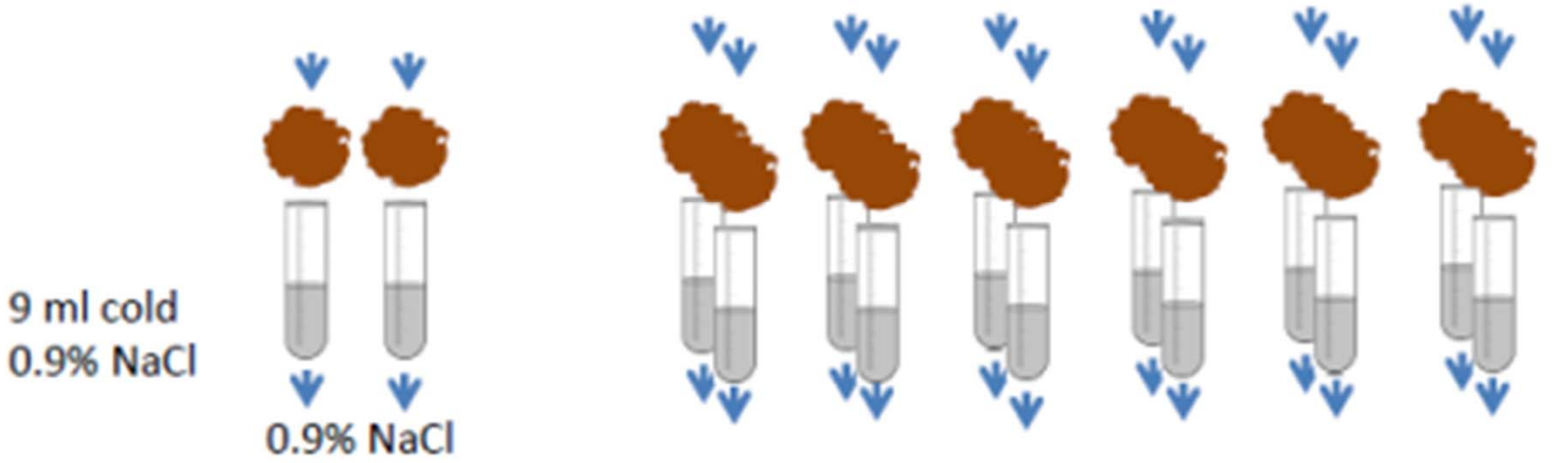
- Three *E. coli* types (*E. coli* CTX-M-1; *E. coli* CMY-2; *E. coli* ATCC 25922)
- Six concentrations (from 10^7 to 10^2 CFU/ml) per each *E. coli* type

Spiking the caecal and meat samples

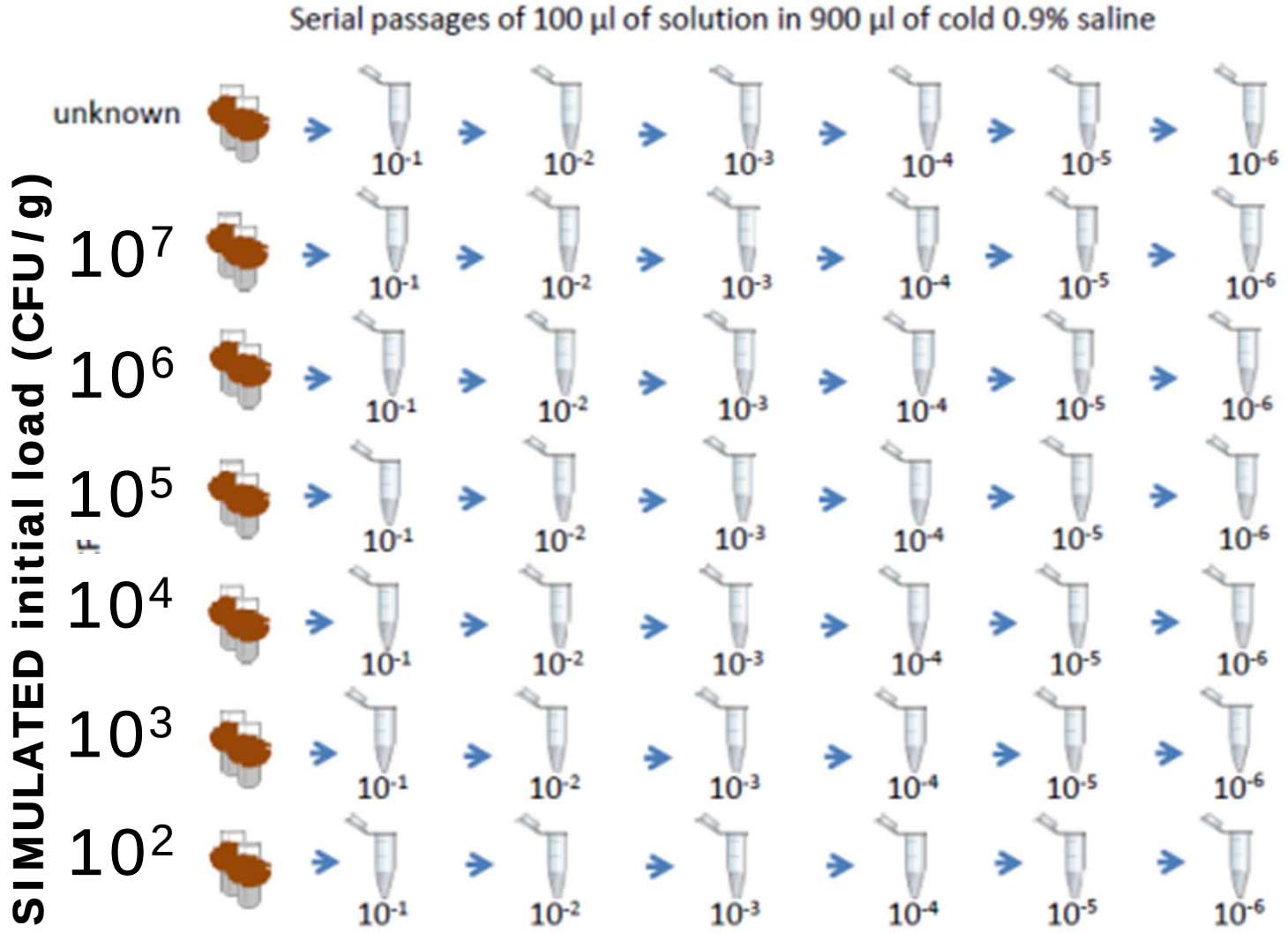
A) 1 g of caecal content or meat in 9 ml 0.9% cold saline - each step is performed in duplicate

B) 1 ml of sterile 0.9% saline is added to one set of tubes

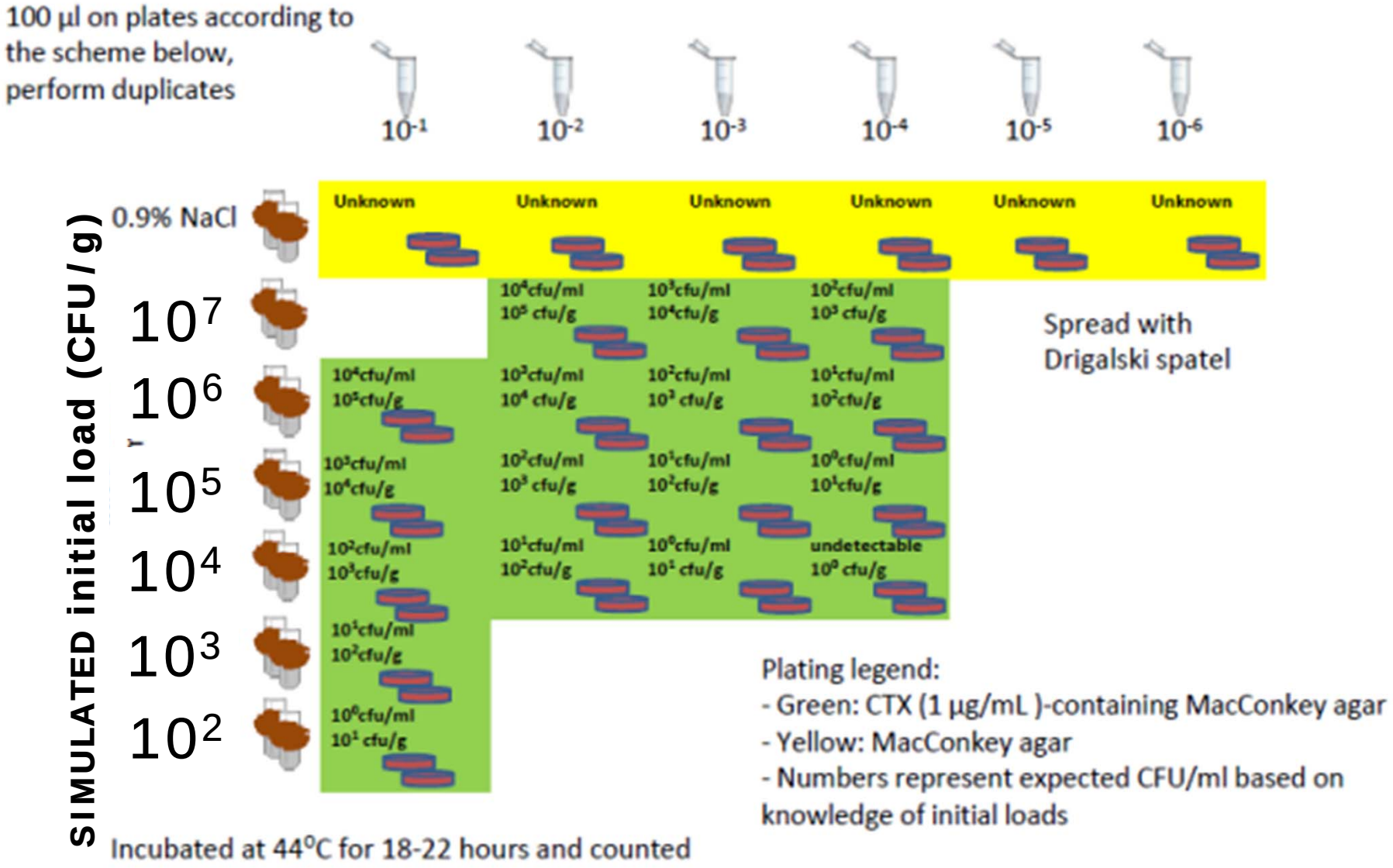
C) 1 ml of the different spiking dilutions is added to different sets of tubes to simulate different initial bacterial loads



Serial dilutions of the samples (original and spiked)



Plating of relevant dilutions



Results and Conclusions

- CTX-M-1- and CMY-2-positive *E. coli* recovered from all matrices spiked (difference for pig caecal)

- Detection limit: ~ 10^3 CFU/g

IV. This protocol is mainly designed to be applied in case of loads of ESBL/AmpC-producing *E. coli* in the range 10^3-10^8 CFU/g, thus is useful in those situation with high prevalence of samples positive for ESBL-/AmpC-producing *E. coli*. Given the current

V. This protocol is not intended for counts of carbapenemase-producing *E. coli* (CPE). At present, CPE occur at low prevalence in food animals and meat in EU Member States, thus highly sensitive methods such as selective enrichment are currently recommended for detection of CPE. Selective enrichment methods alter the relative proportion of bacteria within samples and therefore are not suitable to downstream calculations of CFUs.

THANK YOU FOR YOUR ATTENTION!