WAGENINGEN
UNIVERSITY & RESEARCH

Nederlandse Voedsel- en
Warenautoriteit
Ministerie van Economische Zaken

Wageningen Food Safety Research (WFSR)

Rapid detection and differentiation of *Salmonella* species, *S.* Typhimurium and *S.* Enteritidis by multiplex Real-time PCR

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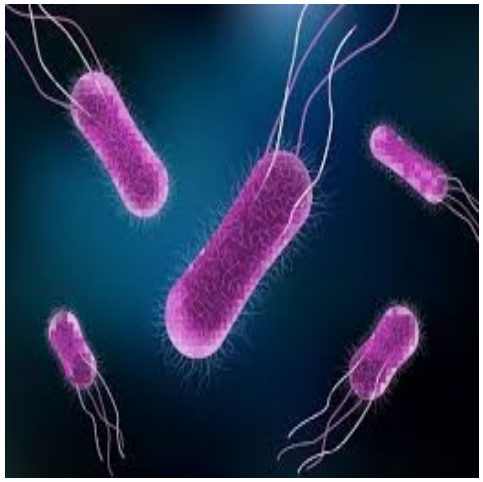
Team bacteriology K&E

Salmonella

Different strategies:

- *Salmonella* spp.
  - processed poultry meat and other food samples

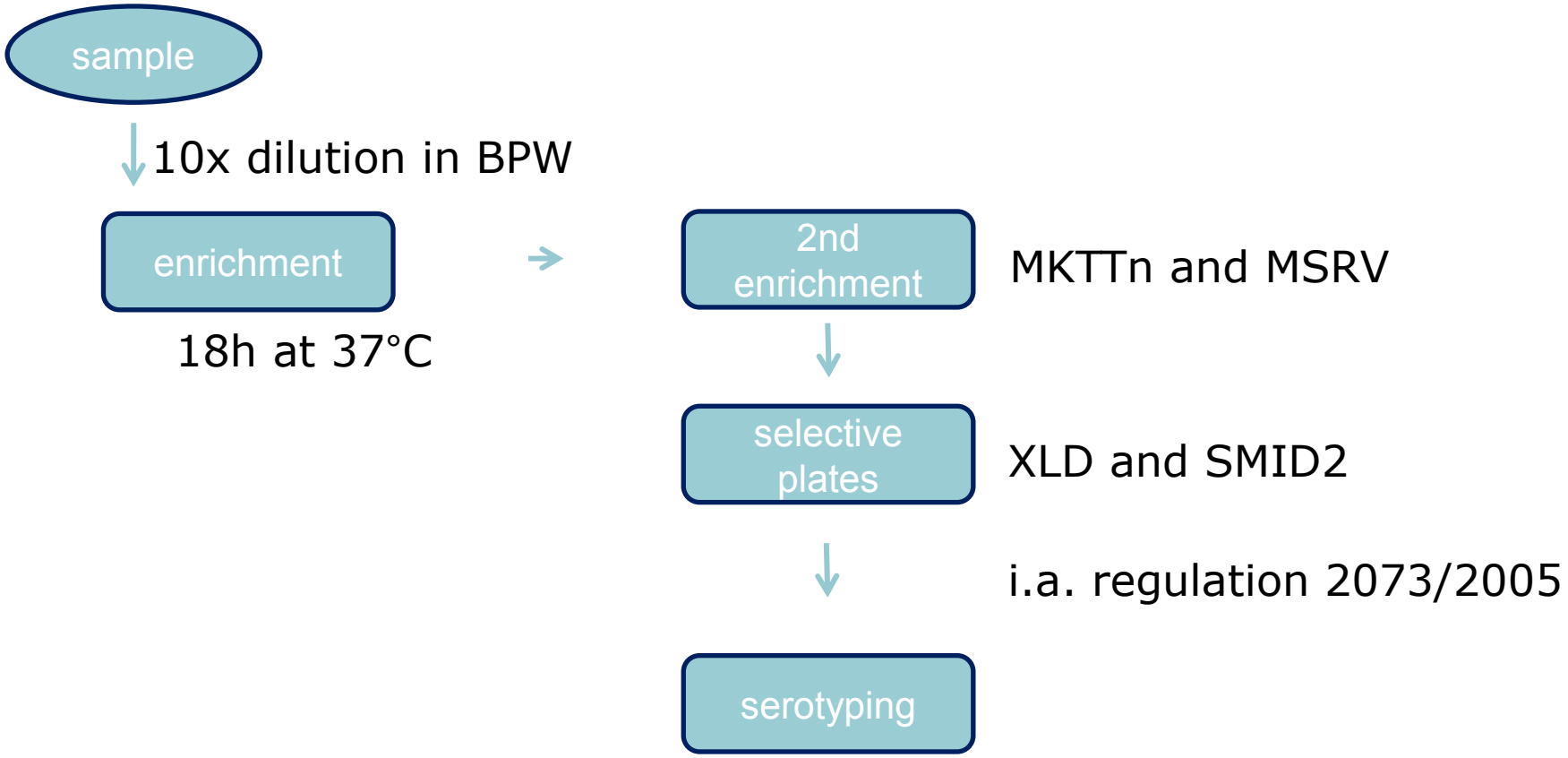


- *Salmonella* top 2 serotypes
  - Enteritidis and Typhimurium
  - unprocessed poultry meat

Farm monitoring:

- 6 serotypes of importance
  - Enteritidis, Typhimurium (incl. monoph.), Hadar, Infantis, Virchow and Java

Salmonella ISO protocol ISO 6579-1



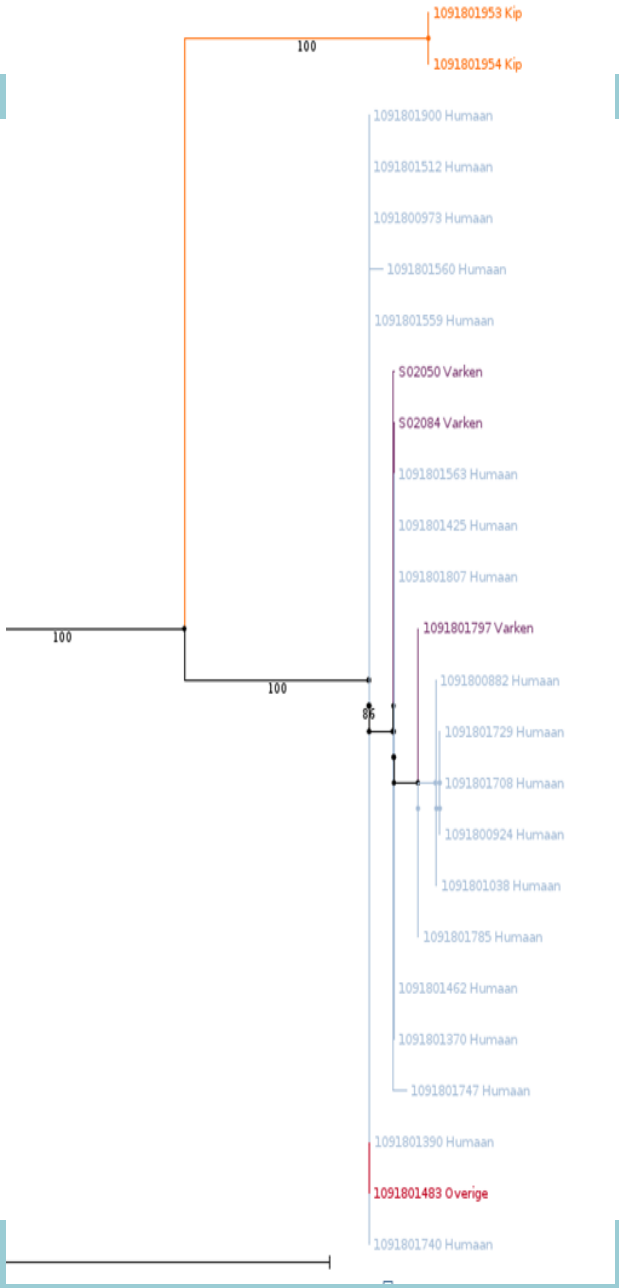
Salmonella serotyping (WFSR approach)

- serotyping by Check&Trace (over 300 serotypes)
  - Includes all main serotypes
  - microarray based detection

- Additional serotype isolates are send to Public health institute (RIVM)
  - SE and ST are additionally typed by MLVA

Salmonella WGS

- Use of Whole-genome Sequencing analysis
- Quick identification of source
- Collaboration with RIVM (human – food)



Salmonella monitoring 2018

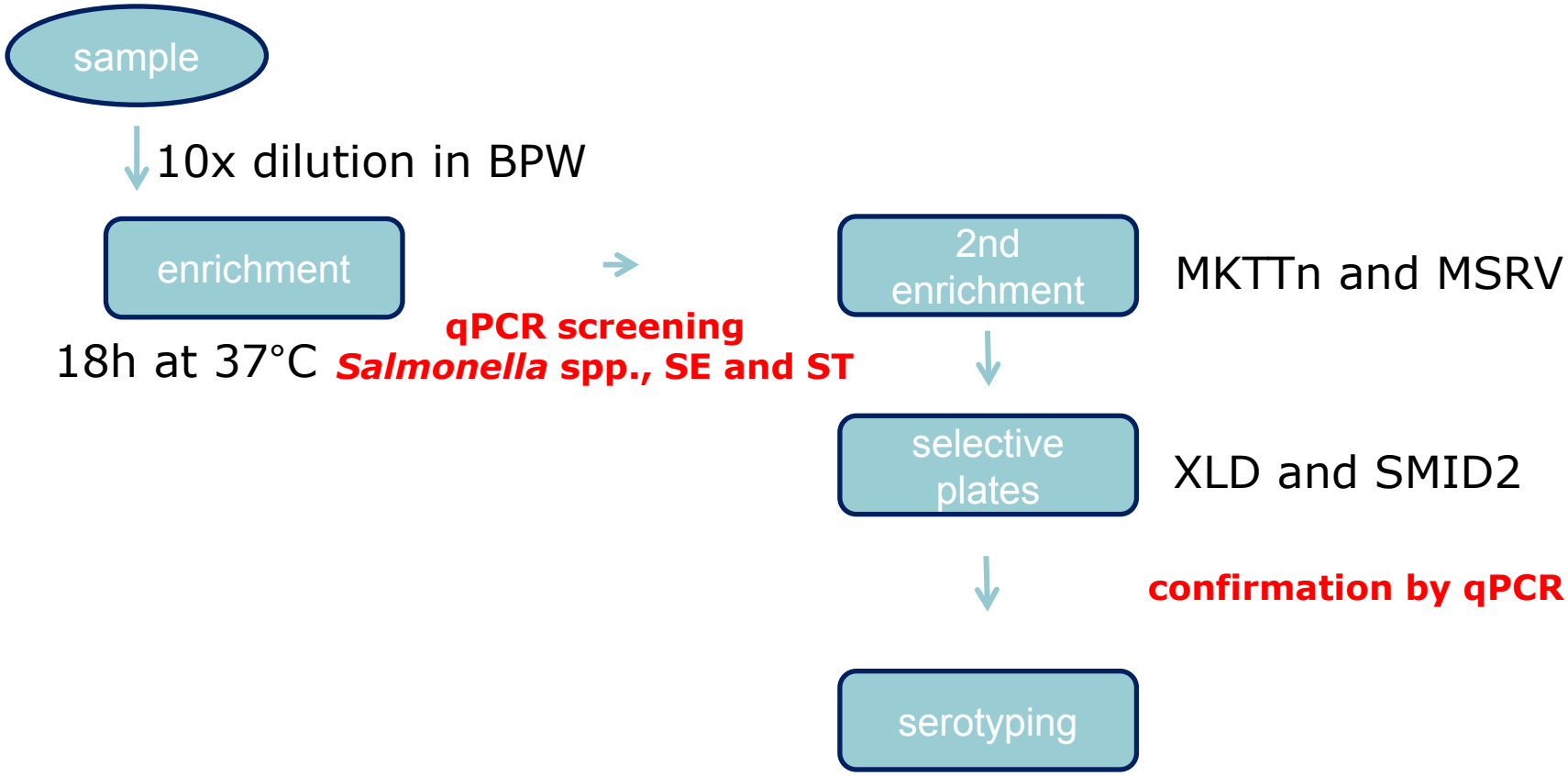
- Almost 12000 samples tested – 370 positive (3%)

- Meat
  - porcine, bovine, poultry, small ruminants, exotic meat
- Vegetables, herbs and spices
- Fish and sea products
- National and import

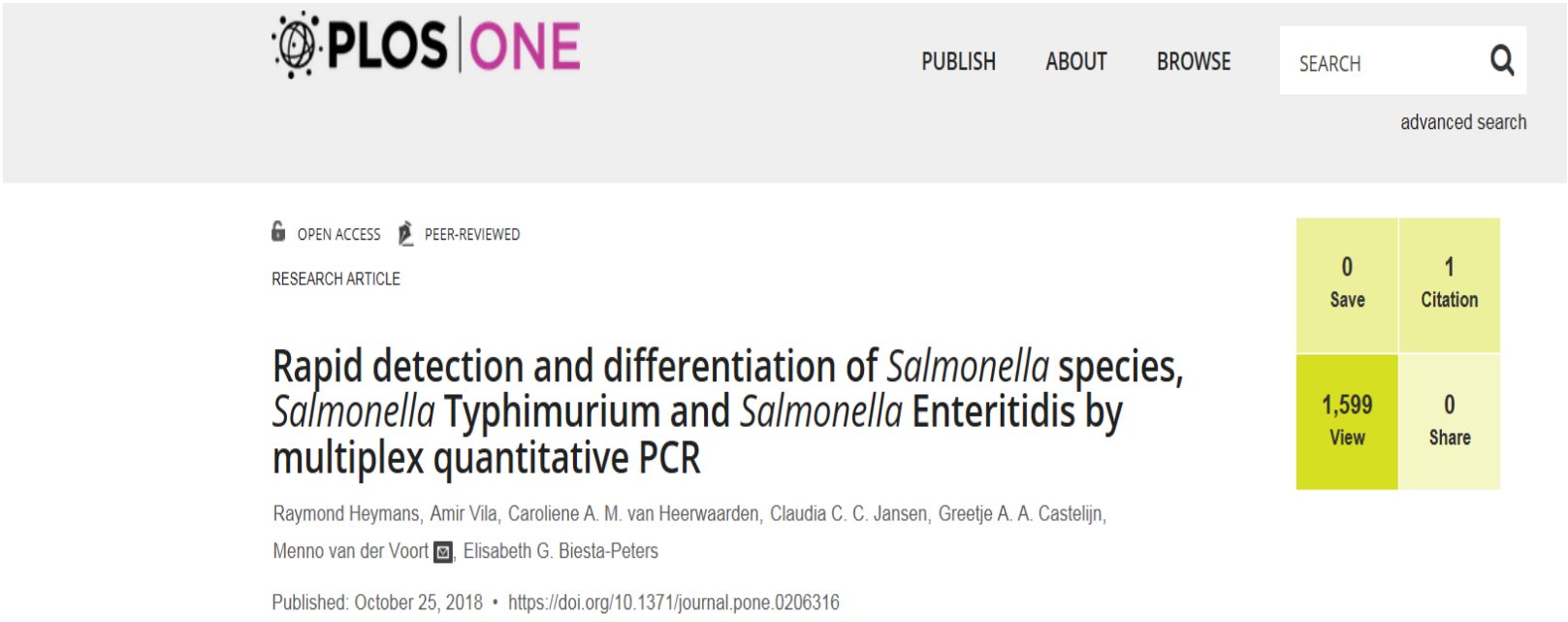
→ retail, warehouse, slaughterhouse

→ conventional, organic

Salmonella ISO protocol ISO 6579-1



Publcation of PCR



Primer & probe design

Target	Gene	Function	PCR product size(bp)
Salmonella spp.	*invA*	Invasion protein	95
S. Typhimurium (ST)	STM4200	Putative phage tail fiber protein	101
S. Enteritidis (SE)	SEN1392	Predicted phage protein	77

PCR primer/probe specificity

- *InvA* gene for *Salmonella* spp.
  - Target analysed in 30 scientific paper
  - Total 4023 isolates of *Salmonella* spp. and 758 non *Salmonella* spp. isolates screened
  - **Inclusivity: >99,7%,** 11 isolates with different serovars negative
  - **Exclusivity: 100%**

- SEN1392 for SE
  - Analysed in one additional paper
  - Total 156 isolates of SE, 72 ST and 35 non *Salmonella* spp. isolates screened
  - **Inclusivity**: 100%
  - **Exclusivity**: ~100% (one isolates SEN1392 and STM4200 positive)

PCR primer/probe specificity

- STM4200 for ST
  - Total 72 St isolates, 49 isolates of SE and 35 non *Salmonella* spp. isolates screened
  - **Inclusivity**: 100%
  - **Exclusivity**: 94,6%: cross reaction with Derby, Rissen en Goldcoast and one isolate SEN1392 and STM4200 positive

Target	Cross reaction isolates (from 2013)	Poultry	Most
STM4200	5,2% (95/1833)	6% (9/95)	Derby (77/95)

Multiplex qPCR assay and relative accuracy

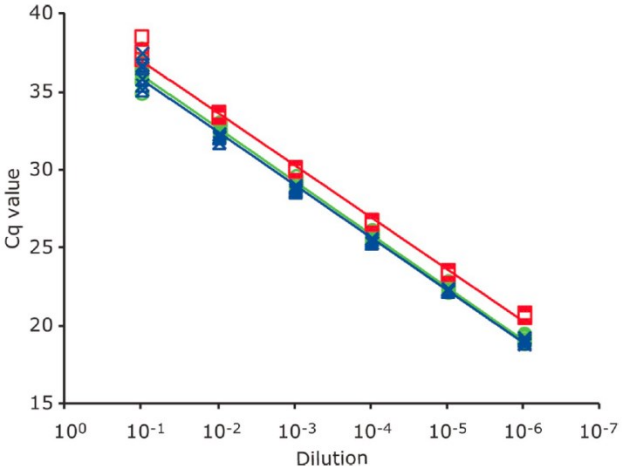


Fig 1. Standard curves for targets *invA*, SEN1392, and STM4200. *invA*: green line and symbols; SEN1392: red line and symbols; STM4200: blue line and symbols.

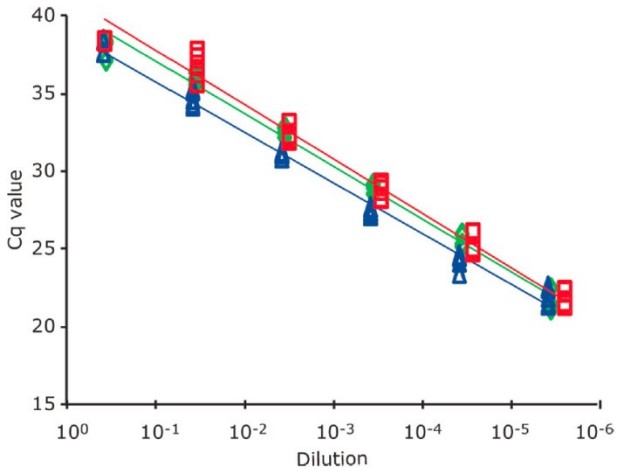


Fig 2. Cell standard curves for target *invA* from S. Typhimurium STM4200 for the matrices BPW, Chicken, and curcuma spice. BPW: Blue line and symbols; Chicken: red line and symbols; curcuma spice: green line and symbols.

Level of detection of multiplex qPCR assay

Matrix	Contamination level (CFU/25g)	Screening using Real-time qPCR method	ISO method
Chicken	4	+ (6/6)	+ (6/6)
Fish (Shrimps)	4	+ (5/6)	+ (6/6)
Powdered milk	11	+ (6/6)	+ (6/6)
Herbs/spices	11	+ (6/6)	+ (6/6)
Egg	11	+ (6/6)	+ (6/6)
Feed	11	+ (6/6)	+ (6/6)
Down	8,5	0 (0/6)	+ (3/6)
Swabs	3	+ (6/6)	+ (6/6)
Minced meat	4	+ (6/6)	+ (6/6)
Boot-socks with chicken feces	43/4	+ (3/6)	+ (5/6)

Conclusions (1)

- qPCR methods specific for *Salmonella* spp., Se and ST were design and validated
  - S spp.: inclusivity: >99,7% and exclusivity: 100%
  - SE: inclusivity: 100%, exclusivity: ≈100%
  - ST: inclusivity: 100%, exclusivity: 94,6% (cross reaction with Derby, Rissen en Goldcoast)
- Three qPCR method can be combined in one multiplex assay with high efficiency, linear relationship with and without matrix
- The multiplex qPCR method is validated for screening of enrichments broths and isolates
- Results are comparable to the ISO method, and allows for detect of low levels (around or below 10 CFU/25g) of *Salmonella* in various (food) matrices.

Conclusions (2)

- By using the multiplex qPCR method, instead of conventional culture methods, for screening of enrichments broths, the analysis time of samples is reduced from 48h to 24h.
  - In 2018 ≈11,000 samples were negative after qPCR screening
- The ability to differentiate between SE and ST makes it a robust tool to easily detect both serotypes as requested by regulation no 2073/2005. However, ST positives must be further confirmed
- This method therefore facilitates effective and faster intervention when contaminated food products are on the market.

Thank you!

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