Wageningen Food Safety Research

Validation of selected *E. coli* isolation method

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Michel Rapallini 10-6-2022

WFSR in short

Food safety and authenticity
Research institute within
Wageningen University & Research

Co-workers
> 350 including PhD students
and foreign guests

History
of > 120 years

Annual turnover
37 M€

Located
on the Wageningen Campus

80% for
Ministry of Agriculture,
Nature and Food Quality

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Our Mission

Safe and reliable food for everyone

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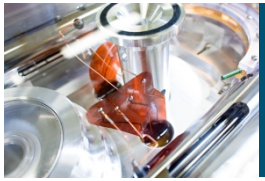
Our activities

Reference institute



Method development

Measuring and detecting substances

Safe food production

Effects of substances on humans and animals

Training and consultancy

Food fraud and composition

24/7

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Monitoring program AMR WFSR 2022

Species	Food samples (n)	Isolates (n)	Panels
Salmonella	10,289	320	EUVSEC3
E. coli	1,534	496	EUVSEC3
Campylobacter	5,135	488	EUCAMP3
ESBL	4,784	226	EUVSEC3/EUVSEC2
*Enterococci**	Monitoring until 2019		EUVENC
MRSA	2,200	180	MIC (EUST)

Survey method isolation commensal *E. coli*

ESBL protocol_survey

Q4 Which method do you use for the isolation of commensal E. coli from faecal and meat samples? Please specify.

Answered: 34 Skipped: 0

#	RESPONSES	DATE
1	We use ISO method 16649-2:2008	4/6/2021 8:20 AM
2	Direct plating on Chromogenic Coliform agar.	4/1/2021 12:56 PM
3	TBX pour plate method, based on ISO 16649-2:2001, but not on faecal samples	3/31/2021 5:30 PM
4	internal prescription using biochemical tests	3/31/2021 11:18 AM
5	SOP based on EURL-AR protocol	3/31/2021 7:22 AM
6	The method reccomended by the EURL-AR	3/30/2021 11:35 AM
7	For caecal content: direct isolation on a selective medium For meat samples: isolation from broth enrichment on a selective medium	3/30/2021 10:19 AM
8	DTU's proposed method	3/30/2021 9:55 AM
9	McConkey or TBX medium and incubated at 44C for 18-24 h. Suspected colonies are	3/30/2021 9:50 AM

Which method do you use for the isolation of commensal *E. coli* from faecal and meat samples?

- 1 We use ISO method 16649-2:2008.
- 3 TBX pour plate method, based on ISO 16649-2:2001, but not on faecal samples.
- 5 SOP based on EURL-AR protocol. MacConkey
- 6 The method recommended by the EURL-AR. MacConkey
- 7 For caecal content: direct isolation on a selective medium For meat samples: isolation from broth enrichment on a selective medium.
- 8 DTU's proposed method. MacConkey
- 9 McConkey or TBX medium and incubated at 44C for 18-24 h. Suspected colonies are inoculated on TBX medium and incubated at 37C for 18-24h. Suspected colonies are purified on Columbia agar supplemented with 5% sheep blood. Identification is done by the OPNG test and Ureum test. Confirmed E. coli pure culture (typical green/blue colonies).

- 10 ISO 16649-1:2018.
- 11 EURL method with MacConkey and blood.
- 13 Meat: NMKL 125, 1:9 in BPW, plate on Violet Red Bile Agar Faecal: Direct plating on Violet Red Bile Agar.
- 16 1:10 dilution sample in saline solution, 10 ml with 90 ml MacConkey broth, 24h 44C. Strike on TBX agar plate 24h 44C.
- 20 Faecal Sample: Direct plating is performed on TBX agar. Meat sample: Pre-enrichment in buffered peptone water, 25 gr of meat + 225ml BPW. After incubation, inoculation with 10 ul loop the surface of a TBX agar plate.
- 21 Faecal samples: direct plating to chromogenic agar. Meat samples: enrichment in BPW without any supplement followed by plating to chromogenic agar.
- 22 Caecal samples: direct plating to chromogenic agar. Meat samples: enrichment in BPW without any supplement followed by plating to chromogenic agar.
- 24 Faecal:Direct plating onto MacConkey No3 agar plates Meat: 10ul of enriched Buffered Peptone Water (BPW), made from a 1 in 10 dilution of 25g meat in 225ml BPW, on MacConkey.

- 26 Same as SWEDRES/SVARM 2016. Dilution 1:10 in BPW, incubation at 37°C overnight. 10 ul spread on MacConkey agar and incubated overnight at 44°C. Lactose-positive colonies with E. coli morphology sub-cultured on horse-blood agar. Confirmed on TBX agar, TSI agar and indole test.
- 28 Pre-enrichment in buffer peptone water (ratio 1/10) and platting on MacConkey agar.
- 29 Sample preparation according to Laboratory protocols from EURL, Isolation of ESBL-,AmpC- and carbapenemase-producing E. coli from caecal/meat samples using MacConkey agar without antibiotic.
- 30 Faecal - plating sample material directly onto MacConkey agar. Meat - we do not do this at the moment. If we do, we use sample material enriched in BPW (same as for ESBL/AmpC/Carba) MacConkey

Comparison MacConkey vs TBX

Method suitable for "one enrichment“ (enrichment in buffer peptone water (ratio 1/10), identical to method for ESBL and Salmonella).

Comparison carried out with *E. coli* isolated from chicken, in case of contamination with multiple species *E.coli* easier to recognize on TBX (Tryptone Bile X-glucuronide)

Flow Diagram Isolation of *E. coli* from food samples

Enrichment

25 g of sample in 225 mL of buffered peptone water

(37°C± 1°C, 18-22 h)

↓

Isolation

Spread a 10-µl loopful of the broth on TBX agar

↓

(44°C ± 1°C, 22-26 h)

Subculture presumptive *E. coli* colonies on blood agar

↓

(37°C± 1°C, 18-22 h)

Identification (optionally)

MALDI TOF MS

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Classification of food categories according

ISO 16140-3 Microbiology of the food chain — Method validation

- Raw poultry and ready-to-cook poultry products
- Raw meat and ready-to-cook meat products (except poultry)
- Fresh produce and fruits (vegetables)
- Raw and ready-to-cook fish and seafoods
- Multi component foods or meal components

Samples bought on supermarket

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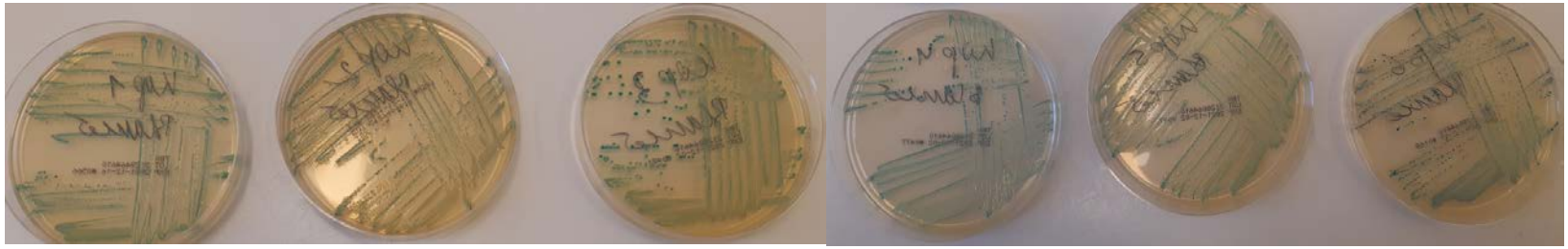
Poultry products



The historical data showed that E. coli was isolated in 95% of fresh poultry meat samples. For this reason, it was decided to replace the food category *raw poultry and ready-to cook poultry products* with *ready-to-eat and ready-to reheat meat poultry products*.

Validation performance characteristics

The following performance characteristics are determined:

Limit of detection

- The spike suspensions were chosen in such a way that the final concentration in the sample was approx. **3**, **10** and **30** CFU/sample and a **blank**. **Six different batches** were examined at each level. In addition, for each spike level, an *E. coli* suspected colony of each TBX plate was analyzed with the MALDI-TOF MS.
- The exact concentration of the **E. coli strain ATCC 25922** with which the samples were contaminated was determined by making a 10 log dilution series of the cell suspension on TSA. These were then incubated for 18-22 hours at 37±1°C, after which the number of colony forming units (CFU) was counted.
- The enrichment medium (BPW) of the blanks and low contamination (3 CFU) were also **cooled for 72 hours**, after which they were spread on TBX plates, to allow a possible cooling step of the enrichment in the procedure.

Validation

Trueness

The trueness is determined from **the results of the proficiency tests** in which participation will be held 1x per year from 2022 onwards.

Robustness

The robustness of this method was determined based on the **results of the first line controls** (**PPC, BPC and NPC**) included in the study. The PPC (positive process control) was added to a sterile matrix in each sample series at approximately the level of detection. If critical factors are present in which the level of detection is lower, this will result in the PPC being rejected and therefore also the samples that were used in that batch.

Selectivity

For the selective isolation of E.coli, TBX medium was used and the **in- and exclusivity** is determined. For the **inclusivity**, a **literature review** was carried out and for the determination of the exclusivity, various bacterial strains were **incubated on TBX plates**. At least 50 positive pure cultures (inclusiveness) and **30 negative pure cultures (exclusivity)** were analyzed.

Results Limit of detection

Food categorie	Limit of detection (cfu / 25g)
Fresh produce and fruits (vegetables)	3
Raw meat and ready-to-cook meat products (except poultry)	3
Multi component foods or meal components	4
Raw and ready-to-cook fish and seafoods	4
Ready-to-eat and ready-to reheat meat poultry products	2

Results Selectivity

- For the inclusivity, a literature review was carried out. (100%)
- For the determination of the exclusivity, various bacterial strains were incubated on TBX plates. (96,7%)

Strain	TBX colony color	Strain	TBX colony color
Acinetobacter baumannii	White	*Salmonella enterica typhimurium*	White
Bacillus cereus	No growth	*Salmonella enterica Enteritidis*	White
Cronobacter sakazakii	White/Yellow	*Shigella flexneri*	White
Cronobacter muytjensii	White	***Shigella sonnei***	**White-Green/Blue**
Edwardsiella tarda	White	*Staphylococcus aureus MRSA*	No growth
Enterococcus faecalis	No growth	*Staphylococcus aureus*	No growth
Enterococcus faecium	No growth	*Staphylococcus epidermidis*	No growth
Ewingella americana	No growth	*Staphylococcus saprophyticus*	No growth
Klebsiella oxytoca	White	*Staphylococcus bovis*	No growth
Klebsiella Pneumoniae	White	*Vibrio parahaemolyticus*	No growth
Listeria monocytogenes	No growth	*Vibrio vulnificus*	No growth
Listeria innocua	No growth	*Vibrio cholerae*	White/Yellow
Morganella morganii	White	*Yersinia entercolitica*	No growth
Proteus mirabilis	White	*Mycobacterium engbaekii*	White/Yellow
Pseudomonas aeruginosa	White/Yellow	*Pantoea agglomerans*	No growth

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Conclusions

- The limit of detection of *E. coli* on TBX is about 3 cfu/25g per food category. It is also possible to cool the enrichment medium for 72 hours.
- The inclusiveness and exclusivity of TBX medium is respectively 100% and 96.7%. The robustness of the methodology is good.

Thank you for your attention

Any questions?

www.wur.eu/food-safety-research

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