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Detection of *E. coli* producing β-lactamases in fresh pork, veal and beef meat in Belgium: results of a small scale study

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NRL Antimicrobial resistance in Food-Belgium

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Goal of the study

To compare the performance of the method described in the EU-legislation with the method accredited in Belgium

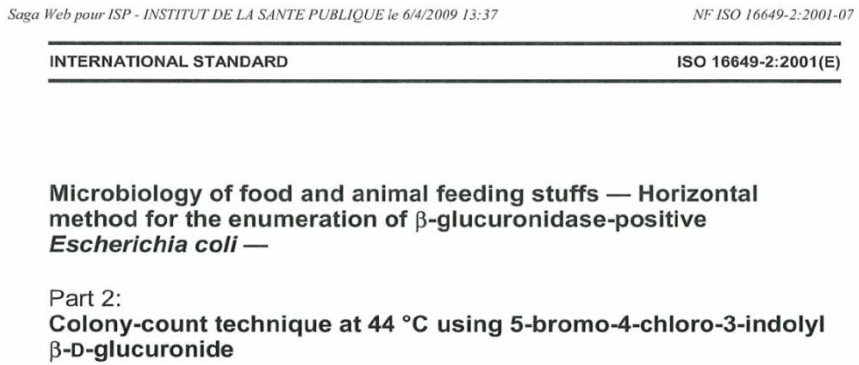
Small scale study

- Number of meat samples n=40
  (fresh meat purchased at retail)
- Selective vs non selective enrichment
- Use of TBX vs McConkey for isolation

Why we use TBX for *E. coli* isolation?

Reference methods in food labs:

ISO 16649 : Harmonized method in food, feed and environmental samples for the enumeration of *E. coli*. The isolation medium of choice is TBX.



Saga Web pour ISP - INSTITUT DE LA SANTE PUBLIQUE le 6/4/2009 13:37 *NF ISO 16649-2:2001-07*

INTERNATIONAL STANDARD **ISO 16649-2:2001(E)**

Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of β-glucuronidase-positive *Escherichia coli* —

Part 2:
Colony-count technique at 44 °C using 5-bromo-4-chloro-3-indolyl β-D-glucuronide

ISO TS 13136: Detection and isolation of STEC : The isolation medium of choice is TBX.

4.6 Isolation

If the presence of a STEC is suspected, the isolation is attempted. If one of the serogroups specified in the scope of this Technical Specification is detected, a serogroup-specific enrichment (e.g. IMS) can be performed followed by plating on to tryptone–bile–glucuronic agar (TBX) or a specific selective medium if available (see Annex F, Notes 2 and 3) in order to facilitate the isolation of the STEC from the background flora.

TRYPTONE BILE X-GLUCURONIDE (TBX) MEDIUM (7692)

Intended Use

Tryptone Bile X-Glucuronide (TBX) Medium is used in the isolation and identification of *E. coli* in food.

Product Summary and Explanation

Tryptone Bile X-Glucuronide (TBX) Medium is a modification of Tryptone Bile Agar.[1] Tryptone Bile Agar was developed to improve the detection of *E. coli* in foods.[2,] TBX Medium is enhanced by the addition of a chromogenic agent, X-glucuronide, detecting glucuronidase activity. The presence of the enzyme β-D-glucuronidase differentiates most *E. coli* spp. from other coliforms, and is the same enzyme used in the MUG reaction.[3] X-glucuronide reacts slightly differently and when released into the medium is insoluble, accumulating within the cell.

MacConkey Agar

Use: Moderately selective culture medium for the detection of coliform organisms and enteric pathogens

GROWTH

LACTOSE POSITIVE
examples

- *Escherichia coli* (most strains)
- *Klebsiella pneumoniae*
- *Entrobacter cloacae*

LACTOSE NEGATIVE
examples

- ***Salmonella enterica* ssp. *enterica***
- ***Shigella* spp.**
- *Proteus* spp.
- *Citrobacter freundii* (some strains)
- *Morganella morganii*
- *Providencia* spp.

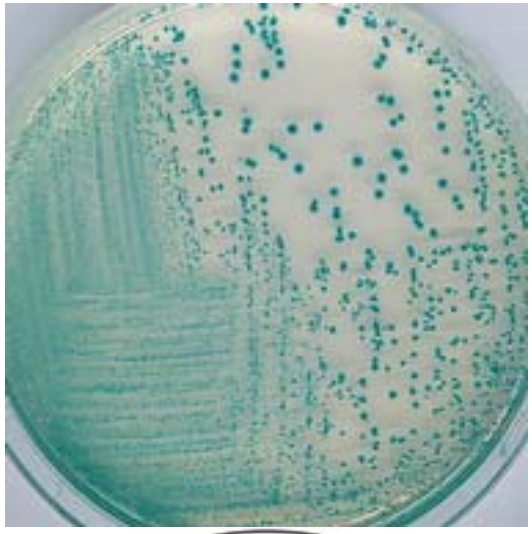
INHIBITION OF GROWTH

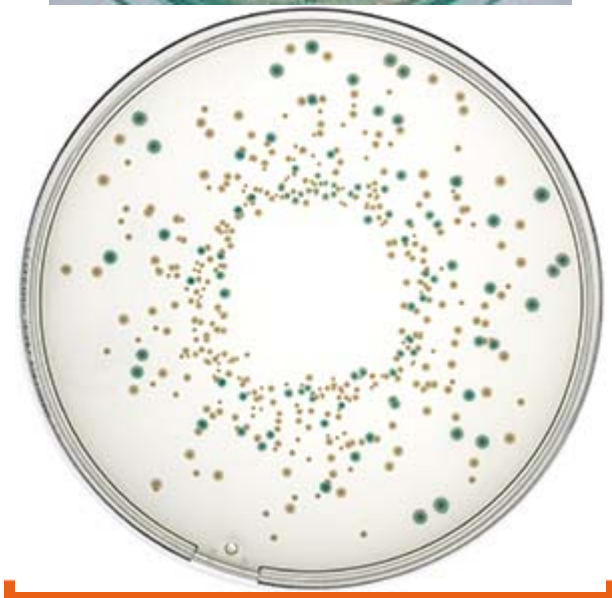
- staphylococci
- streptococci
- enterococci

Crystal violet and **bile salts** in medium inhibit growth of Gram-positive microorganisms

BACTERIUM	TYPICAL GROWTH ON MACCONKEY AGAR
Escherichia coli	Pink to rose-red. Colonies may be surrounded by a zone of precipitated bile.
Enterobacter cloacae	Pink, mucoid.
Klebsilla pneumoniae	Pink, mucoid.
Proteus	Colorless.
Pseudomonas aeruginosa	Colorless to pink.
Salmonella and *Shigella*	Colorless.
Gram-positive bacteria	No growth to slight growth (pale pink).

Species that grow on TBX at 37° C





Expected Cultural Response: Cultural response on Tryptone Bile X-Glucuronide (TBX) Medium at 35 ± 2°C and examined for growth at 18 – 24 hours incubation.

Microorganism	Growth	Reaction
Enterobacter aerogenes ATCC® 13048	growth	white
Escherichia coli ATCC® 25922	growth	blue
Escherichia coli ATCC® 35218	growth	blue
Escherichia coli ATCC® 11775	growth	blue
Escherichia coli O157:H7 ATCC® 35150	growth	white
Klebsiella pneumoniae ATCC® 13883	growth	white
Salmonella choleraesuis ATCC® 13076	growth	white
Shigella flexneri ATCC® 12022	growth	white

The organisms listed are the minimum that should be used for quality control testing.

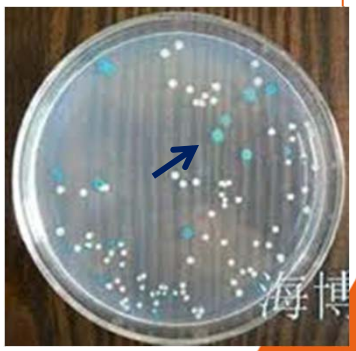
Comparision between EU-legislation and Belgian protocol for verification of resistance/identification *E. coli*

EU Legislation protocol

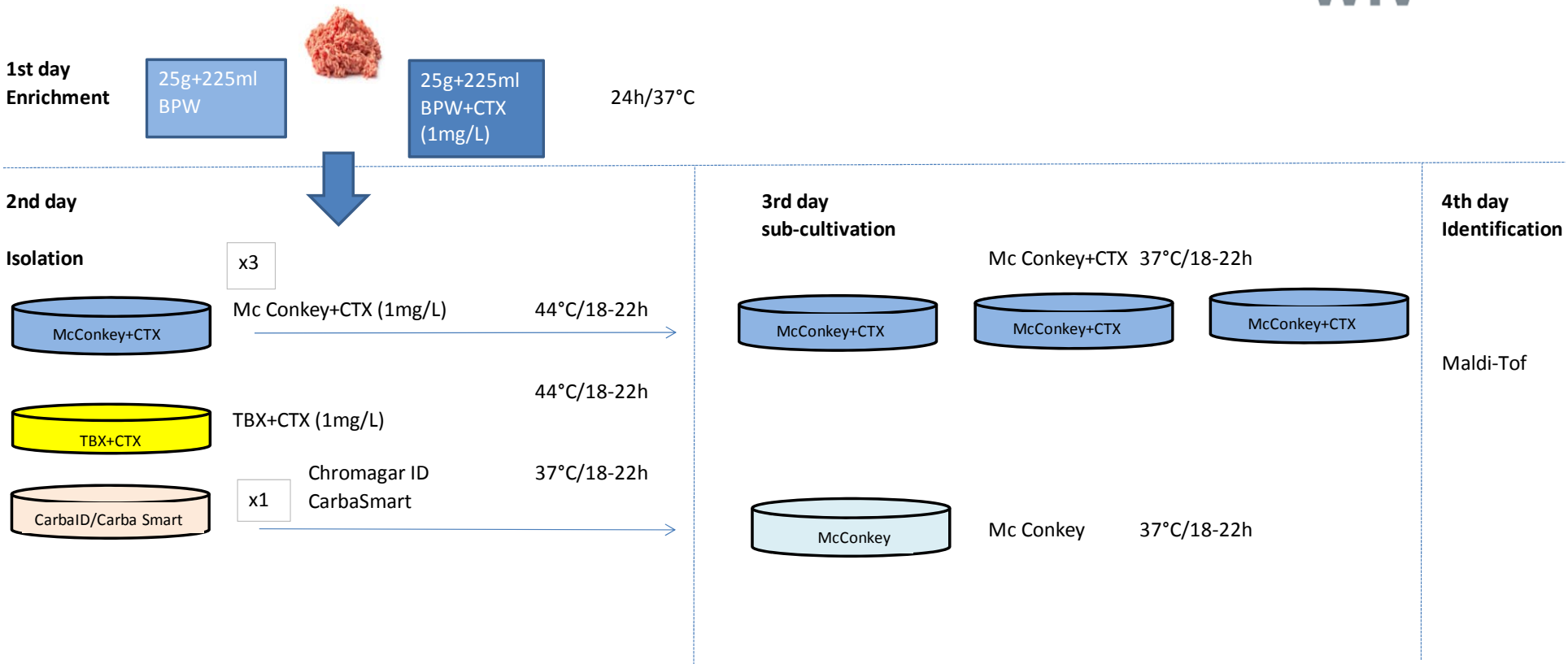
- Non-selective pre-enrichment
- Isolation of suspected colonies on selective **McConkey agar supplemented** with 1mg CTX/L
- Selection of **3** suspected colonies and **subcultured on McConkey+CTX** for purification
- confirmation of **3 suspected colonies** of *E. coli* (Maldi-Tof, TBX?)
- **Cost** of verification of resistance/identification (according to step 3, 4 & 5 EURL protocol) : expensive and up to 3 repeats
- More experience needed
- **Time** consuming

Belgian protocol

- Non-selective pre-enrichment
- Isolation of suspected colonies on selective **TBX agar supplemented** with 1mg CTX/L
- Selection of **1** typical colony of *E.coli*
- Cost: cheap
- Easy to recognize
- **Time** –saving



Validation: detection of *E. coli* BLSE/AmpC/Carba in beef, veal and pork (meat at retail)



veal	12
pork	11
beef	11
mixed pork/beef	4
mixed pork/veal	2

Results

i) Detection of ESBL

	BPW		BPW+CTX	
	McConkey+CTX	TBX+CTX	McConkey+CTX	TBX+CTX
Number of samples	40	40	40	40
Number of samples *E. coli* ESBL positive	7	7	5	5
Number of samples *E. coli* ESBL plus other bacteria	1	0	3	0
Number of samples where only *Acinetobacter* was isolated	4	0	6	0
Number of samples where *Citrobacter braakii* was isolated			1	

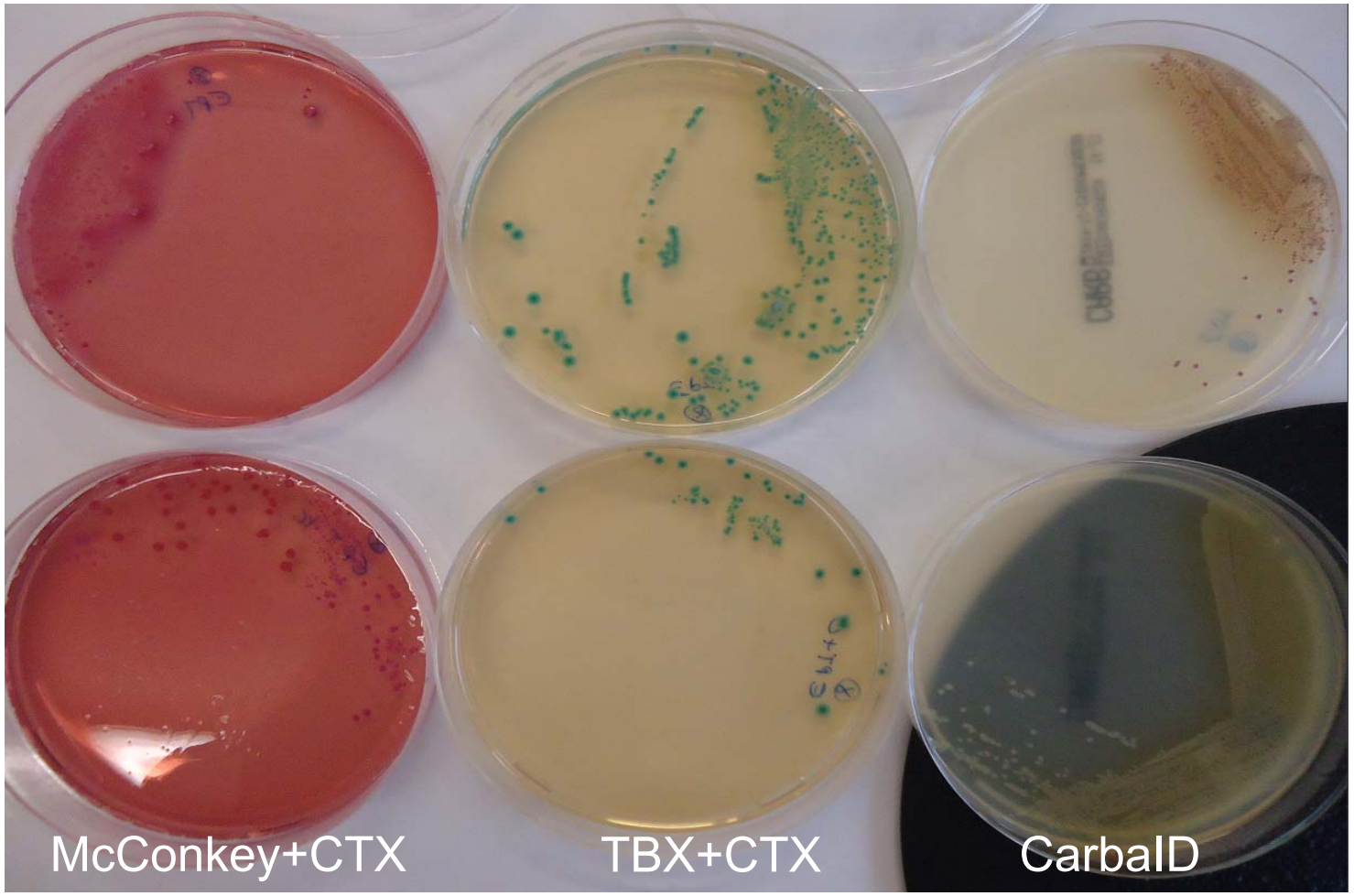
ii) Non specific flora

	McConkey		TBX	
	BPW	BPW+CTX	BPW	BPW+CTX
E.coli/Acinetobacter	2	1	0	0
Acinetobacter	6	4	0	0
Citrobacter	1	0	0	0

iii) Per matrix in BPW

matrices	n
Veal	4
Pork/beef (mixed)	2
Pork	1

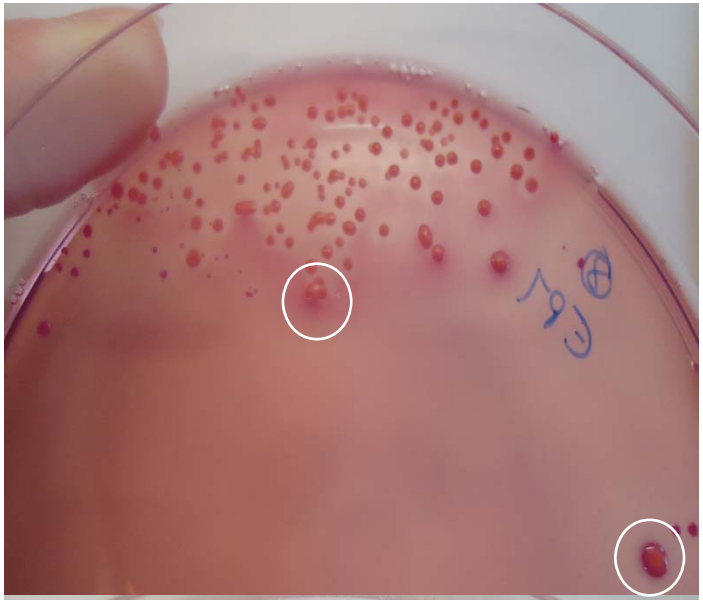
Minced veal :*E. coli* ESBL isolated from both enrichment broths



BPW

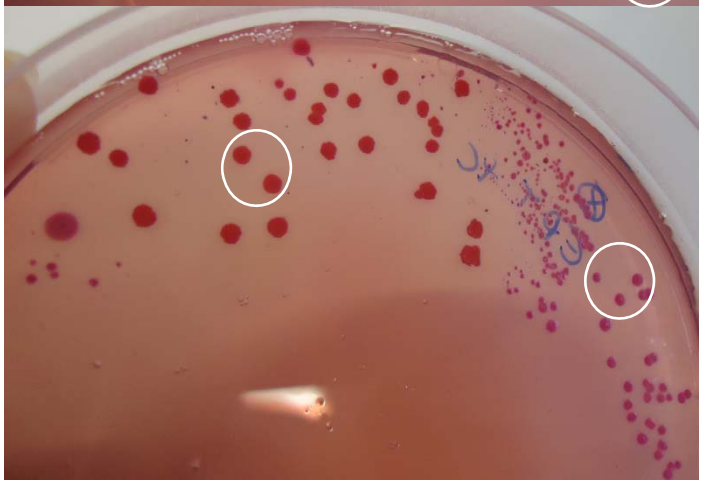
BPW+CTX

veal



BPW

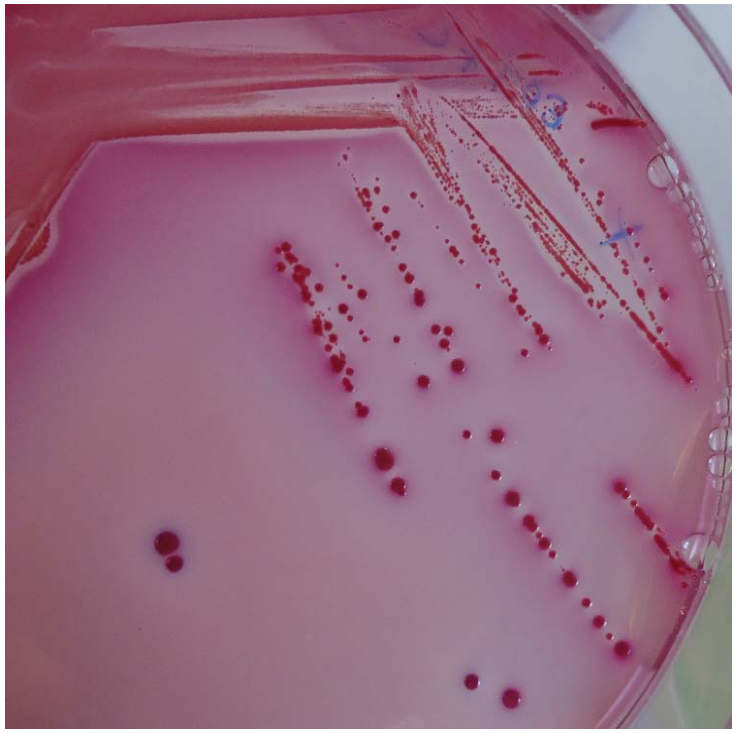
E. coli
ESBL



BPW+CTX

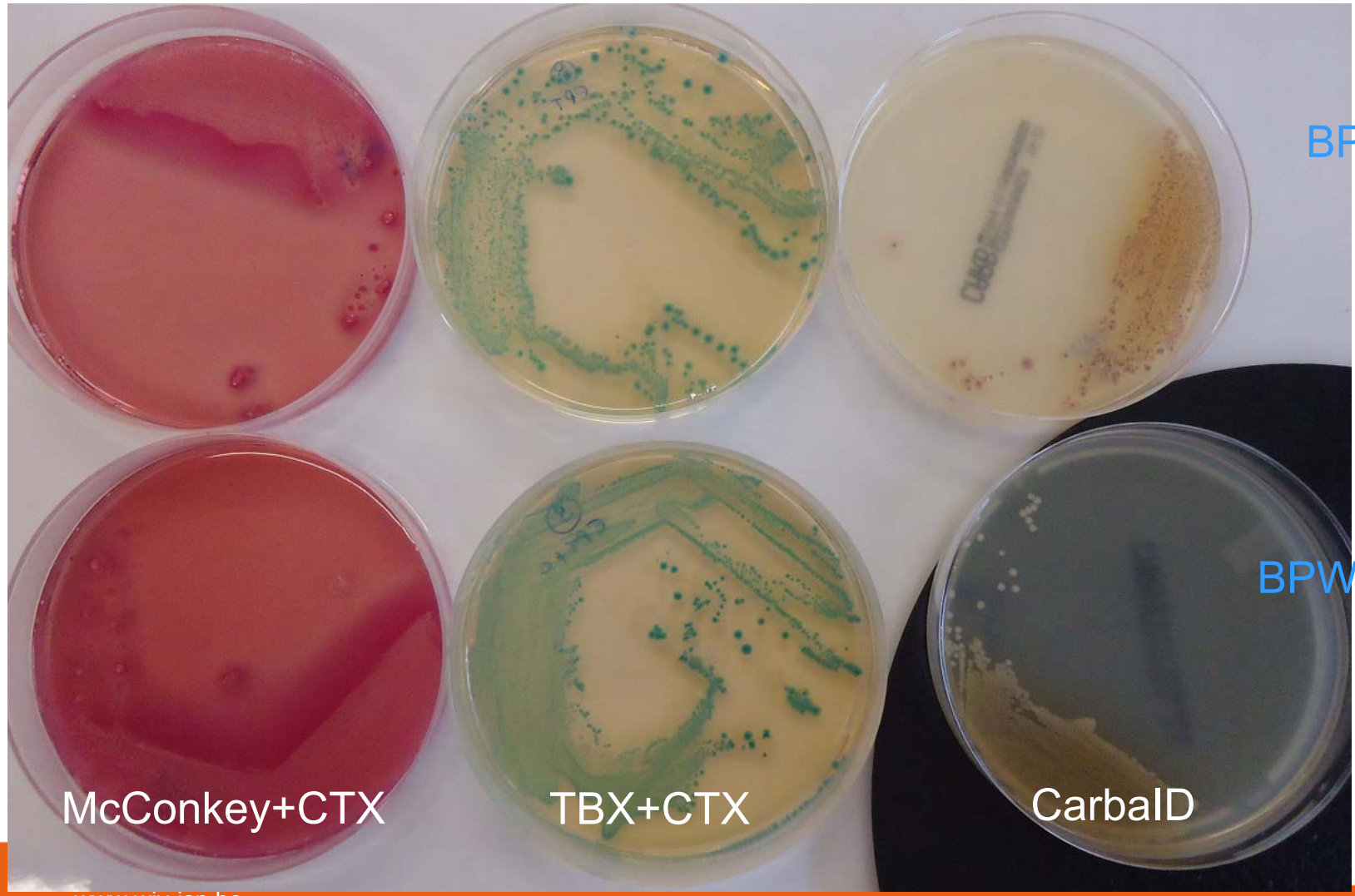
E. coli
ESBL

Positive control

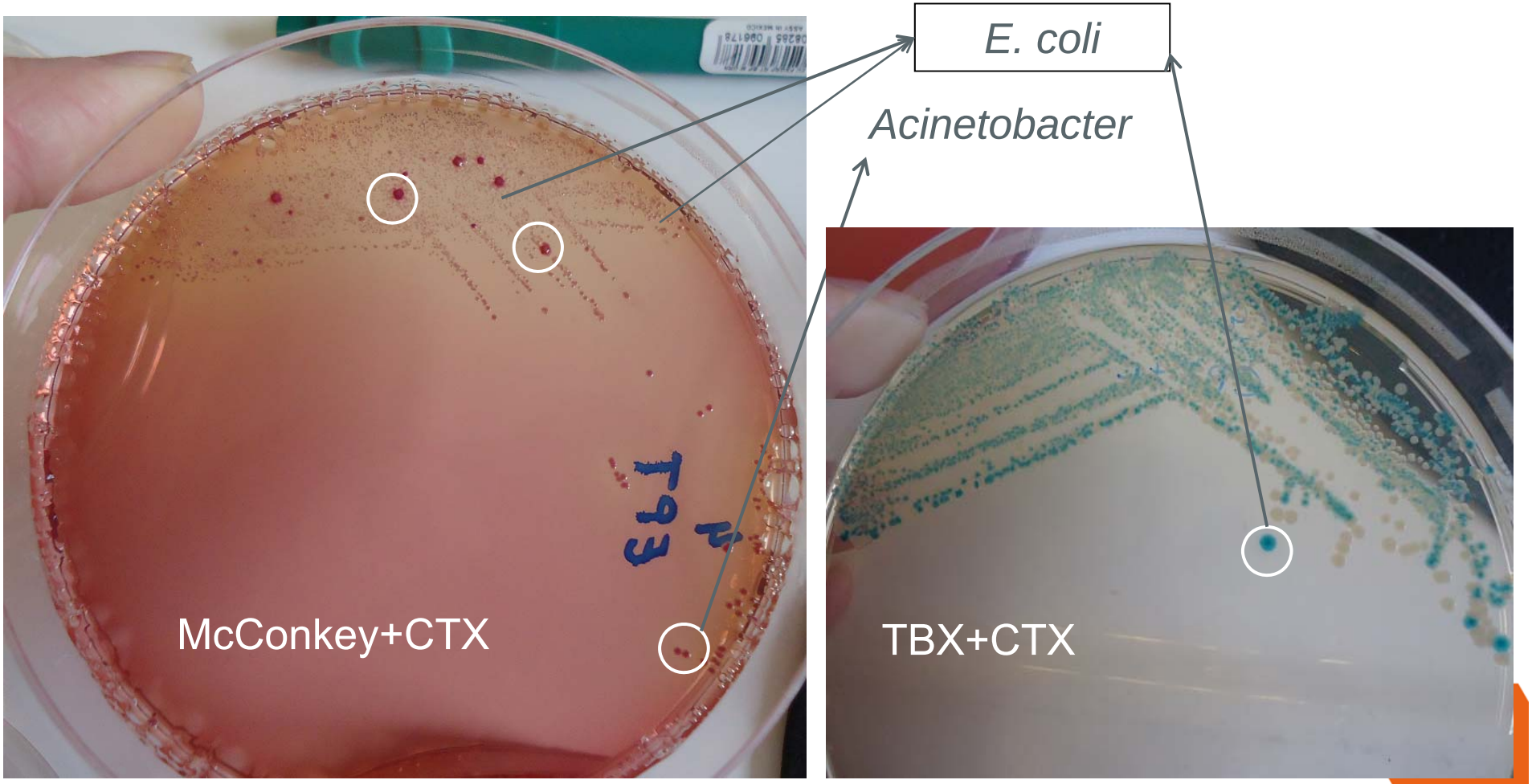


Typical and non typical *E. coli* colonies

Minced veal :*E. coli* ESBL isolated from both media

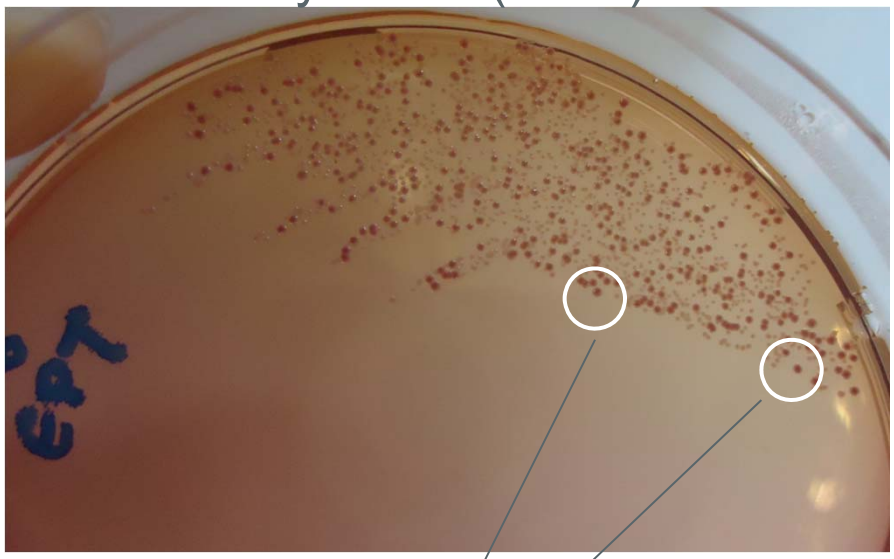


E. coli and *Acinetobacter* isolated from minced beef/veal



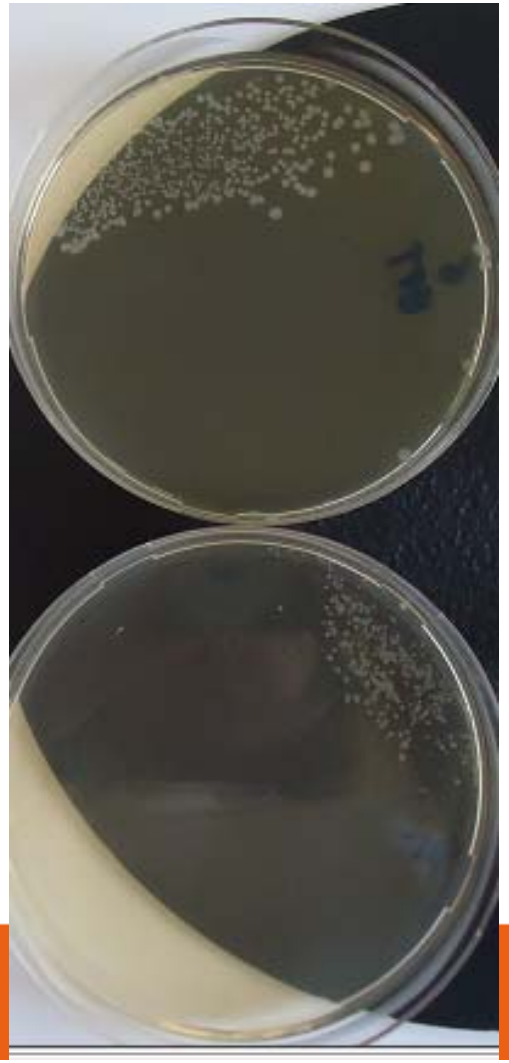
Suspected but non confirmed as *E. coli*, isolated on McConkey vs TBX

Acinetobacter on McConkey+CTX (BPW)



Subcultivation and ID by Maldi-Tof

TBX+CTX (BPW)



Conclusions

- Pre-enrichment with CTX is not advised :more positives detected when non selective pre-enrichment was used.
- ESBL could be detected in the same range when **McConkey** and **TBX** media were used
- When McConkey agar was used, typical and non-typical colonies were identified as *E. coli* ESBL
- Presumptive *E. coli,* that were not confirmed as *E. coli*, were more frequently isolated when using McConkey agar (n=3) (similar coloured colonies were isolated that belonged to other species)
- In case of the use of TBX: confirmation step not necessary