# SADCMET Water PT evaluation workshop

# Microbiology Proficiency Testing

# 1st round

Dr. Katrin Luden

# Topics

- Methods used and results obtained
- General challenges of standardized methods
- Used laboratory equipment

# Methods and results

## *E. coli* / coliform bacteria

| CFU/100 ml | method |
|---|---|
| **22.000.000** | **pour plate, brilliance chromogenic selective Agar (Oxoid), incubation at 36°C** |
| 106 | AWWA 9222D Membrane filtration; feacal Coliform procedure;**44.5 °C**; Biolab m-FC agar HG000C92.500; E. coli absent by SABS 221-1990) Inocculation in Tryptone water with possible E. coli and incubate at **44.5°C** for 24 hours Confirm with Kovacs reagent for pr |
| 0 | MFM ISO 9308-2, 44°C; MLSB |
| 22.500 | MF mEndo Agar LES (Difco); 35 +-2 °C |
| 276 | BMM-S11-08; Mc Conkey agar (Oxoid); 30°C |
| >300 | ISO 9308-1; 37°C; Lactose TTC agar |
| **3.000** | **MF ISO 9308;37°C M-Endo agar les** |
| 1.600 | MPN; 35°C Lauryl Tryptose broth; E. coli present isolated on Eosin Methylene Blue (EMB) agar; EC broth at 44.5°C; Methods described in Standard Methods for the examination of Water and wastewater 20th edition 1998 |
| **1.425.000** | **Colilert-18** |

# Methods and results

## *E. coli* / coliform bacteria

| CFU/100 ml | method |
|---|---|
| 3.000 | |
| 17 | |
| 1.150 | standard methods 20th Edition MF; 9125D; 44.5°C; MFC Agar, Brilliant green bile; lactose broth |
| >300 | violet red bile agar (unleserlicher Rest) |
| 46.000 | ISO 9308-1; 37°C; MPN; Lauryl-sulphate broth, brilliant green bile broth |
| **210** | **ISO 9308-1:2000; 37+-1°C; Violet red bile Agar** |
| 18.000 | pour plate violet red bile agar; 37°C |

# Methods and results

## TPC

| CFU/ml | method |
|---|---|
| **210.000** | ISO 6222:1999, pour plate; yeast extract agar Merck; 36°C |
| 21.700 | ISO 6222 pour plate method 35°C; Merck yeast extract agar Cat. No. 1.13116.0500 |
| 4 | ISO 6222 pour plate, 37°C; Nutrient agar |
| **2.950.000** | **pour plate; TGEA (Oxoid)** |
| 706 | BMM-S11-07; Plate count agar (oxoid); 30°C |
| 323.000 | ISO 6222; 37°C; yeast extract agar |
| 100 | ISO 6222 and ISO 8199 pour plate; 37°C |
| 31.000 | plate count agar pour plate method; 35°C |
| 550.000 | |
| **78.000.000** | **Standard methods 20th Edition Pour plate 9215B; plate count agar** |
| >300 | plate count agar (unleserlicher Rest) |
| **51.000.000** | **ISO 6222:1999 at 22°C and 37°C; yeast extract agar** |
| 842 | ISO 8199:2005; standard plate count agar |
| 370.000 | pour plate; standard plate count agar;37°C |
| 15.000 | Standard plate count procedure 36°C 24h and 22°C 72 h; nutrient agar; all samples diluted using 1/4 strength ringers solution |

# What are coliforme bacteria?

Lactose metabolism / gas formation

„historic“

# ISO 9308-1 (reference method)

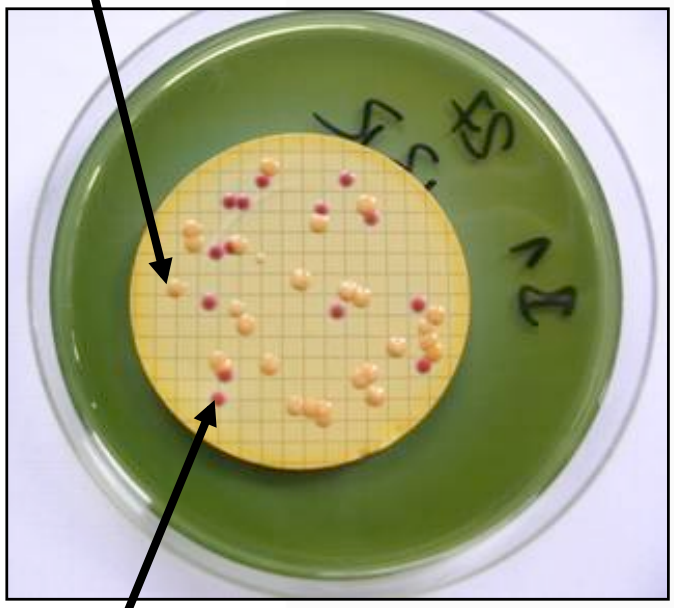
Detection: **metabolites** and **enzyme activity**

**Coliform bacteria:**

- acid formation from Lactose
- Oxidase-negative

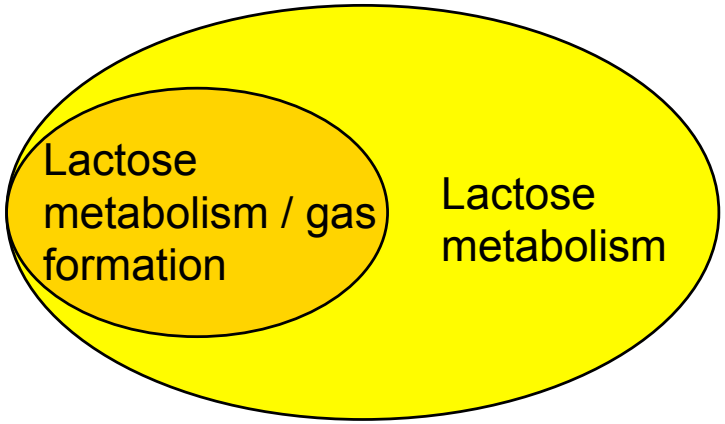
***E. coli***:

- Indole production at 44°C




**Duration**: ca. 48 hours or more for positive results

# What are coliforme bacteria?




„historic“ ISO 9308-1

# Colilert®-18

Detection of specific **enzymeactivities**

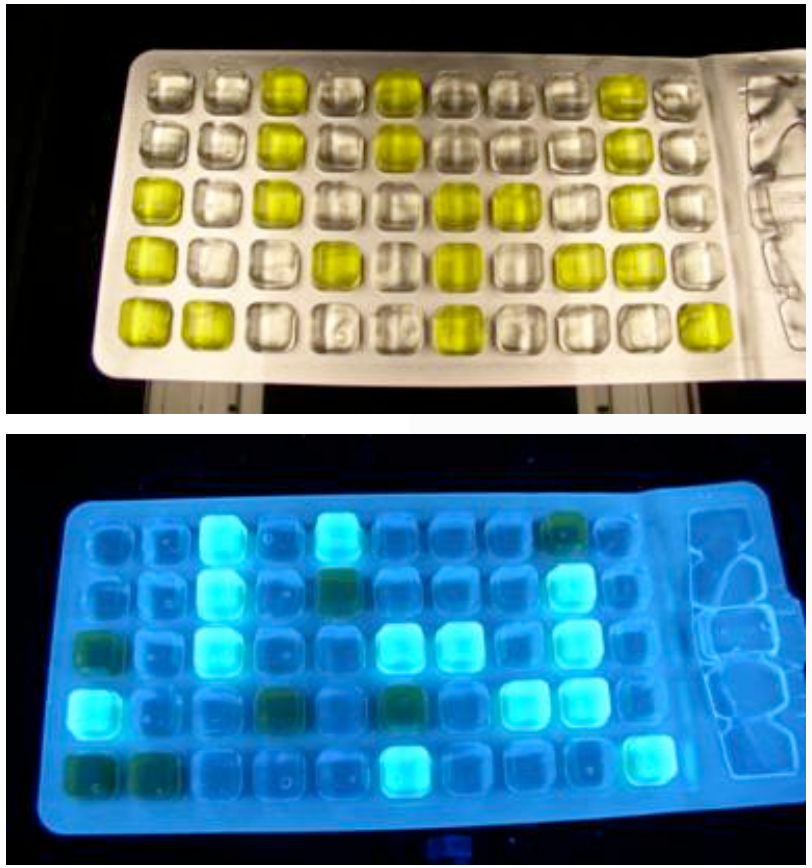
**Coliforme bacteria:**

***β-Galactosidase***

Lactose + $H_2O$ → Glucose + Galactose

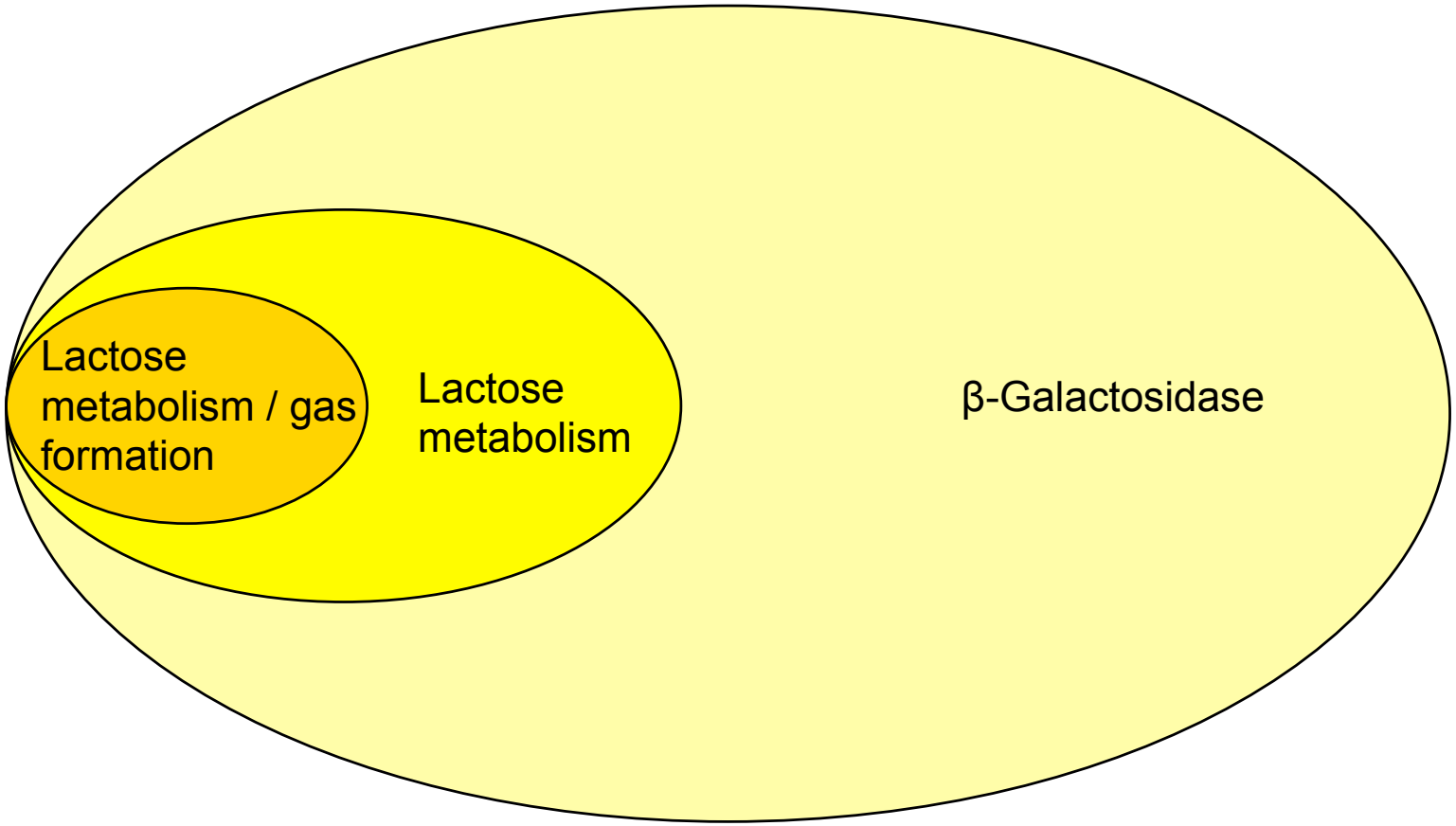
***E. coli***:

Additionally ***β-Glucuronidase***



**Duration**: 18 hours

# What are coliforme bacteria?




„historic“ ISO 9308-1 Colilert®-18

# What are coliforme bacteria?

| “historic” | ISO 9308-1 | Colilert®-18 |
|---|---|---|
| Escherichia | Escherichia | Escherichia |
| **Klebsiella** | **Klebsiella** | **Klebsiella** |
| **Enterobacter** | **Enterobacter** | **Enterobacter** |
| **Citrobacter** | **Citrobacter** | **Citrobacter** |
| | **Yersinia** | **Yersinia** |
| | **Serratia** | **Serratia** |
| | **Hafnia** | **Hafnia** |
| | **Pantoea** | **Pantoea** |
| | **Kluyvera** | **Kluyvera** |
| | | **Cedecea** |
| | | **Ewingella** |
| | | **Moellerella** |
| | | **Leclercia** |
| | | **Rahnella** |
| | | **Yokenella** |

**environmental** and **fecal** coliform bacteria

# Problems

- Test principles are not identical
  - equivalence considers mean of results (statistics)
- Coliform bacteria detected by one method might not be coliform bacteria by the other method
- how to deal with the differing results of the two “equivalent” methods e.g. samples are analyzed by different labs
- Independent of the method detection of coliform bacteria results in non-compliance to the EU drinking water directive (limit: 0 CFU/100 ml)

# Colony count

Traditionally used in check monitoring for drinking water

| "TVO 1990"<br>Limit 100 CFU/ml | ISO 6222<br>No abnormal change |
|---|---|
| Medium without yeast<br>incubation for 2 days<br>20°C and 36°C | Medium with yeast<br>Incubation for up to 3 days<br>22°C and 36°C |

These „eqivalent" methods use:

Different substrates

Different time

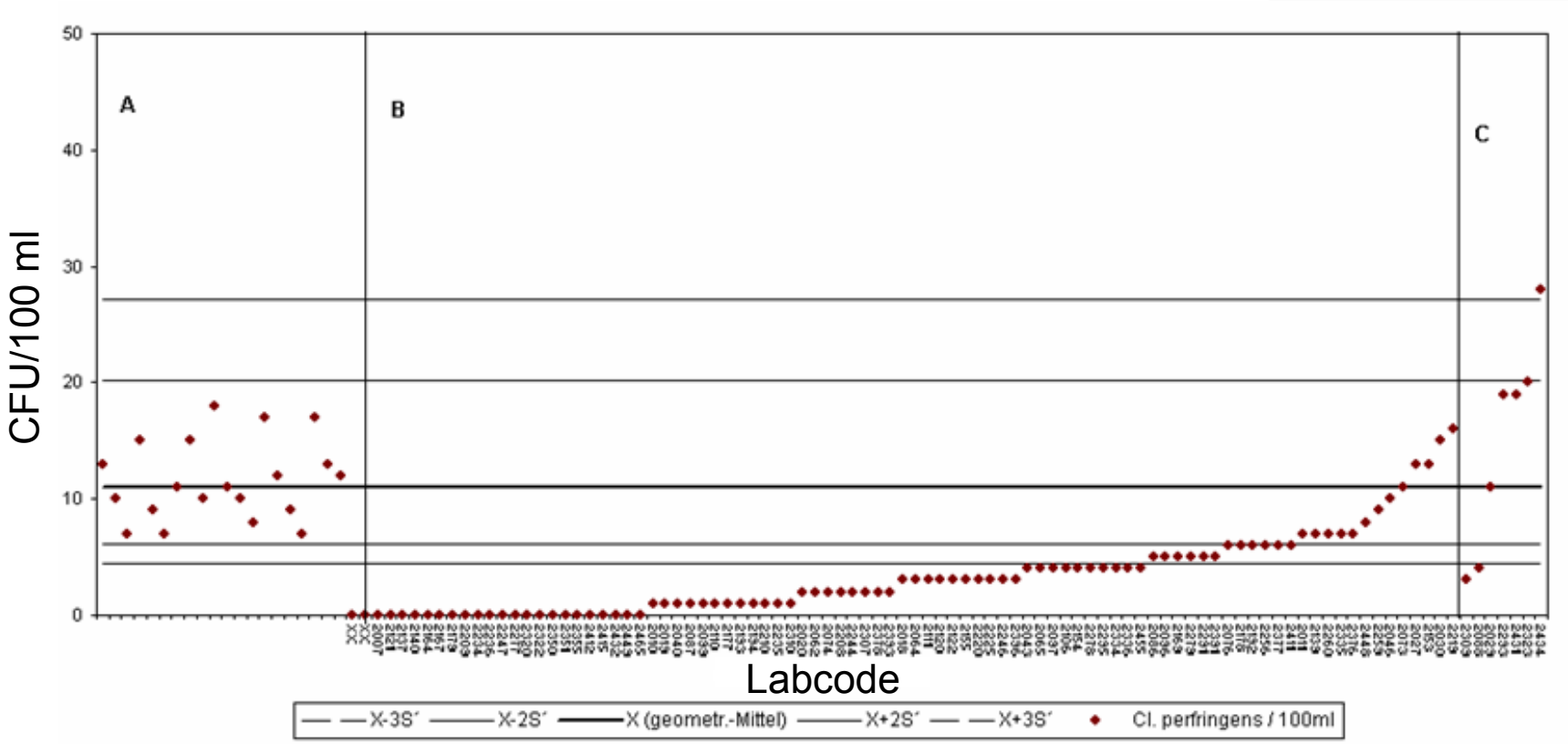
Different temperatures

# Conclusions

- BE AWARE!
- Most microbiological methods are convention methods
- If you make a change in method carefully consider the consequences
- Look closely at what methods are suitable for your purpose (is there a mandatory/reference method)
- If you want comparability e.g. export purposes it might be good to use methods from international Standards

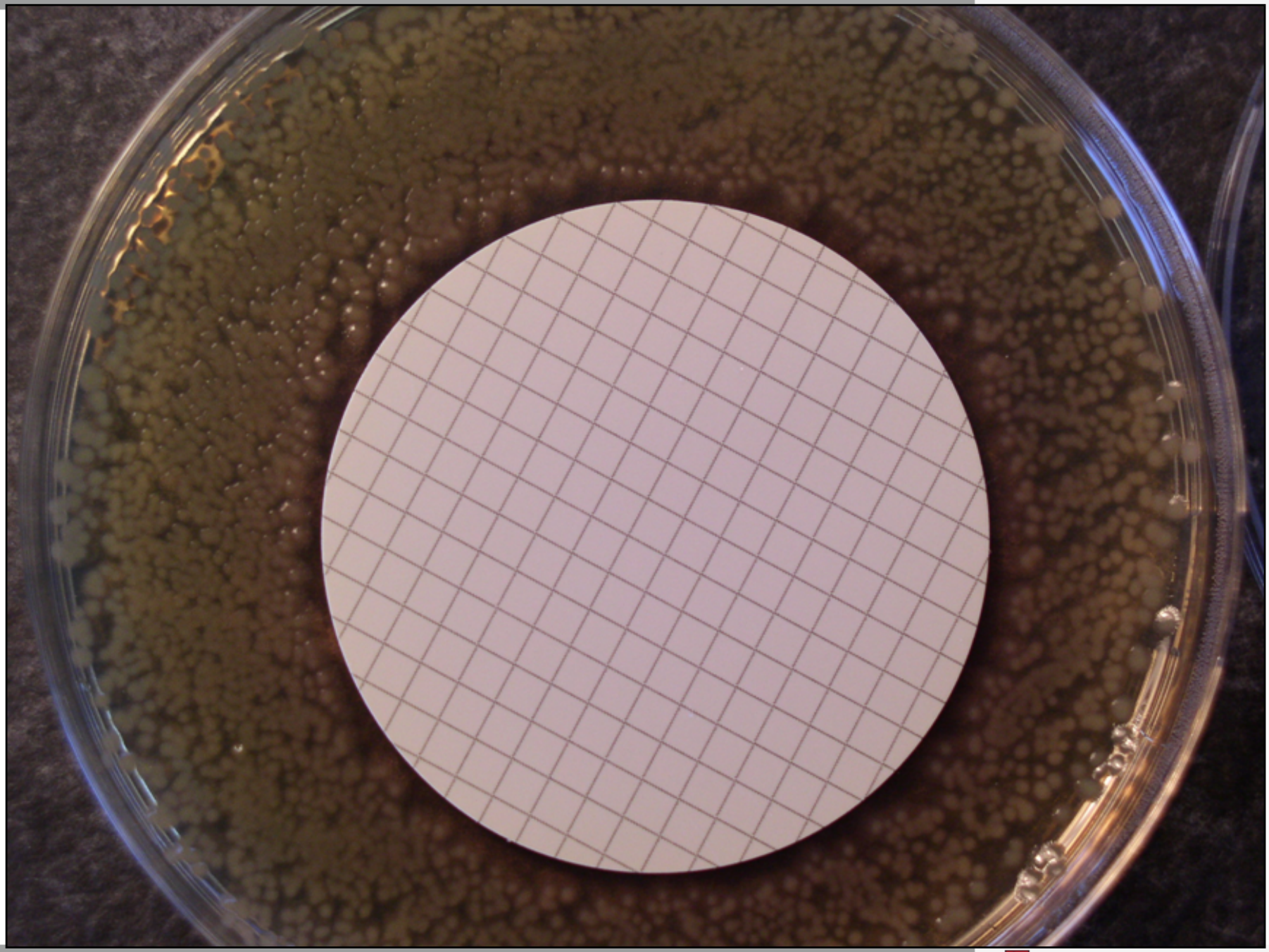
# *Clostridium perfringens*

PT 2-2004



# *Clostridium perfringens*

| Medium/method used | Recovery rate |
|---|---|
| Columbia blood agar / membrane filtration | 100 % |
| Columbia blood agar / membrane filtration - no shaking of the sample | 39 % |
| DEV-Nutrient agar / pour plate method | 78 % |
| mCP Agar / Filtration (24 hours TNT) | 43 % |
| mCP Agar / Filtration (24 hours 4°C) | 43 % |
| mCP Agar / Filtration (PT participants mean) | 11 % |

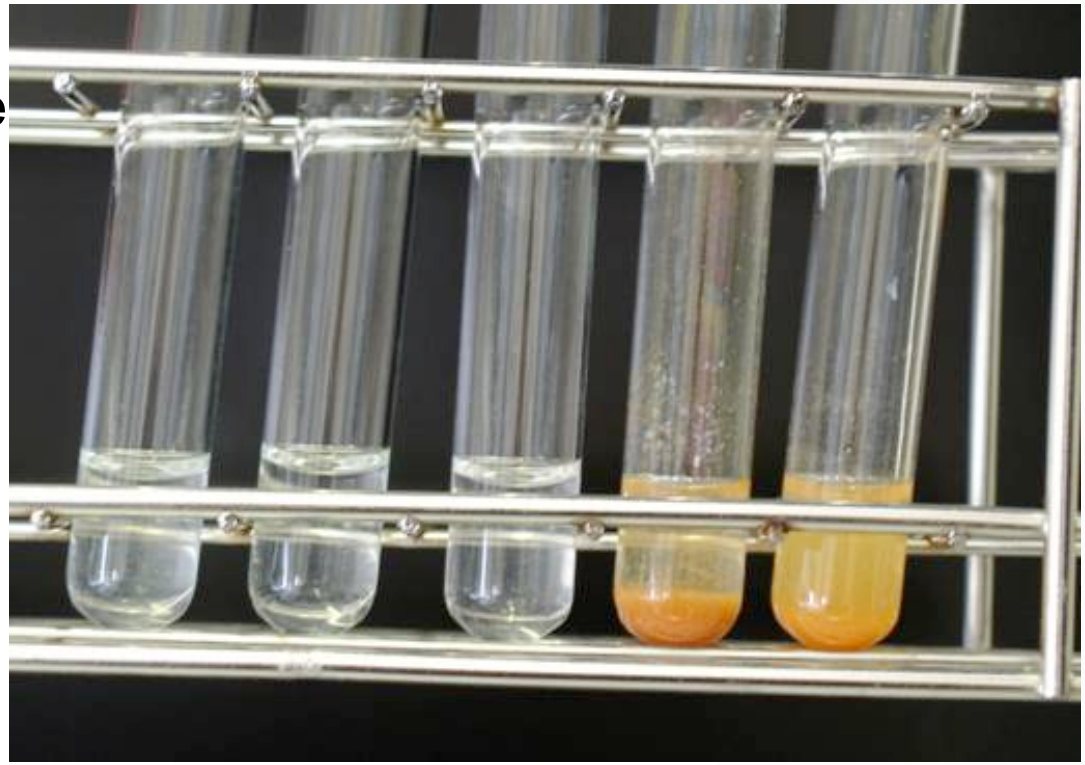


# *Pseudomonas aeruginosa*

In PT 1-2007
30 % of labs failed because they reported a numerical result for *P. aeruginosa* when the strain provided was not a *P. aeruginosa*

**Possible explanation**:

problems with confirmation reaction - Ammonia formation from acetamide



# *E. coli*

A pure culture of *E. coli* was used in PT 4-2004

False results:

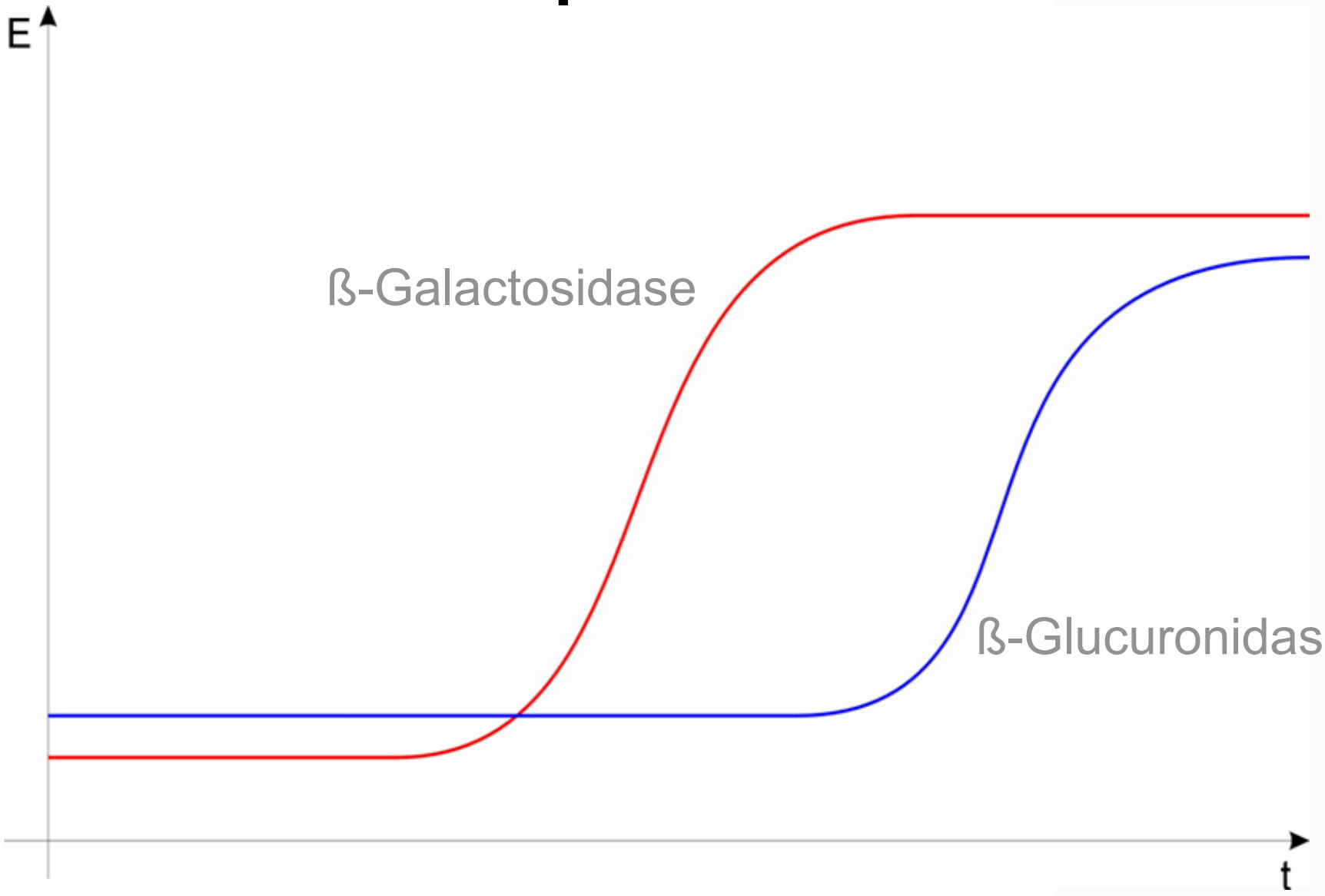
ISO 9308-1: 2,4 %

Colilert®-18: 18,8 %

| ISO 9308-1 (n = 246) | | |
|---|---|---|
| ***E. coli*** | **Coliform bacteria** | **delta** |
| 61 | 68 | 7 |
| 0 | 25 | 25 |
| 30 | 54 | 24 |
| 15 | 16 | 1 |
| 21 | 22 | 1 |
| 24 | 25 | 1 |

| Colilert (n = 80) | | |
|---|---|---|
| ***E. coli*** | **Coliform bacteria** | **delta** |
| 0 | 66 | 66 |
| 66 | 70 | 4 |
| 62 | 70 | 8 |
| 74 | 78 | 4 |
| 0 | 78 | 78 |
| 0 | 83 | 83 |
| 74 | 83 | 9 |
| 30 | 43 | 13 |
| 48 | 50 | 2 |
| 48 | 53 | 5 |
| 50 | 53 | 3 |
| 53 | 59 | 6 |
| 19 | 21 | 2 |
| 24 | 25 | 1 |
| 27 | 45 | 18 |

# Explanation?



# Conclusions

- Interaction between filter and nutrient agar might be unfavorable for growth of the target organism
- Performance of the material combinations used has to be checked (even if supplied with a certificate)
- Negative controls are as important as positive controls

# Way forward

- Finish Report of 1st PT round
- Test packaging material and courier systems
- Announcement of 2nd Microbiology PT
- Look at stability of the strain at higher temperatures
- Leaflet for promotion of the PT scheme
- Add more information to accompanying letter
- Labeling of packages
- …