Qualitative analysis of food for pathogens

Presence / absence tests

pre-enrichment

↓

allow multiplication of target organism

selective enrichment

suppress growth of others

↓

to obtain recognizable colonies

isolation on selective media
selective plating

↓

selection of typical colonies

↓

closely related bacteria may give similar colonies

confirmation

Problem: microbial cells which are sublethally injured

sublethal injury

may be caused by

- mild heat processing
- drying
- freezing

not able to multiply until damage has been repaired

further stresses may cause death

media containing selective agents = stress

resuscitation in non-selective media necessary

Quantitative analysis of food for micro-organisms

Enumeration

colony count techniques

micro-organisms multiply to produce colonies visible to the naked eye

surface colony count

on the surface of suitable agar media

spread plate technique

drop plating technique

homogenization of food sample

dilution series

subsurface colony count

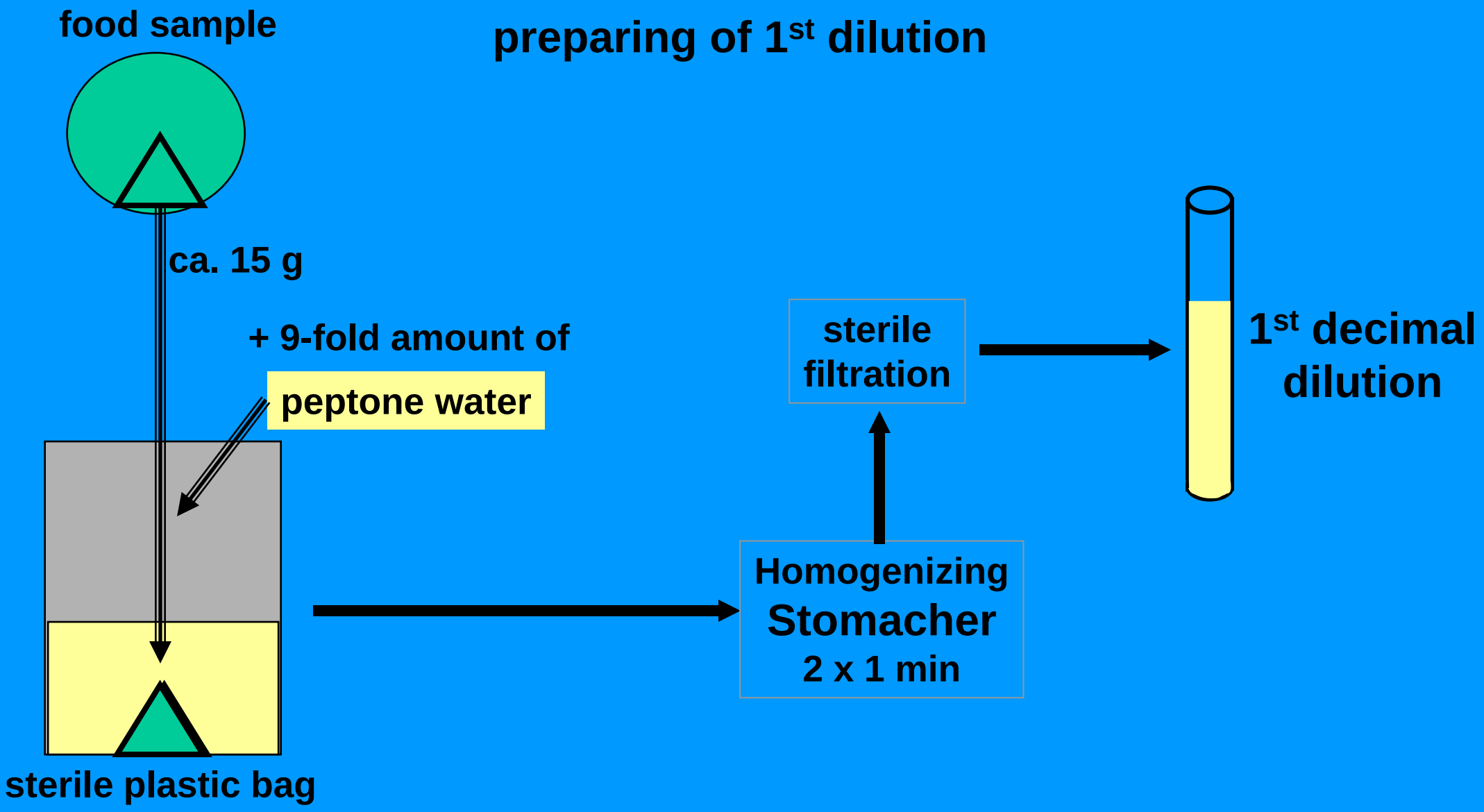
in the depth of suitable agar media which are added in molten state

pour-plate technique

Most Probable Number techniques

decimal dilution series

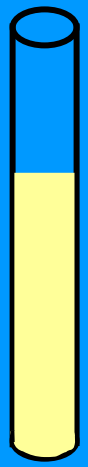
preparing of 1st dilution

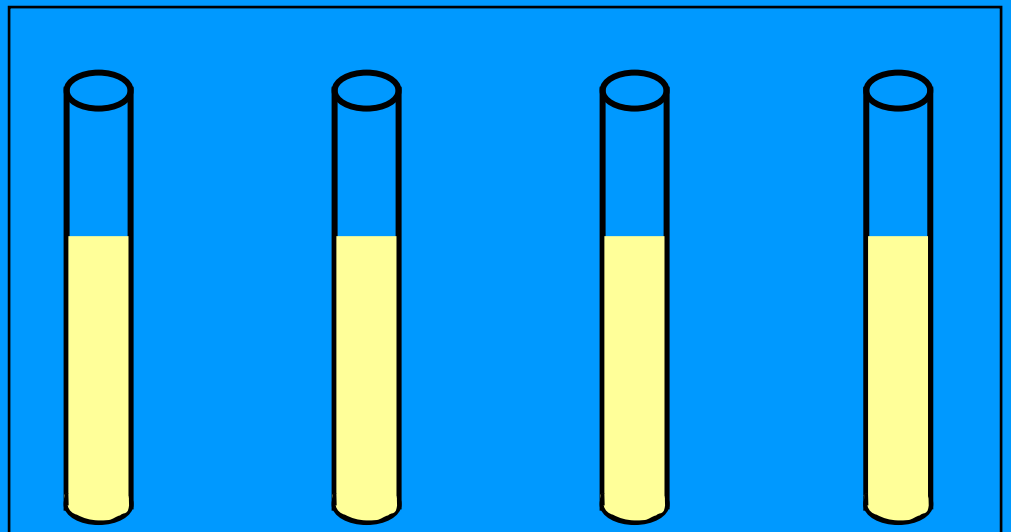


decimal dilution series

preparing of further dilutions

1st decimal dilution 1:10



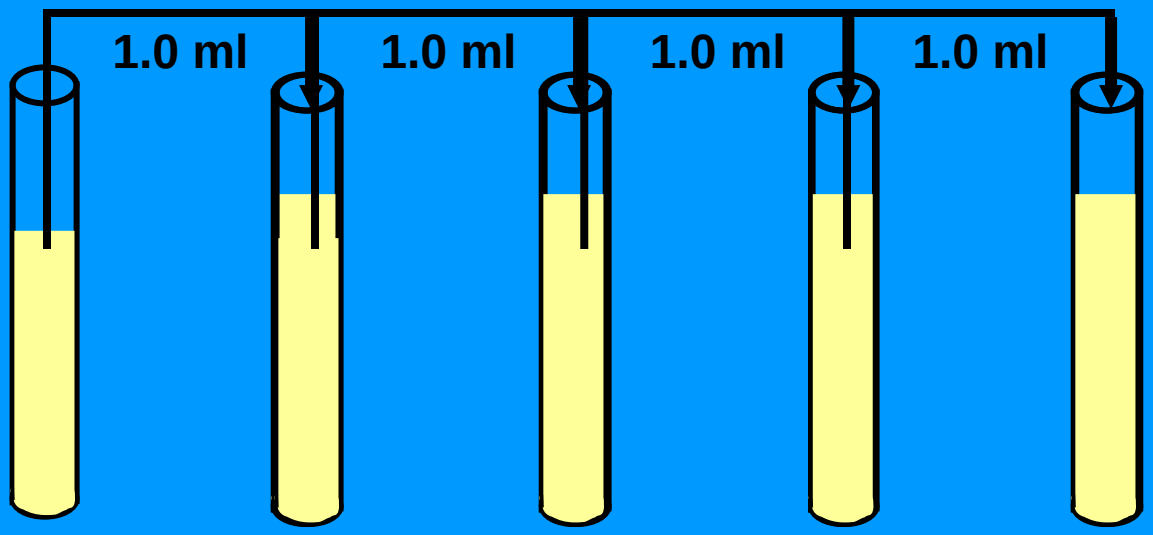


test tubes
containing
9.0 ml peptone water

decimal dilution series

preparing of further dilutions

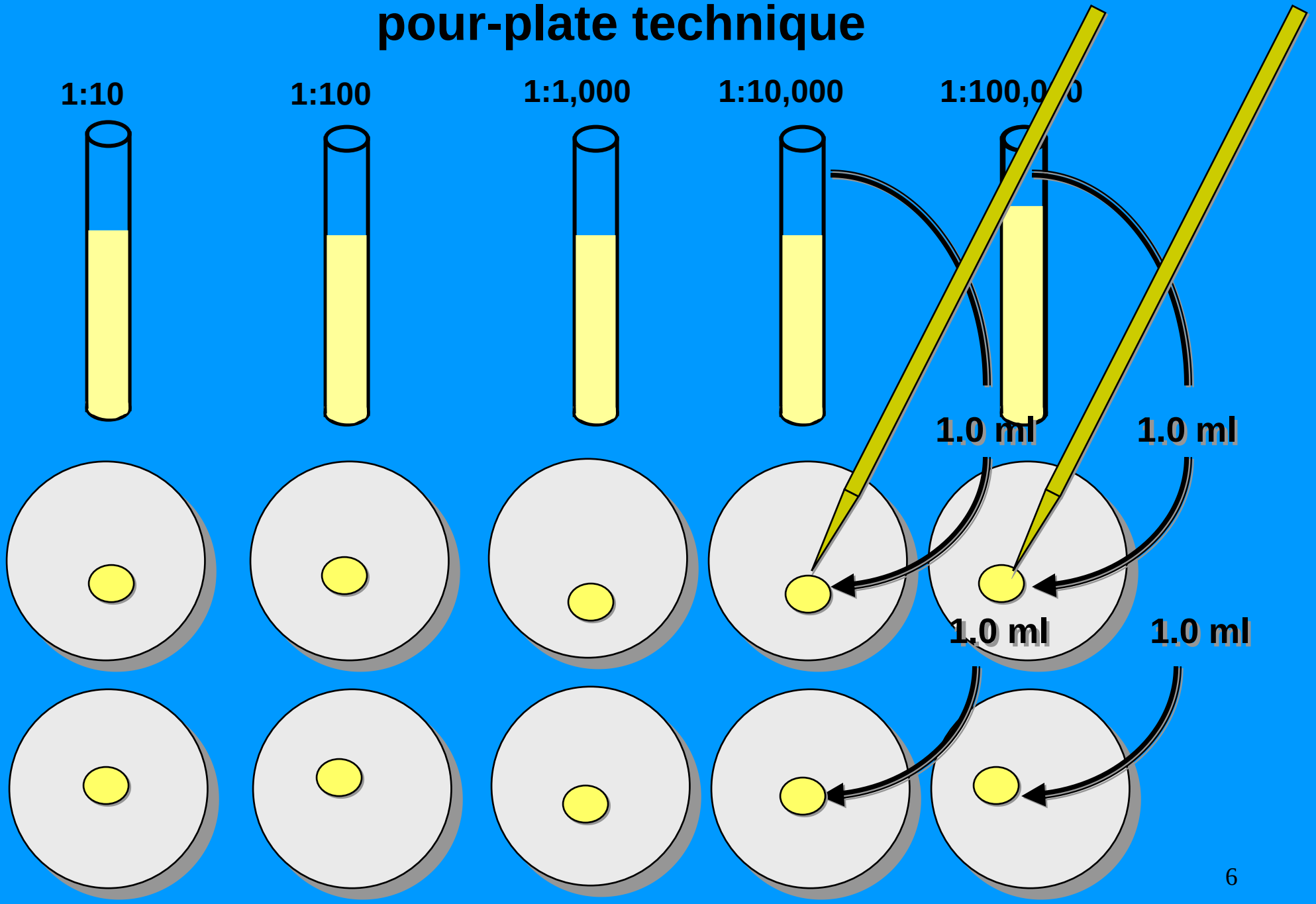
1st decimal dilution	2nd	3rd	4th	5th
1:10	1:100	1:1,000	1:10,000	1:100,000



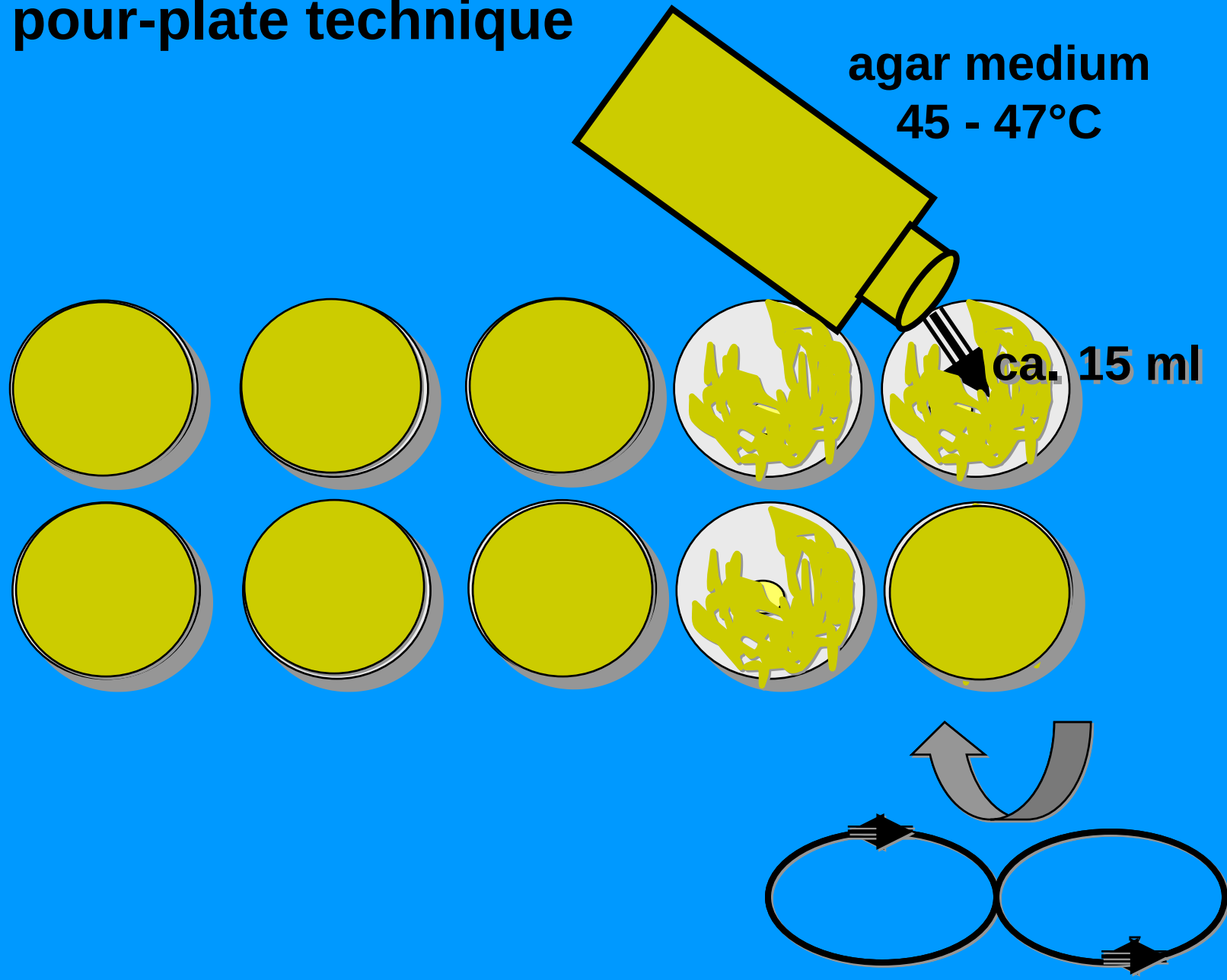
for each dilution step:

use a fresh sterile pipette
mix thoroughly using a whirl mix

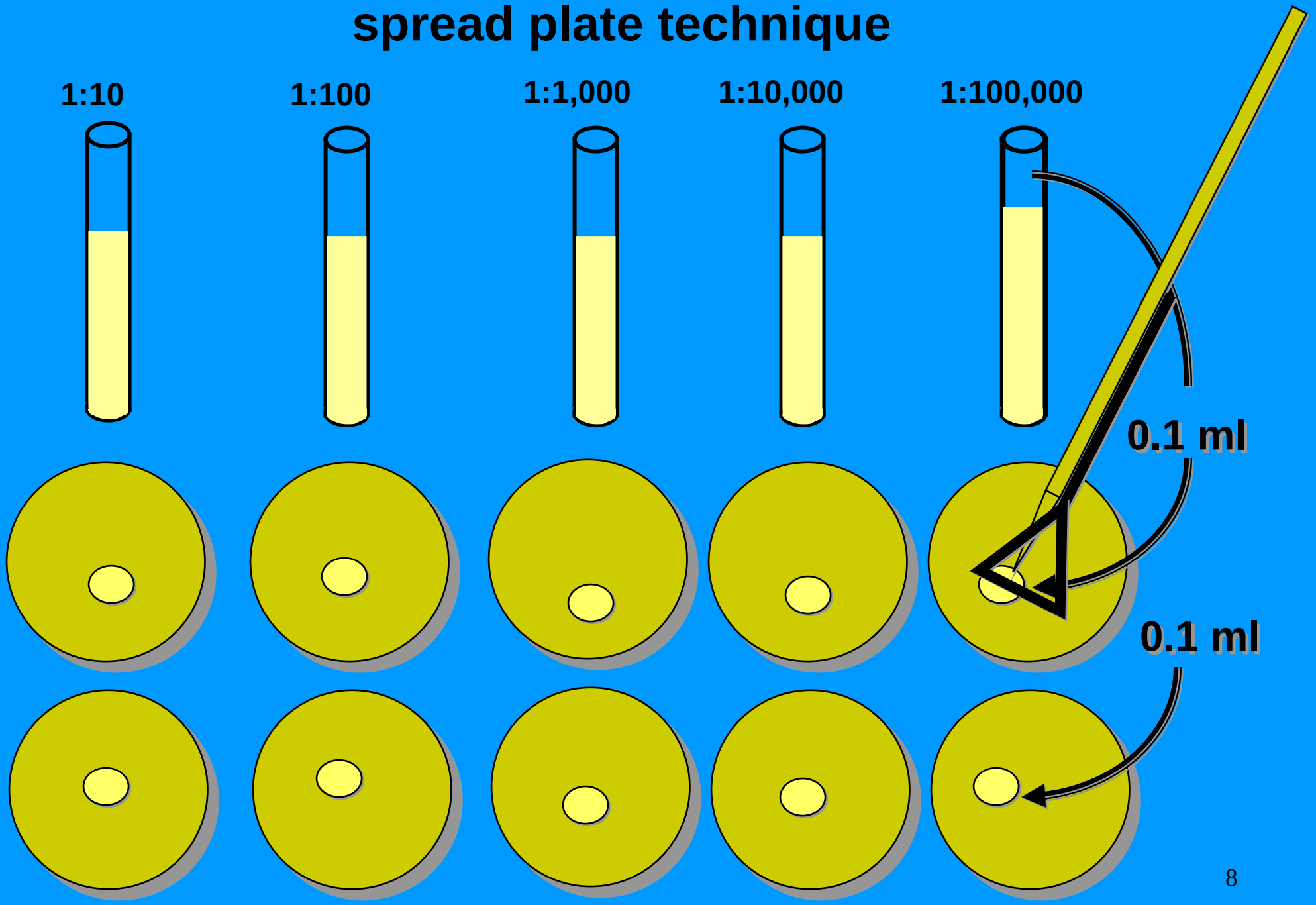
pour-plate technique



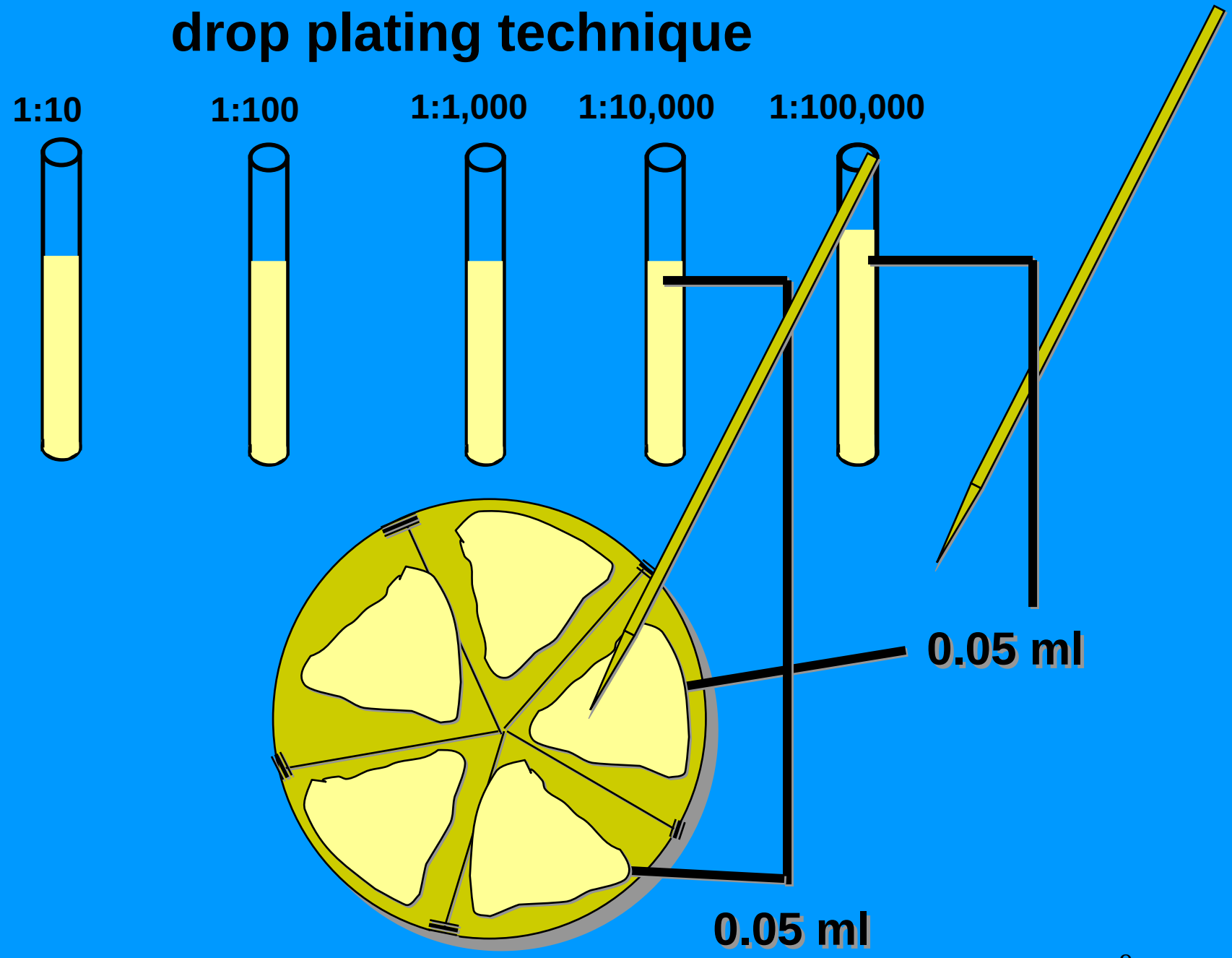
pour-plate technique



spread plate technique

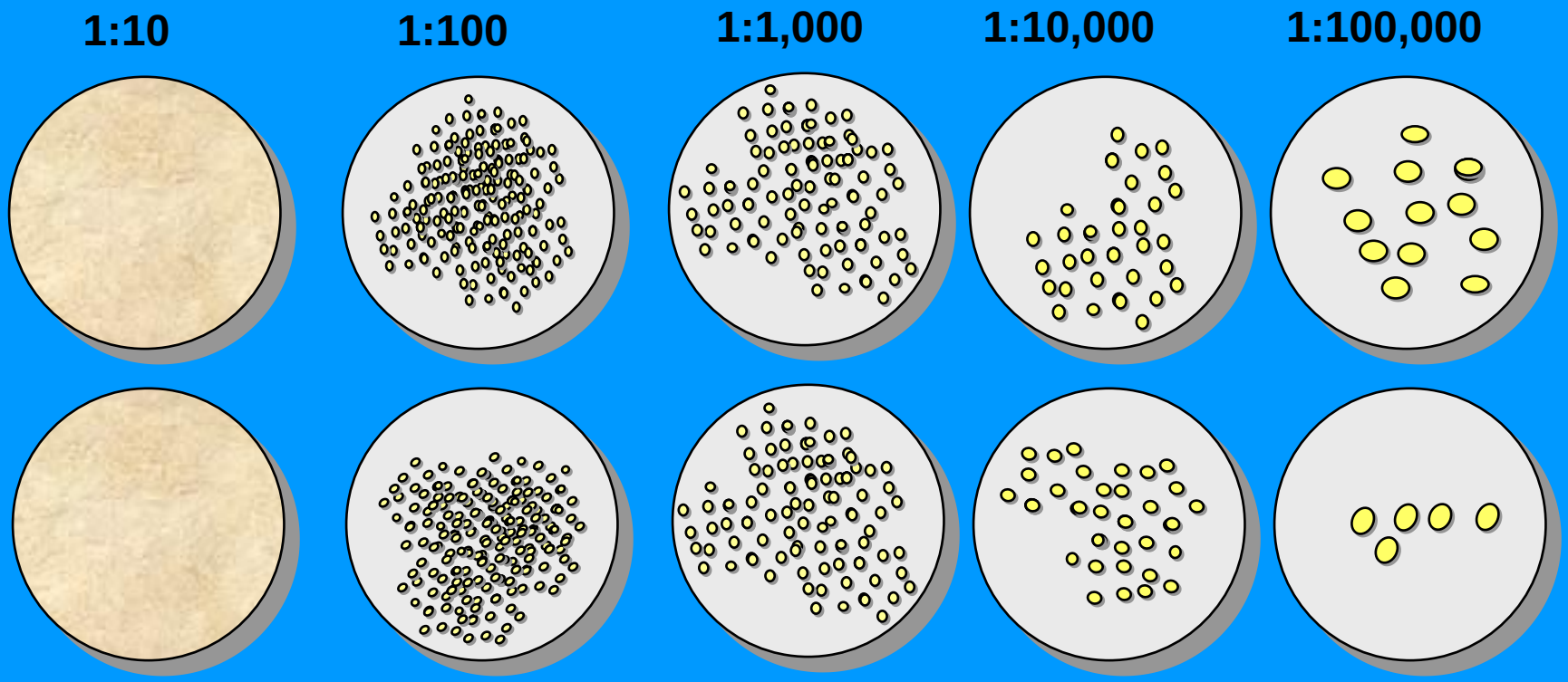


drop plating technique



Counting the plates

Pour plate technique | Spread plate technique

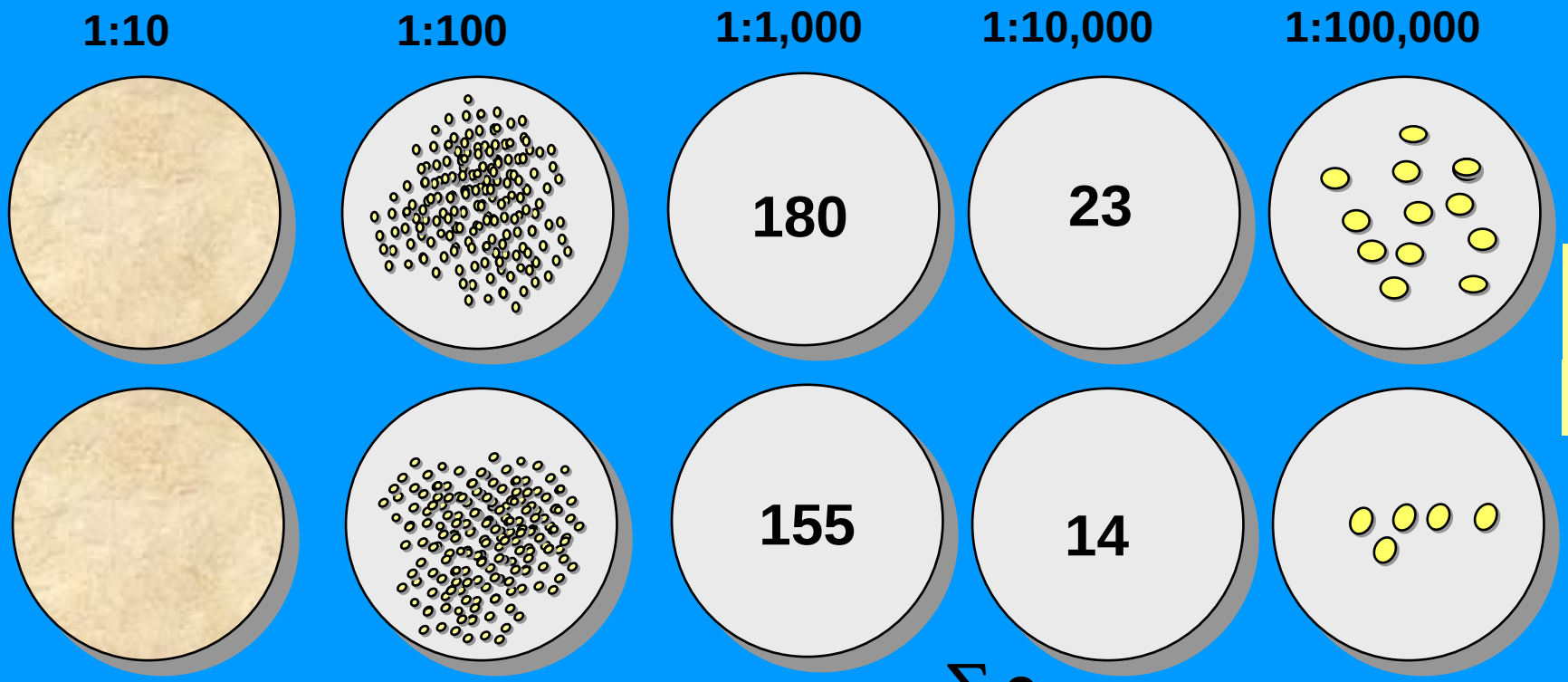


Choose plates containing **less than 300 colonies**
- at greater colony concentration
- growth may be depressed because of
  - overcrowding and microbial antagonism

But **more than 30 colonies**
- at lesser colony concentration
- statistical error becomes overwhelming great

Counting the plates

Pour plate technique | **Spread plate technique**

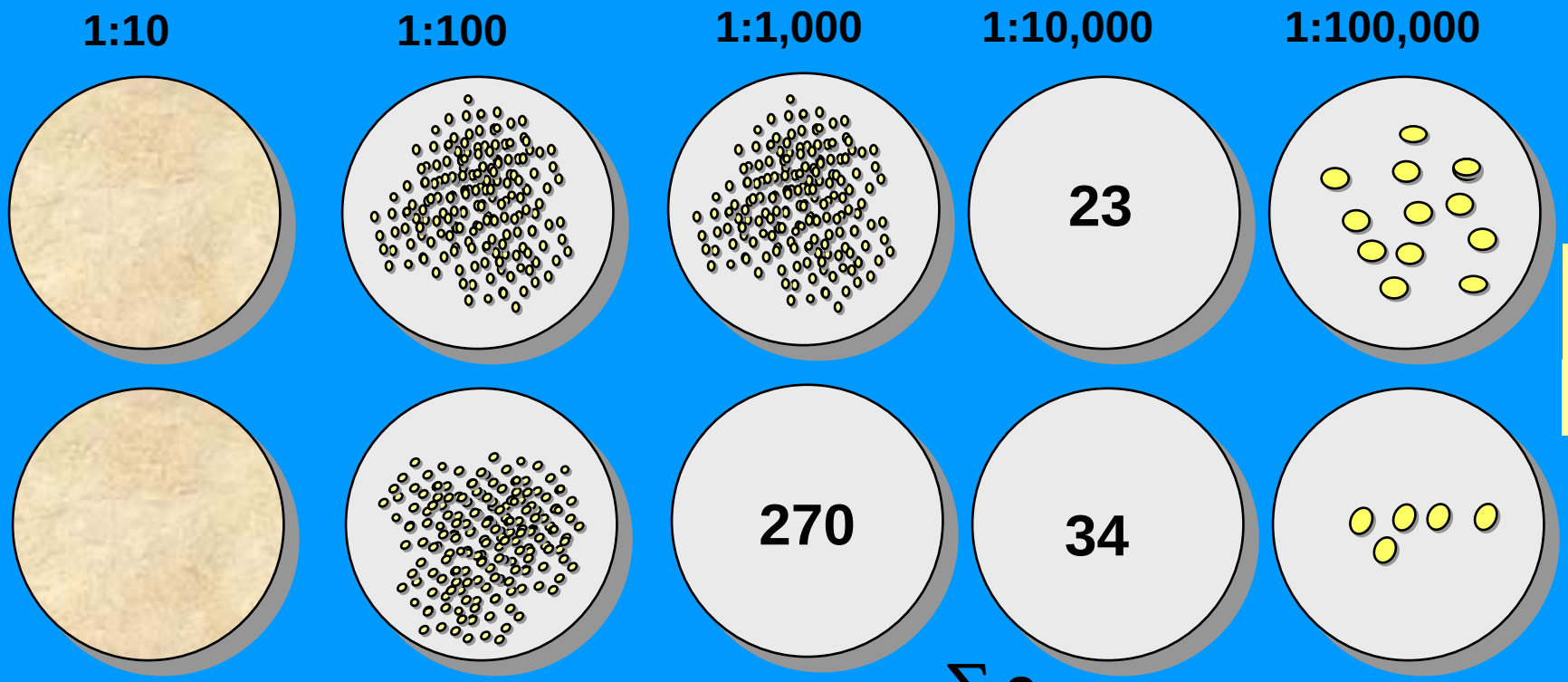


Colony count $N = \dfrac{\sum c}{(n_1 \times 1) + (n_2 \times 0.1) \times d}$

$$N = \frac{180 + 155 + 23 + 14}{(2 \times 1) + (2 \times 0.1) \times \frac{1}{1{,}000}} = \frac{372}{2.2 \times 10^{-3}} = 169{,}091 \text{ cfu / g}$$

Counting the plates

Pour plate technique | **Spread plate technique**



inoculum
1.0 ml
= 1.0 g

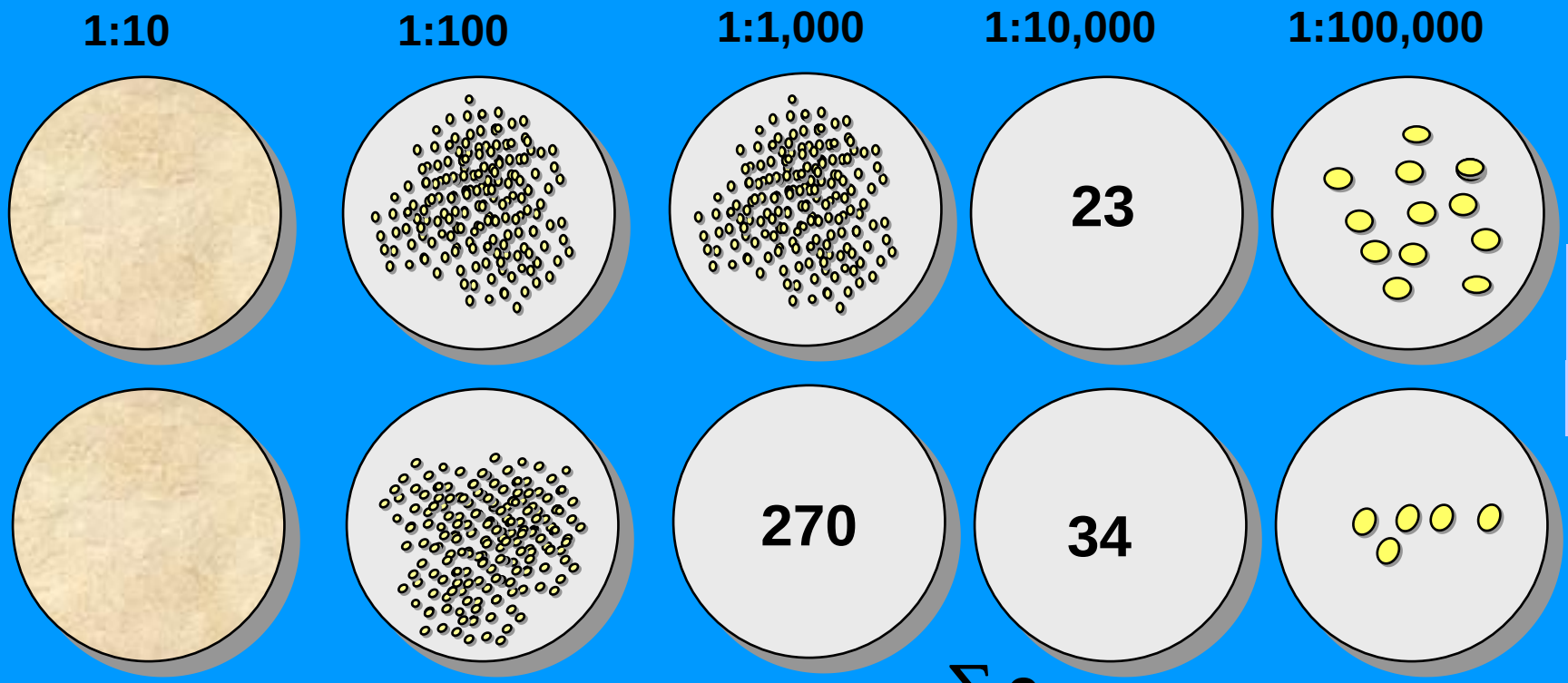
Colony count

$$N = \frac{\sum c}{(n_1 \times 1) + (n_2 \times 0.1) \times d}$$

$$N = \frac{270 + 23 + 34}{(1 \times 1) + (2 \times 0.1) \times \frac{1}{1{,}000}} = 2.3 \times 10^5 \text{ cfu / g}$$

Counting the plates

Pour plate technique | **Spread plate technique**



Colony count $N = \dfrac{\sum c}{(n_1 \times 1) + (n_2 \times 0.1) \times d}$

$$N = \frac{270 + 23 + 34}{(1 \times 1) + (2 \times 0.1) \times \frac{1}{1{,}000}} = 2.3 \times 10^5 \text{ cfu / g}$$

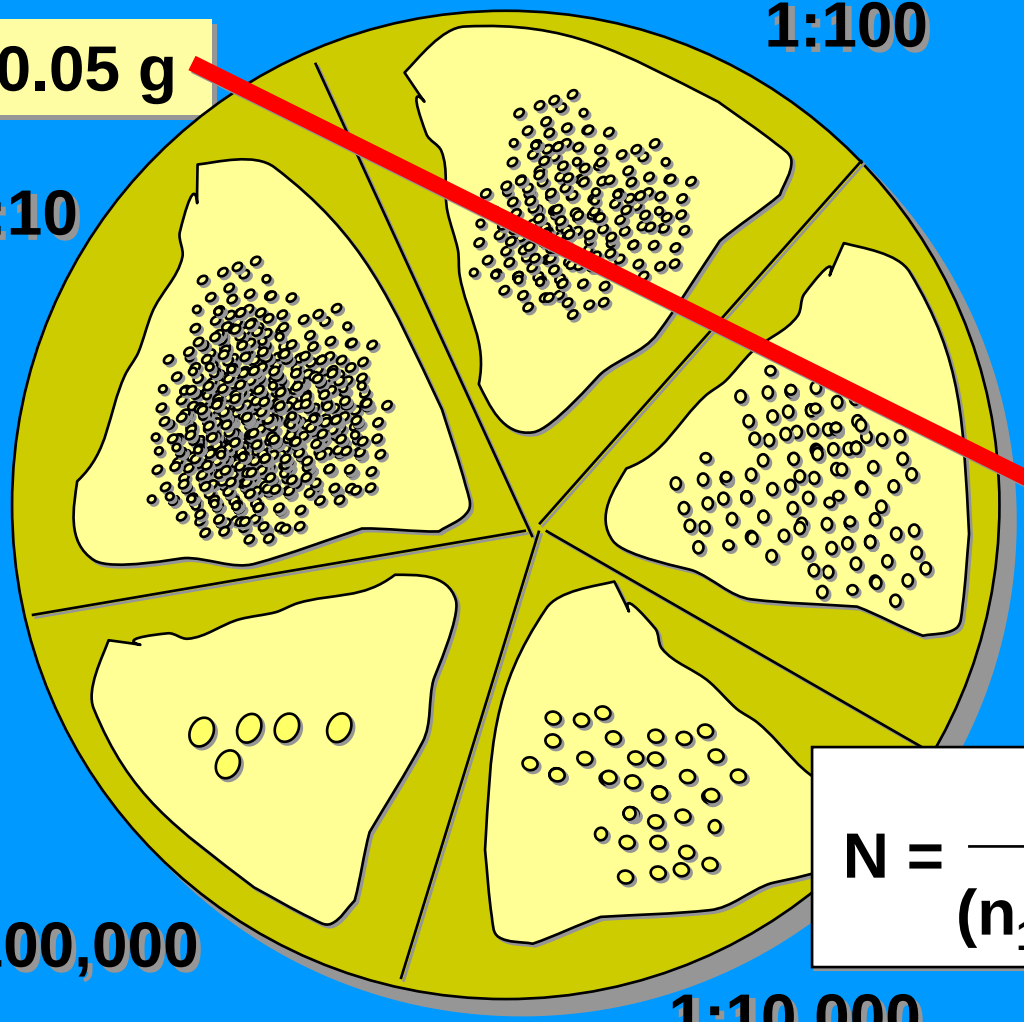
Confidence limits associated with numbers of colonies on plates

colony count	95 % confidence limits	
	lower	upper
3	< 1	9
5	2	12
10	5	18
12	6	21
15	8	25
30	19	41
50	36	64
100	80	120
200	172	228

Counting the plates

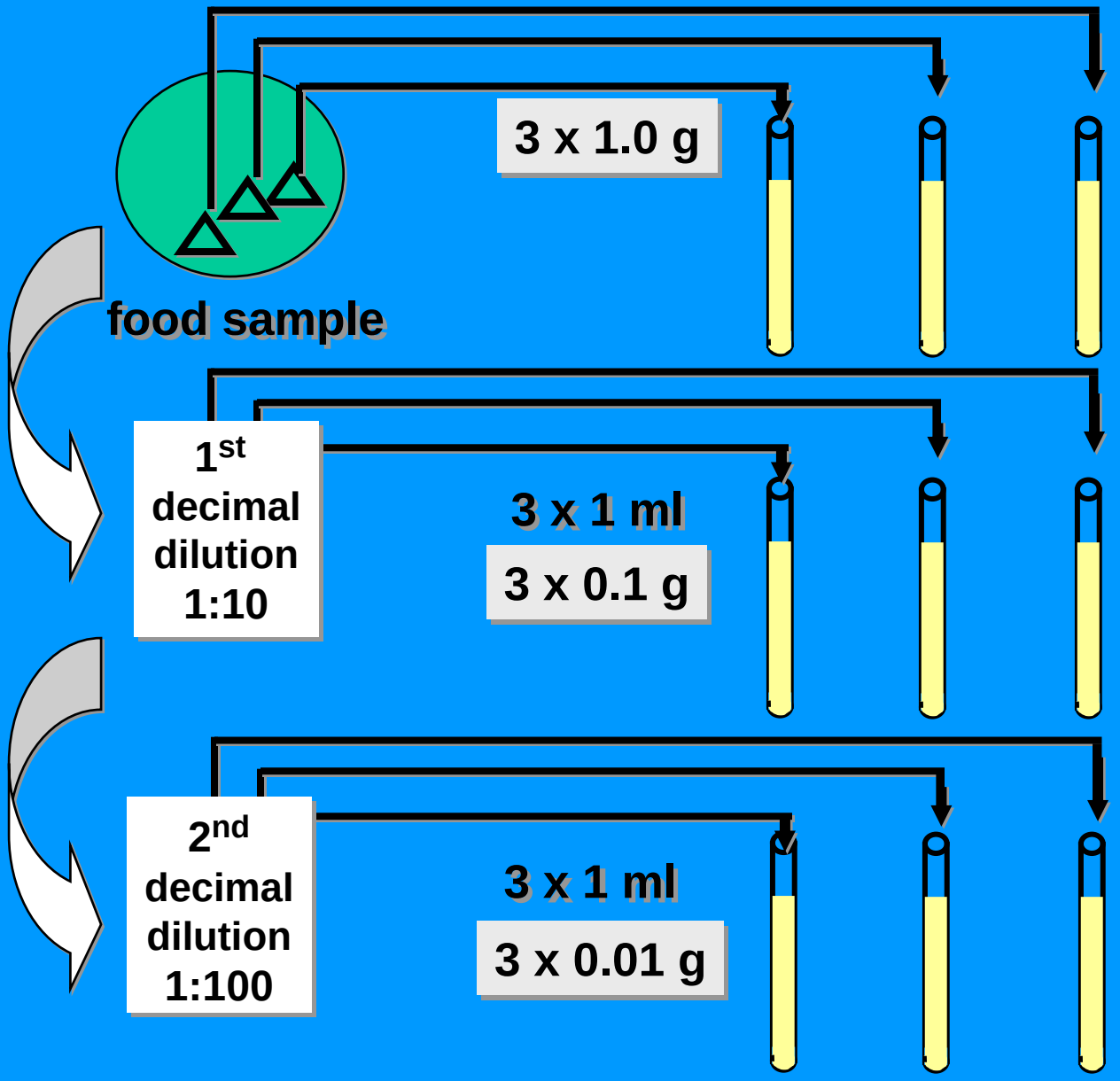
drop plating technique

0.05 ml = 0.05 g



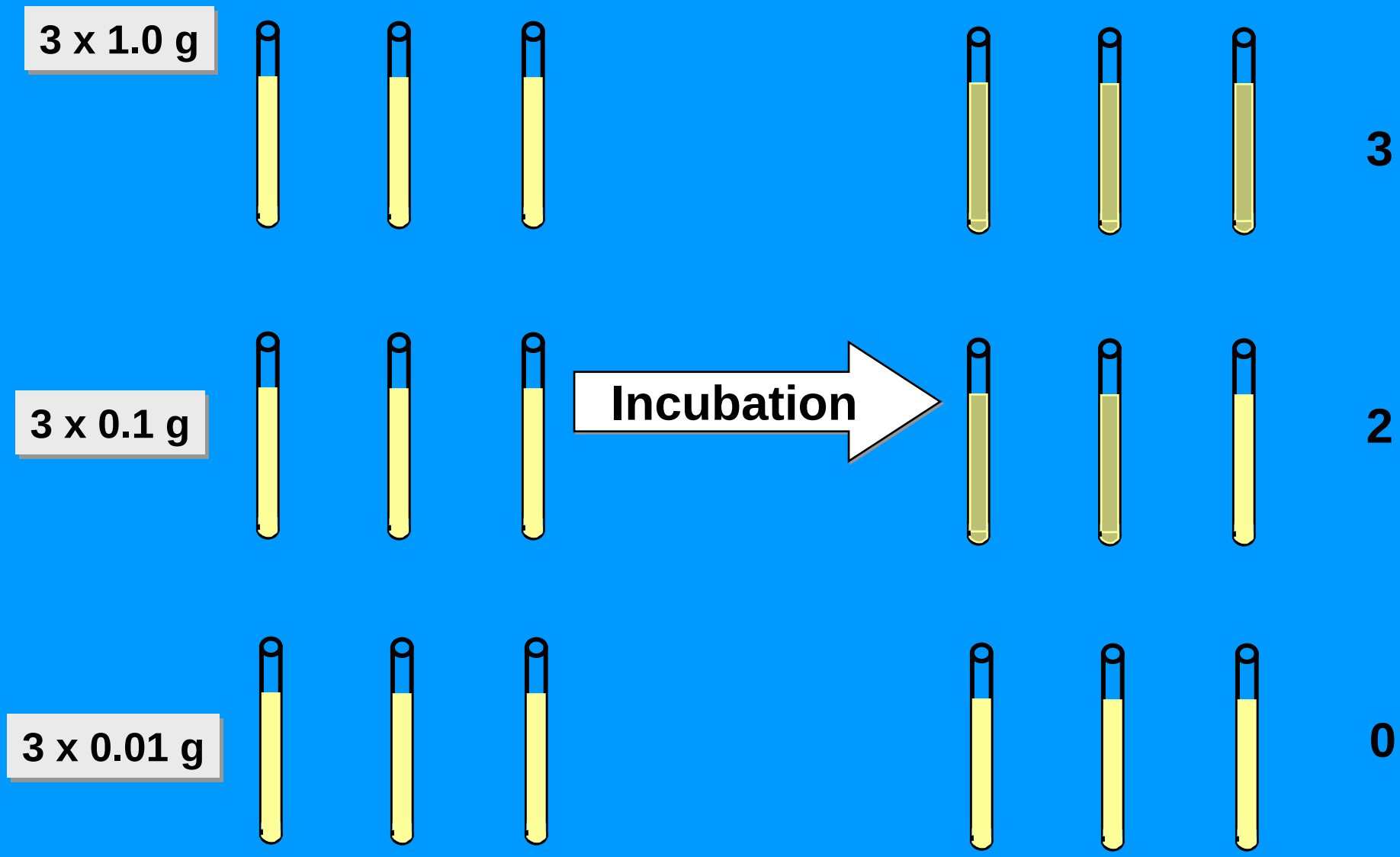
$$N = \frac{\sum c}{(n_1 \times 1) + (n_2 \times 0.1) \times d} \times 20$$

Most Probable Number (MPN) technique



To estimate the number of cfu in food samples, if quantitative detection of low numbers is required

Most Probable Number (MPN) technique



Most Probable Number (MPN) technique

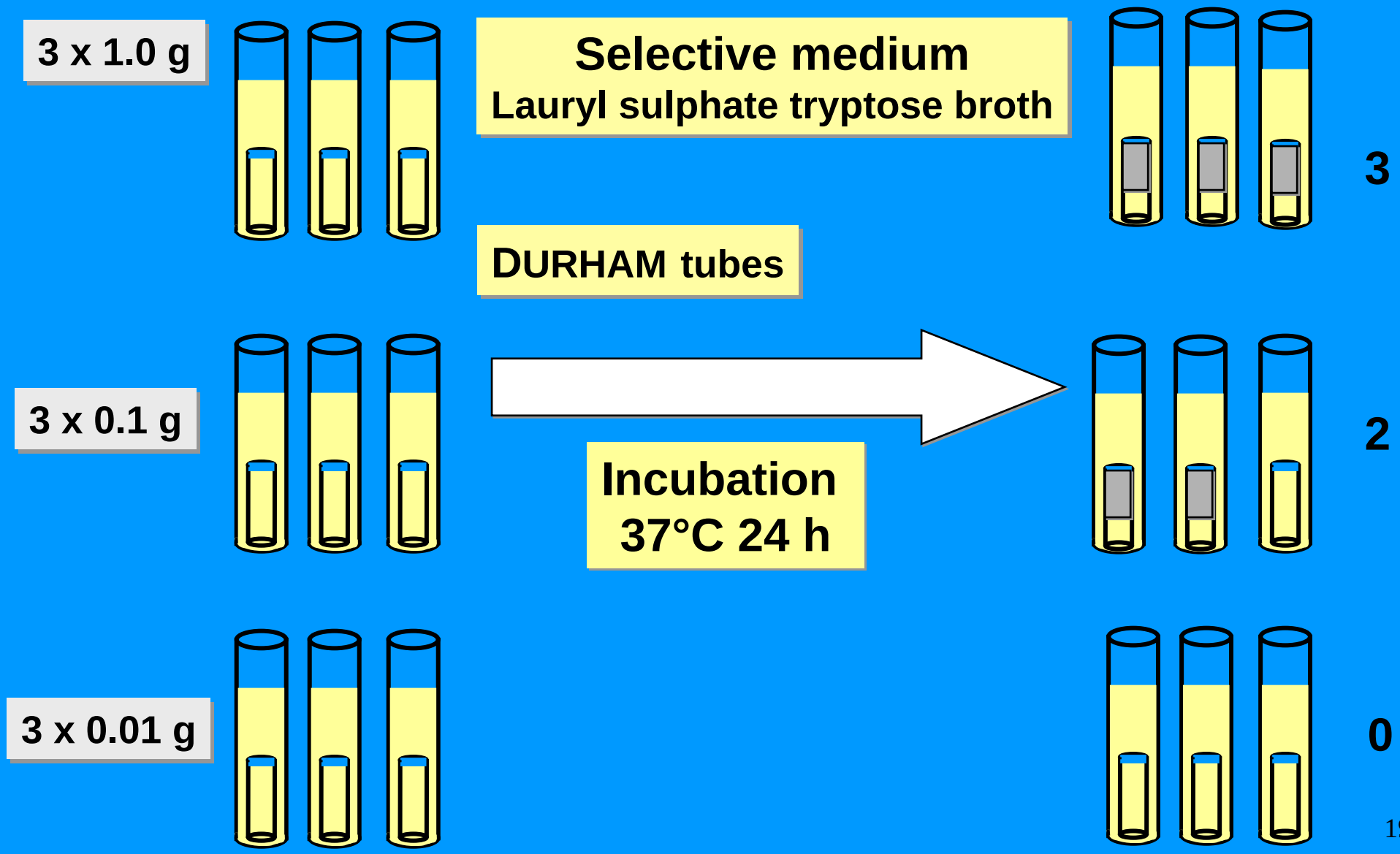
MPN table for 3 x 3

Number of positive tubes inoculated with			MPN / g
1 .0 g	0.1 g	0.01 g	
0	0	0	< 0.3
1	0	0	0.36
1	1	0	0.74
2	0	0	0.92
2	1	0	1.5
2	1	1	2.0
2	2	0	2.1
2	2	1	2.8
3	0	0	2.3
3	1	0	4.3
3	2	0	9.3
3	3	2	110.0
3	3	3	> 110

3 x 1.0 g	3 x 0.1 g	3 x 0.01 g
3	2	0

Most Probable Number (MPN) technique

Example: Enumeration of Coliforms



Genus **Salmonella**

2 Species

Salmonella enterica	*Salmonella bongori*

6 Subspecies

- ***S. enterica* subsp. enterica**
- ***S. enterica* subsp. salamae**
- ***S. enterica* subsp. arizonae**
- ***S. enterica* subsp. diarizonae**
- ***S. enterica* subsp. houtenae**
- ***S. enterica* subsp. indica**

In total ca. 2500 serovars (KAUFMANN-WHITE schema)

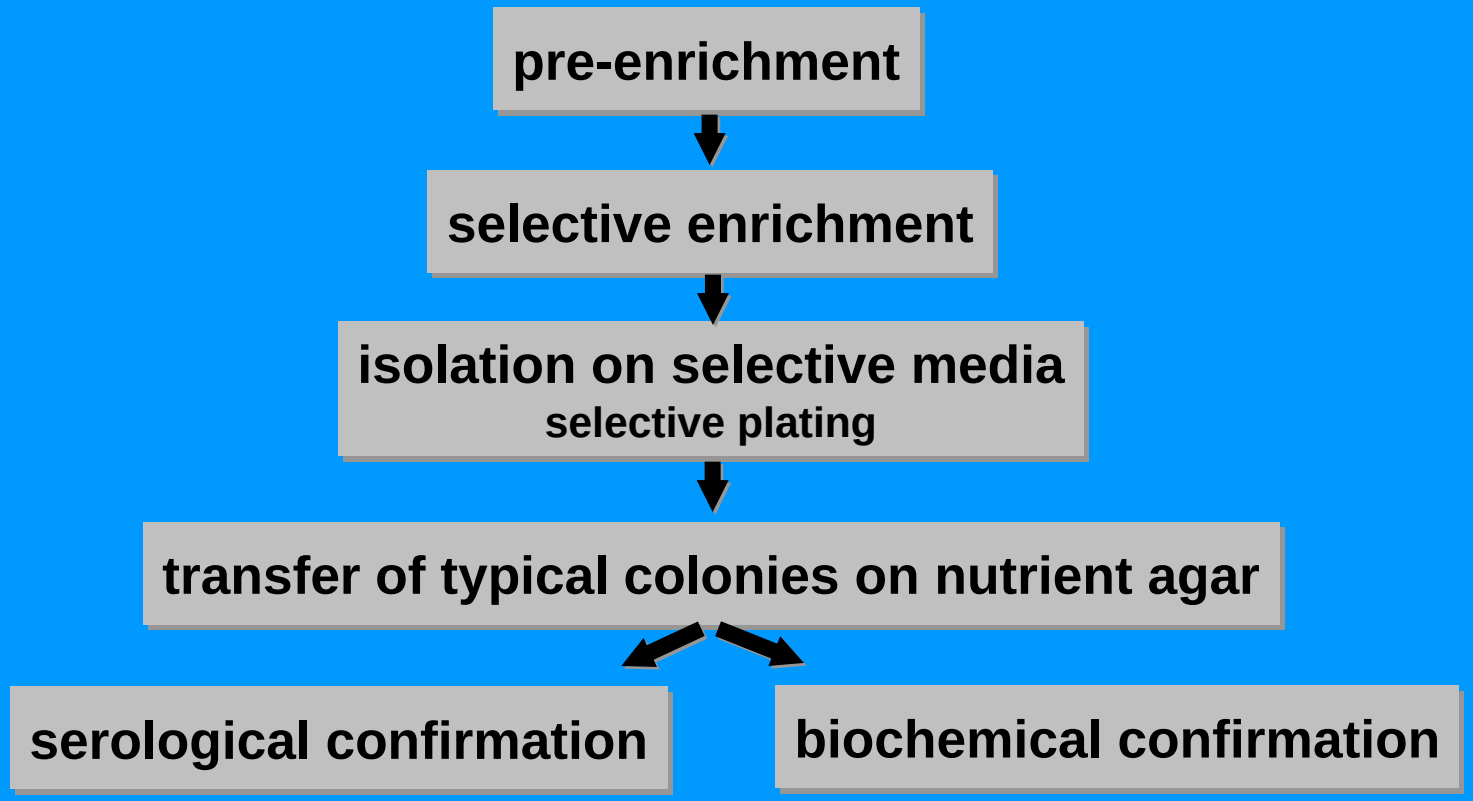
***S. enterica* subsp. enterica serovar typhimurium**

S. typhimurium

***S.* Typhimurium**

detection of *Salmonella*

ISO method 6579:1993



detection of *Salmonella* ISO method 6579 :1993

pre-enrichment — resuscitation of sublethally damaged Salmonellae

25 g sample + **225 ml puffered peptone water**

special pre-enrichment for

spices
chocolate
milk powder

incubation: 20 h at 37°C

⬇

selective enrichment

0.1 ml of PE + 10 ml Rappaport-Vassiliadis broth	10 ml of PE + 100 ml selenite cystine broth
Magnesium chloride $MgCl_2$ Malachite green pH 5,2°C	Attention: selenite toxic for human
Attention: RV-broth does not support growth of S. Typhi	
incubation: 18 - 24 h at 42°C 48 h	incubation: 18 - 24 h at 37°C 48 h

detection of *Salmonella* ISO method 6579:1993

selective enrichment

0.1 ml of PE + 10 ml Rappaport-Vassiliadis broth

10 ml of PE + 100 ml selenite cystine broth

isolation on selective media
selective plating

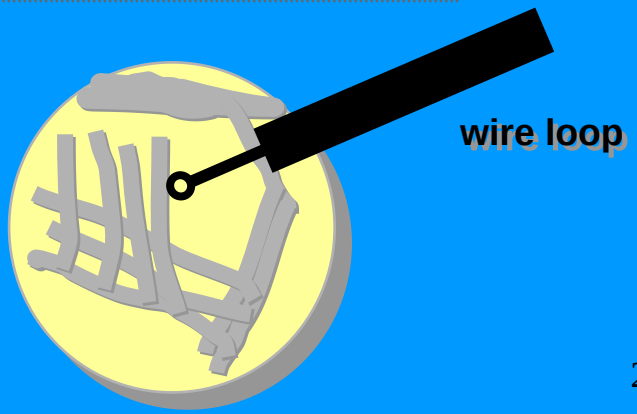
Brillant green Phenol red Lactose Sucrose Agar

Xylose Lactose Desoxycholate Agar

RAMBACH Agar

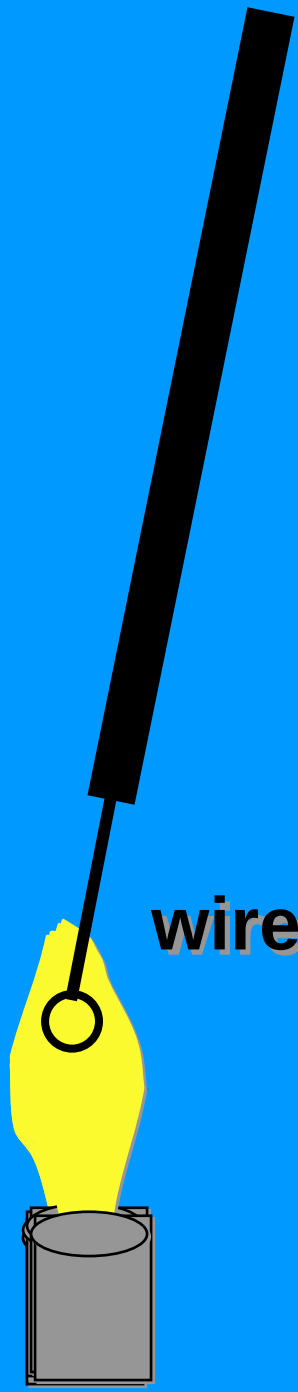
streak plates

separation of mixed culture



streak plates

separation of mixed culture



wire loop

Brillant green Phenol red Lactose Sucrose Agar

incubation: 24 and 48 h at 37°C

Selective agent

Brillant green

Lactose
Phenol red

differential agents

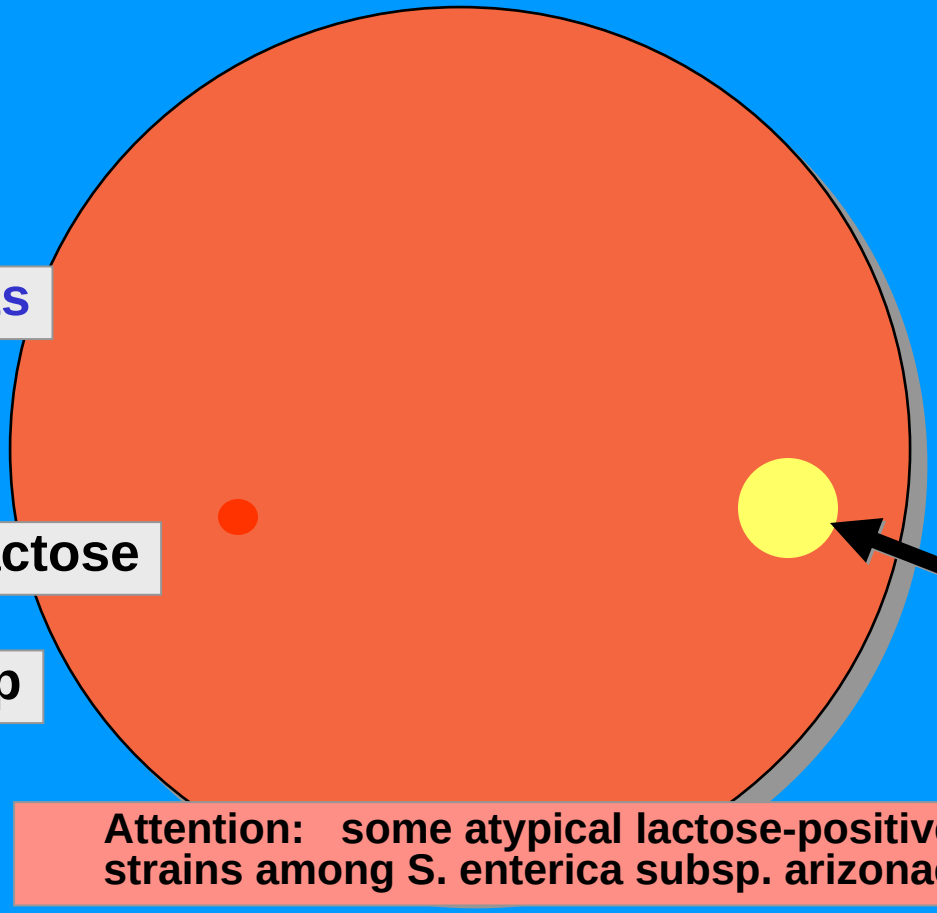
Salmonella

do not ferment lactose

↓

pH does not drop

pH indicator
Phenol red
stays red



coliform bacteria

ferment lactose

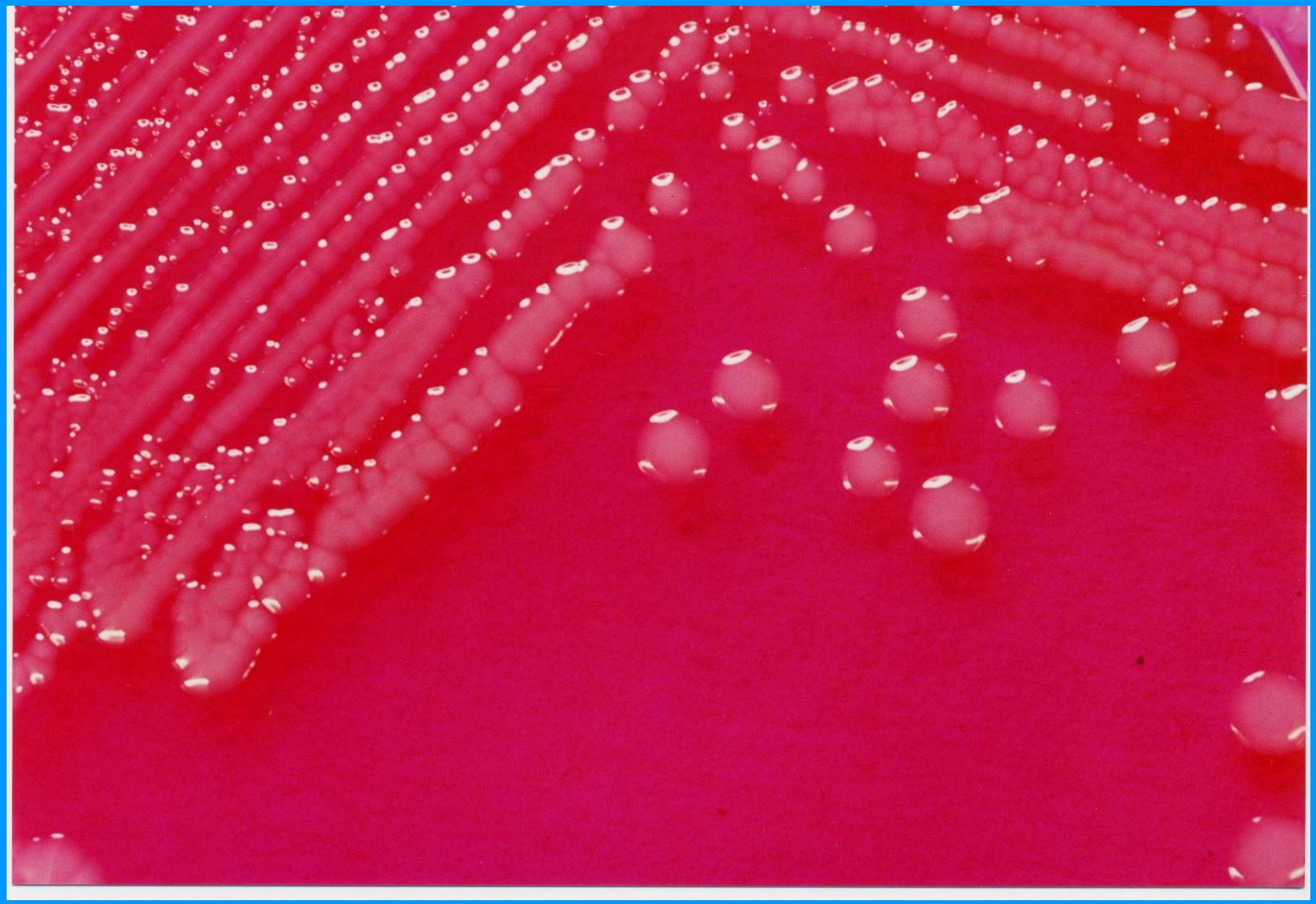
↓

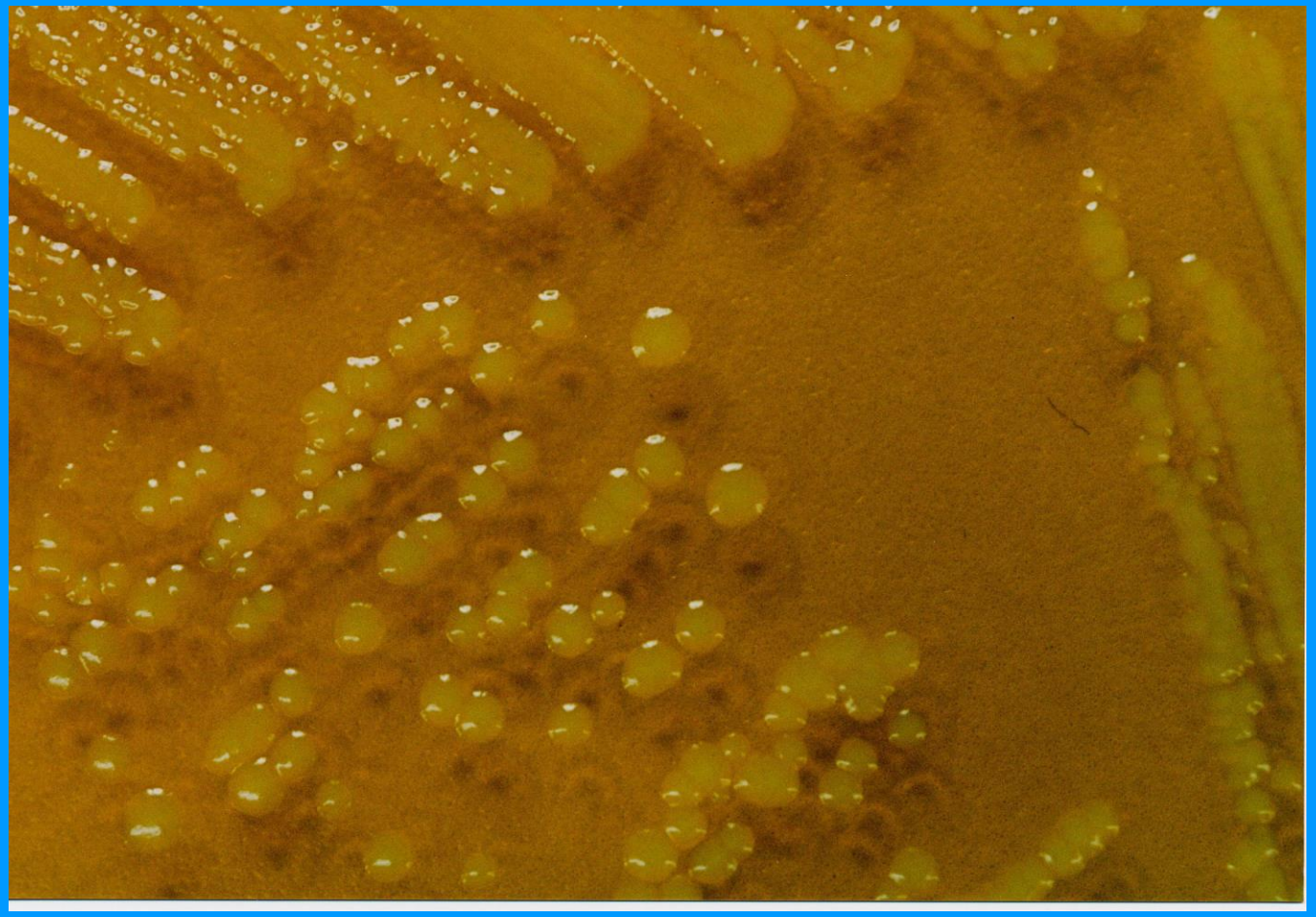
pH drops

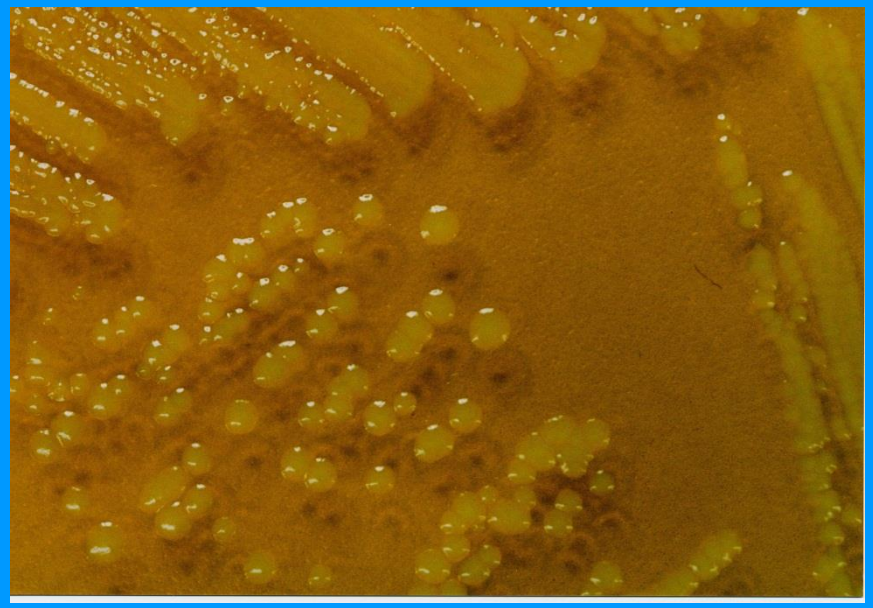
↓

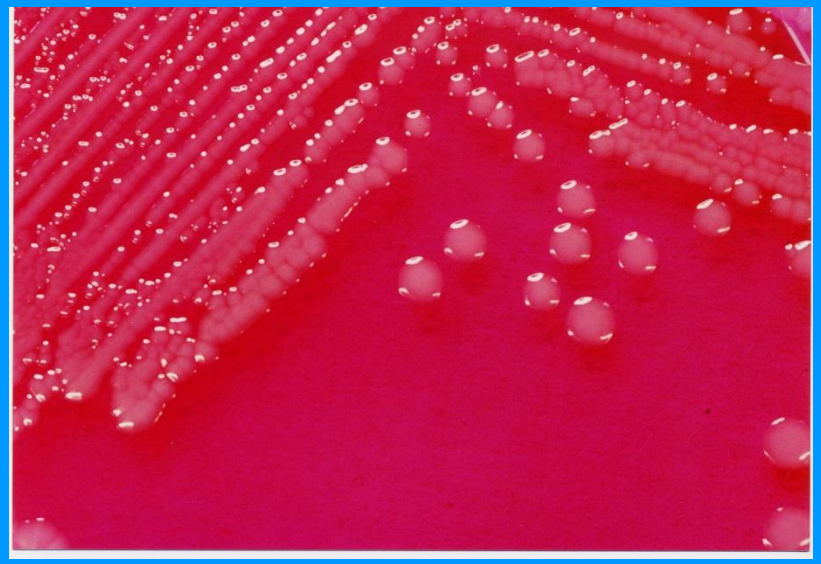
pH indicator
Phenol red
turns to yellow

Attention: some atypical lactose-positive strains among S. enterica subsp. arizonae









Xylose Lactose Desoxycholate Agar

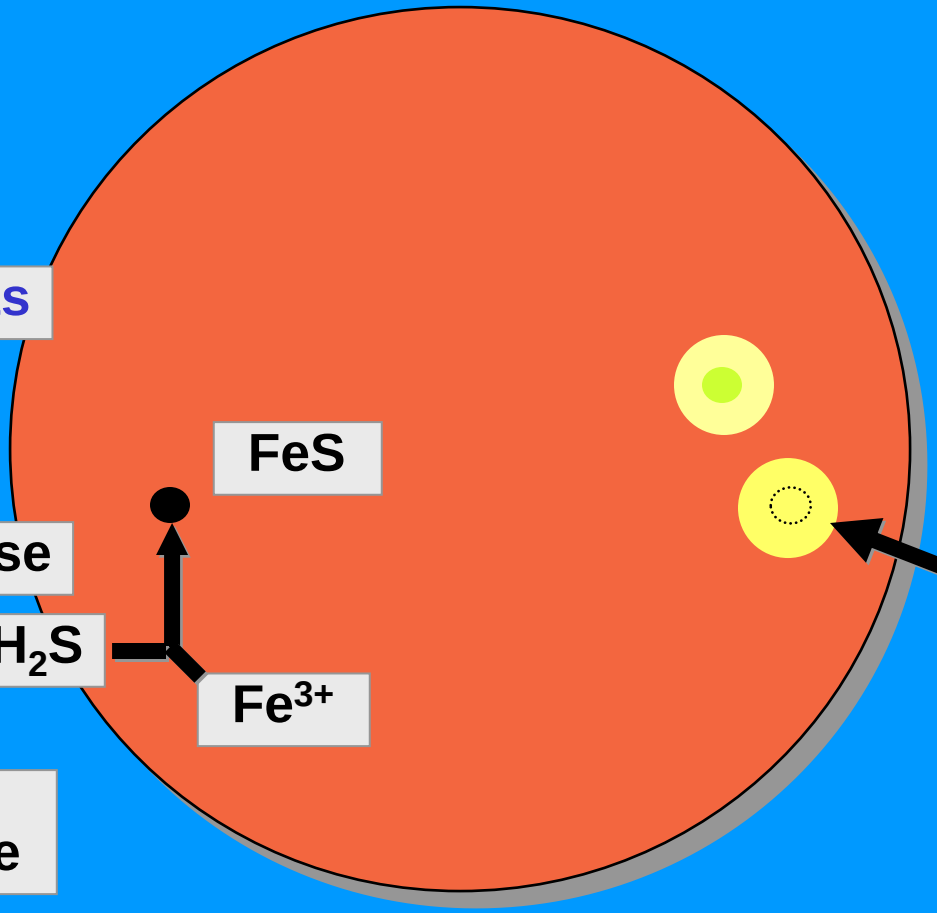
incubation: 24 and 48 h at 37°C

Selective agent

Desoxycholate

Lactose
Xylose
Lysine
Phenol red

differential agents



Salmonella

do not ferment lactose

produce H_2S

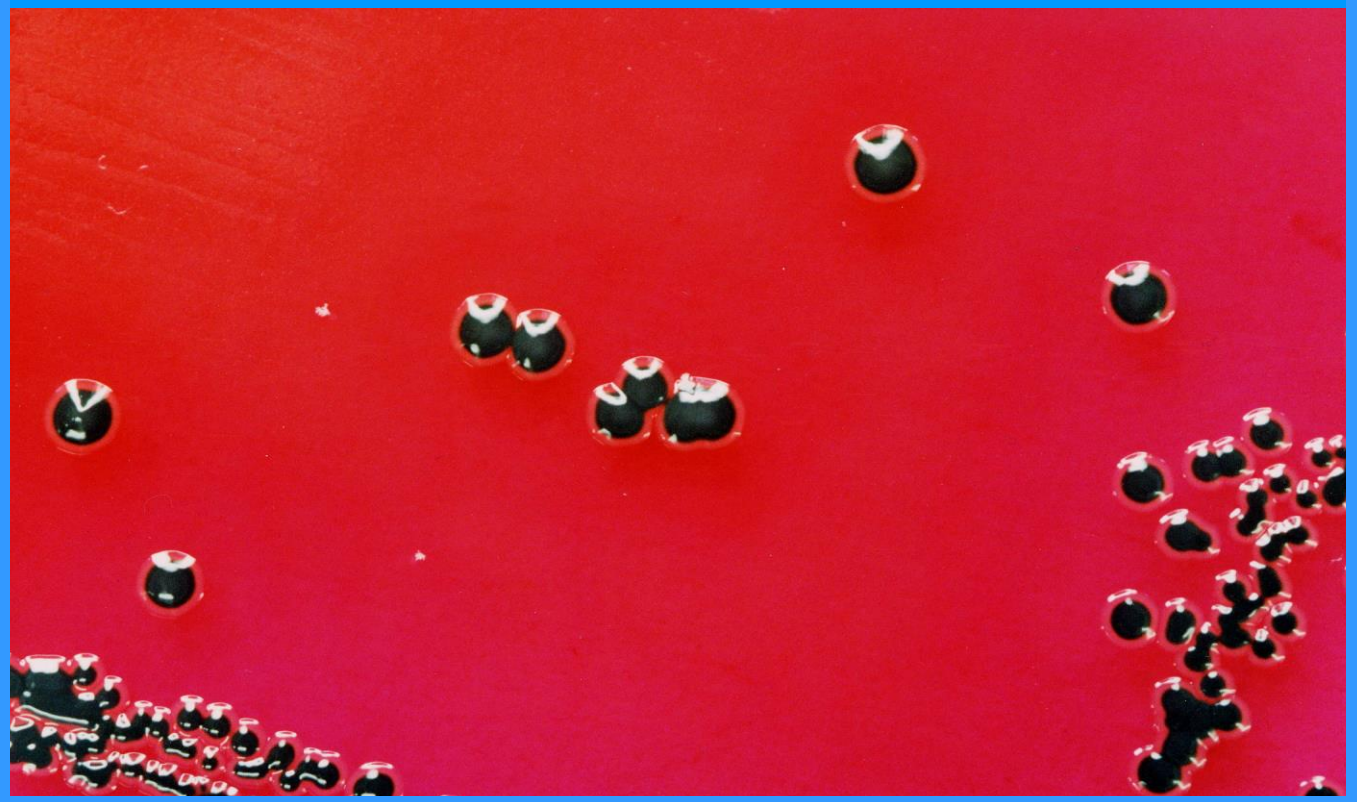
ferment xylose
decarboxylate lysine

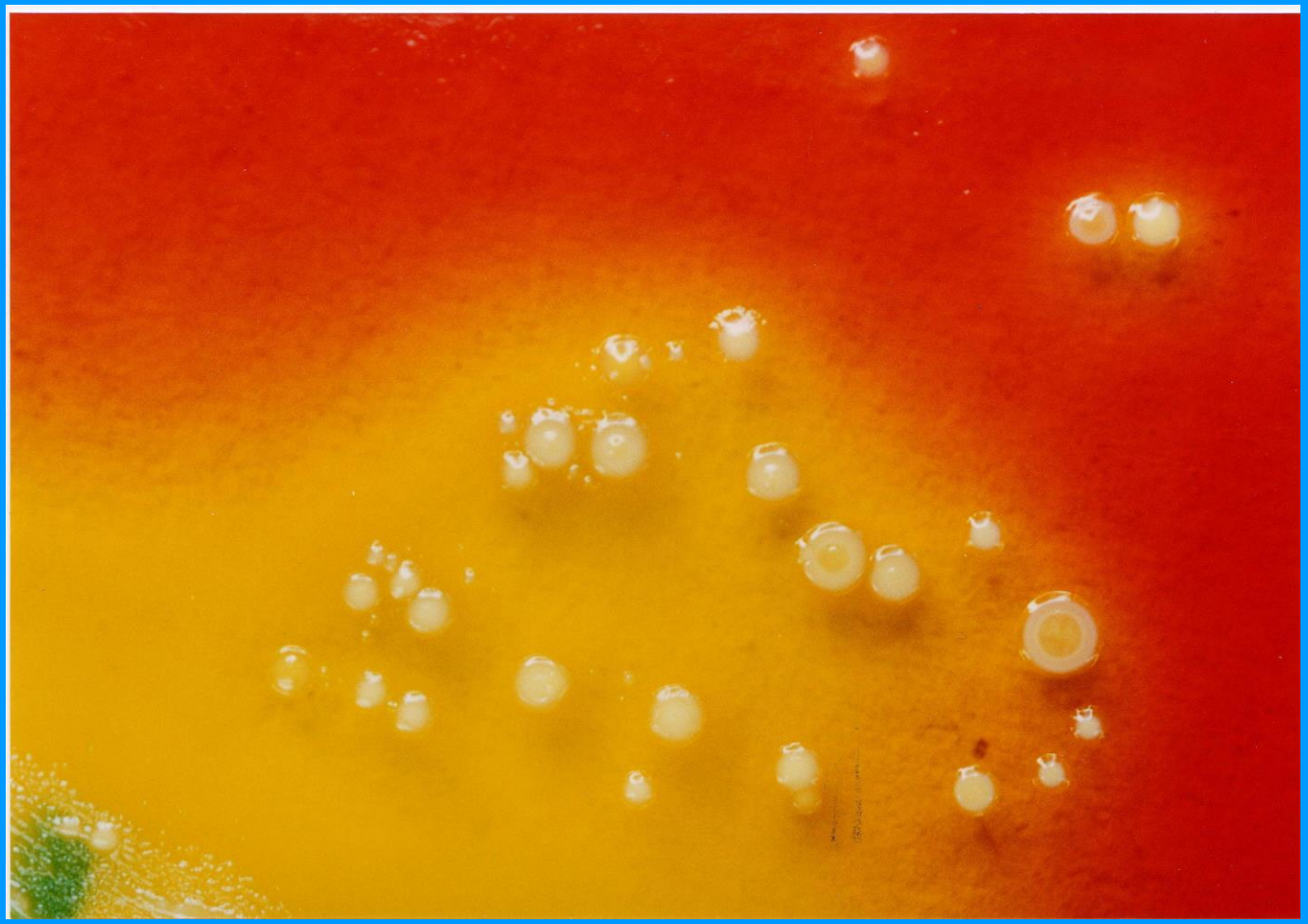
other bacteria

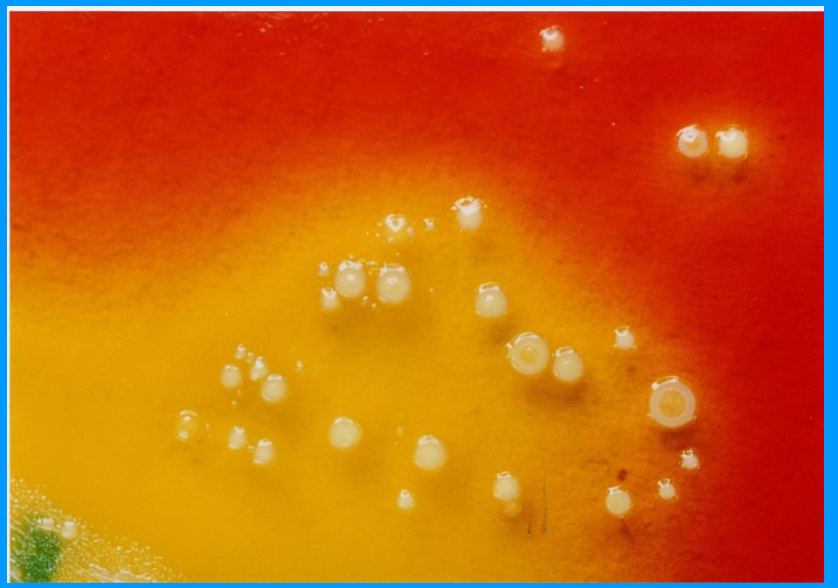
ferment lactose

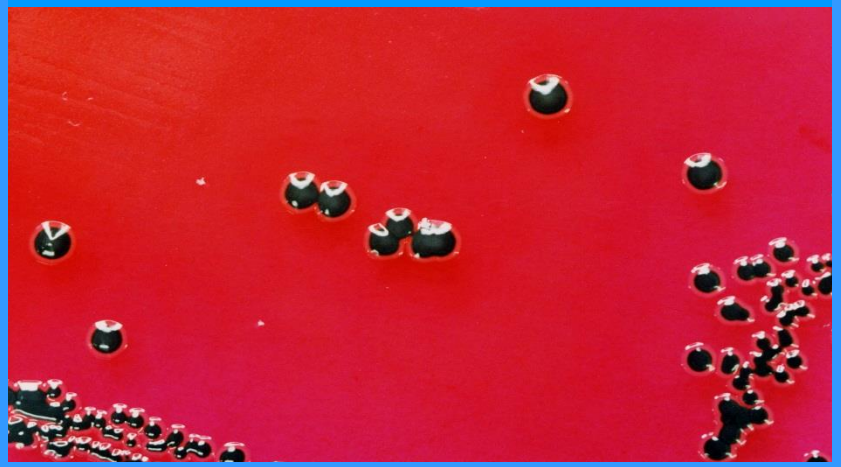
pH drops

pH indicator
Phenol red
turns to yellow





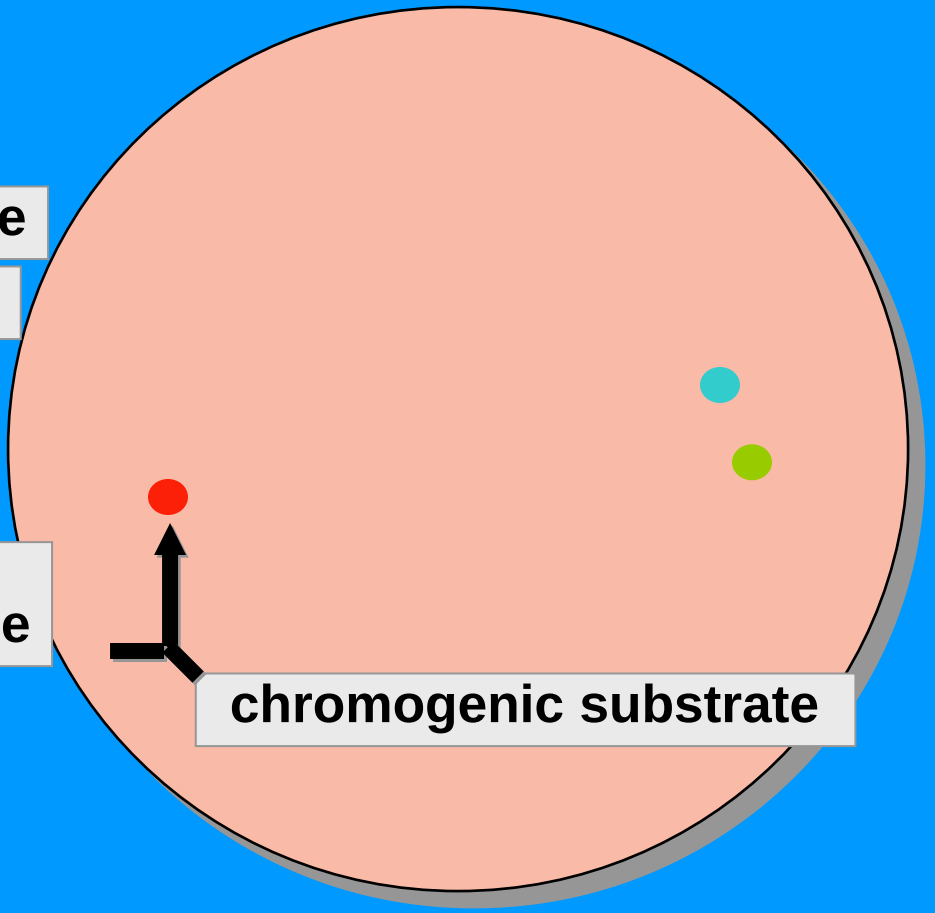




detection of *Salmonella* ISO method 6579:1993

RAMBACH Agar

incubation: 24 and 48 h at 37°C



Propylene glycole

differential agent

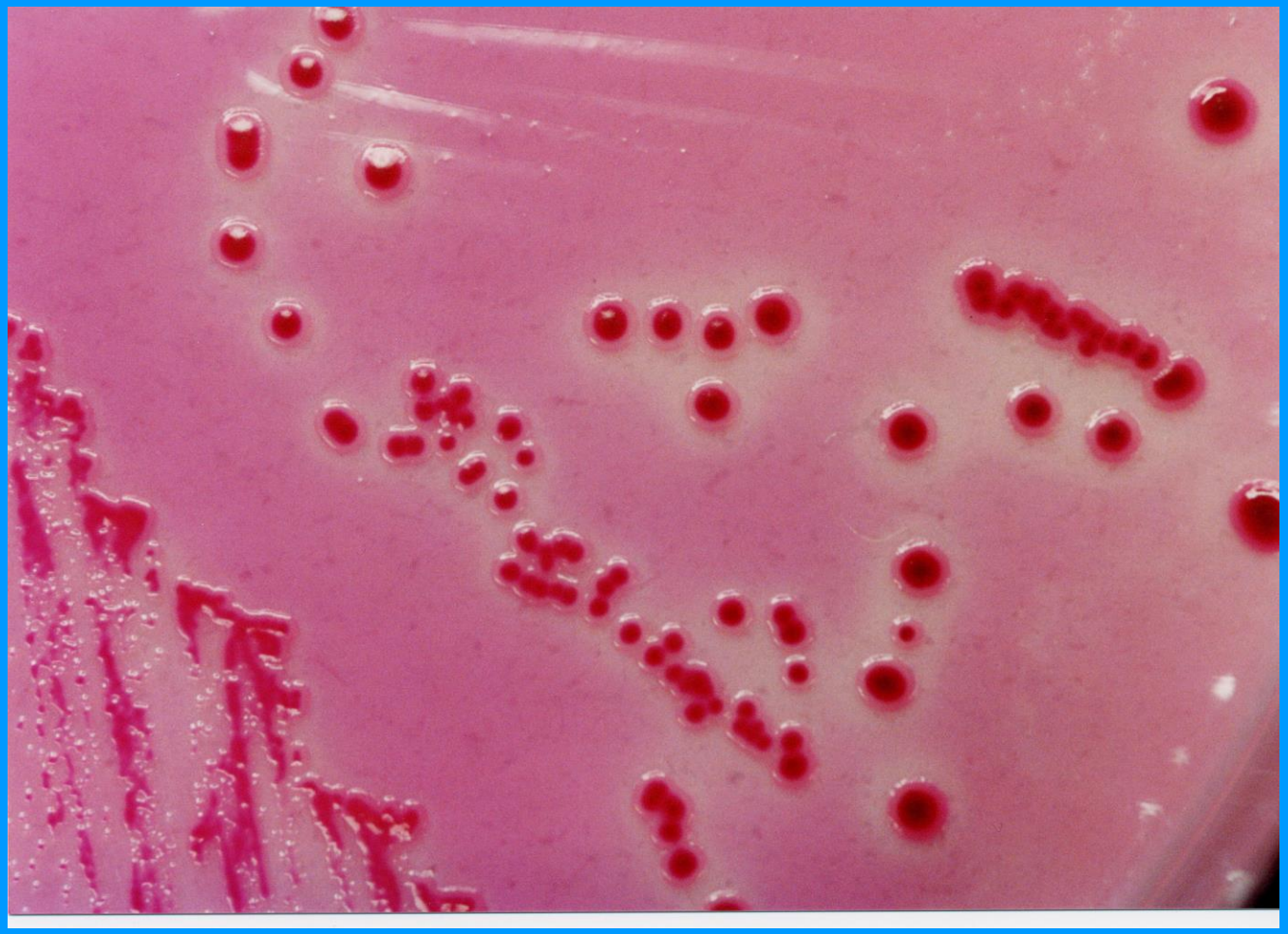
Salmonella

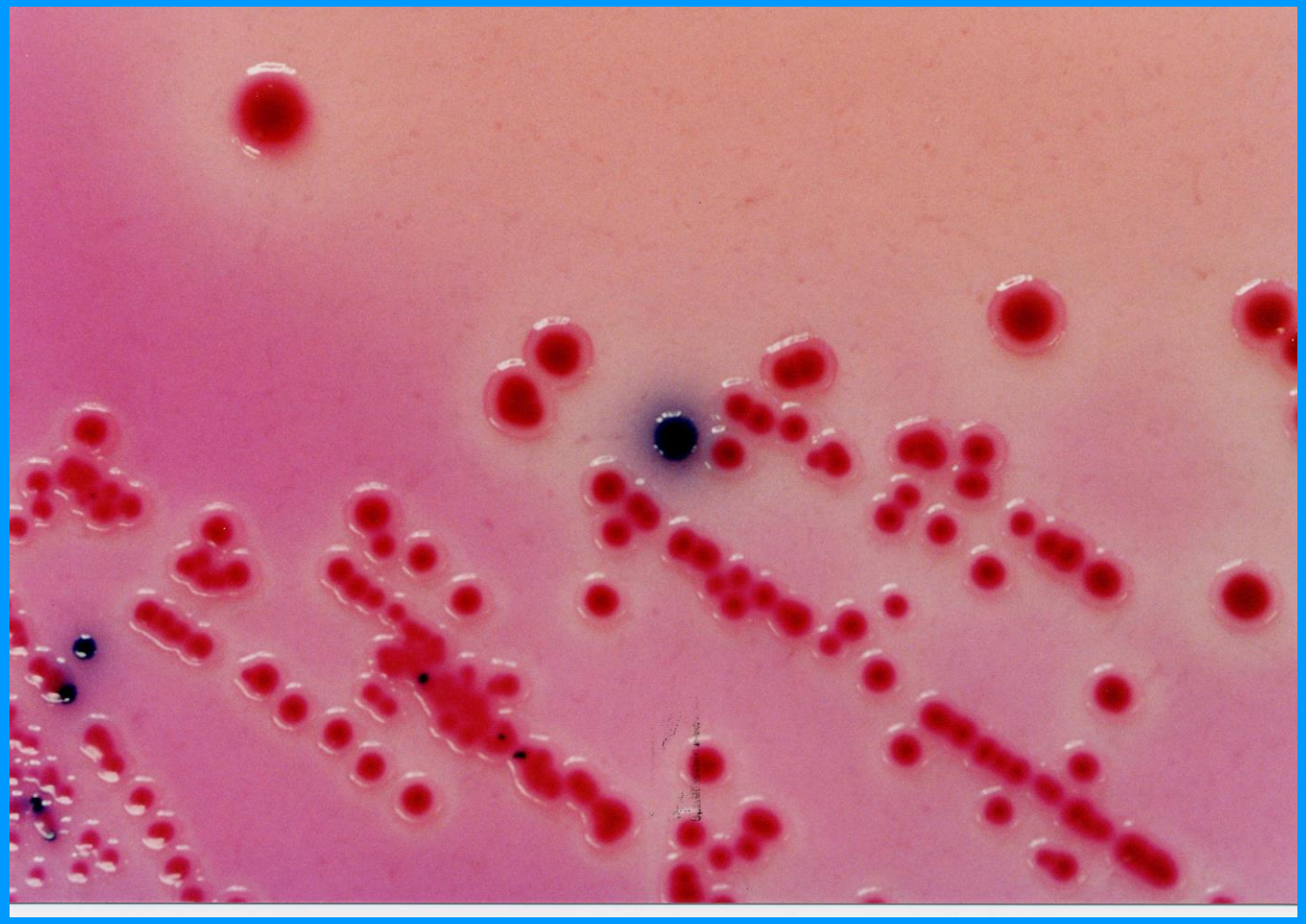
ferment Propylene glycole

other bacteria

do not ferment Propylene glycole

chromogenic substrate





detection of *Salmonella* ISO method 6579:1993

isolation on selective media
selective plating

transfer of typical colonies on nutrient agar — **18 - 24 h at 37°C**

serological confirmation

slide agglutination tests

using polyvalent anti-Salmonella antisera

exclude autagglutination

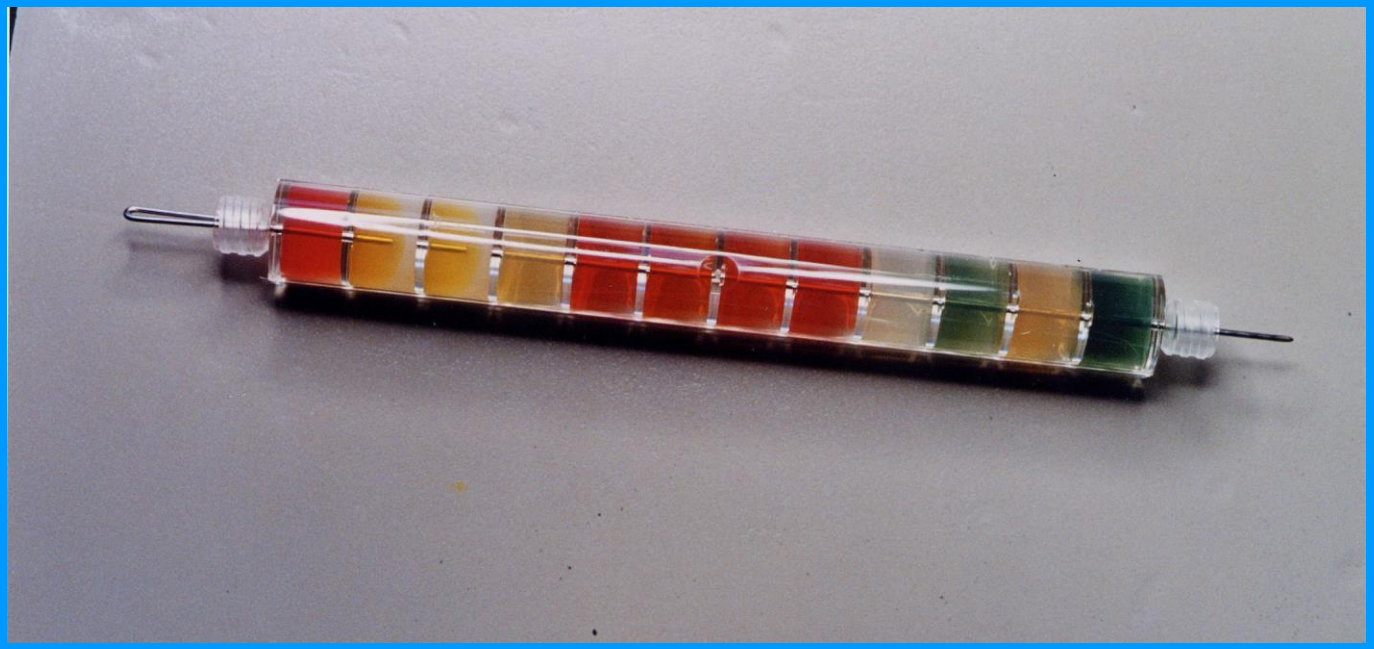
multi-media testing systems

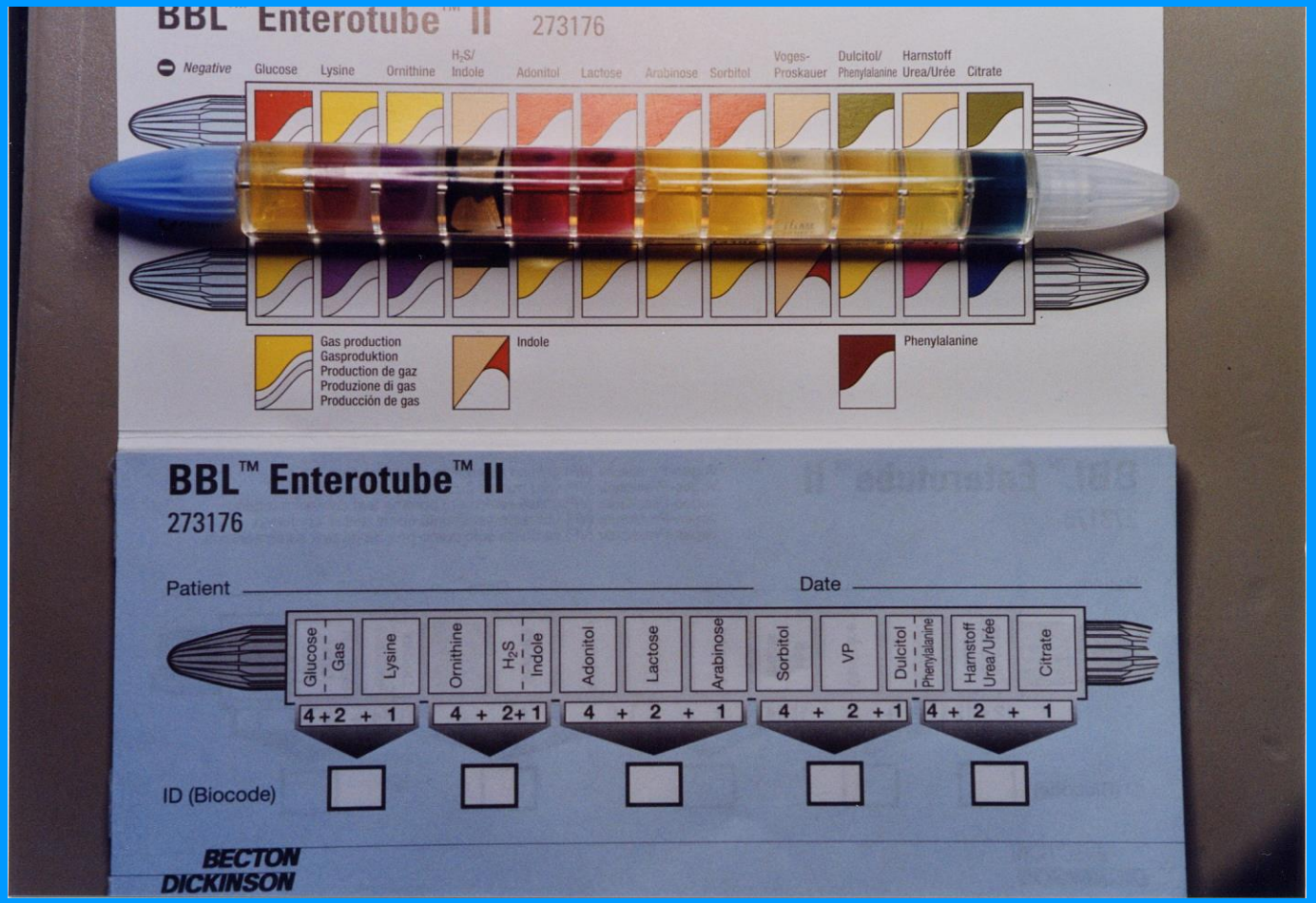
API 20 E **Enterotube II**

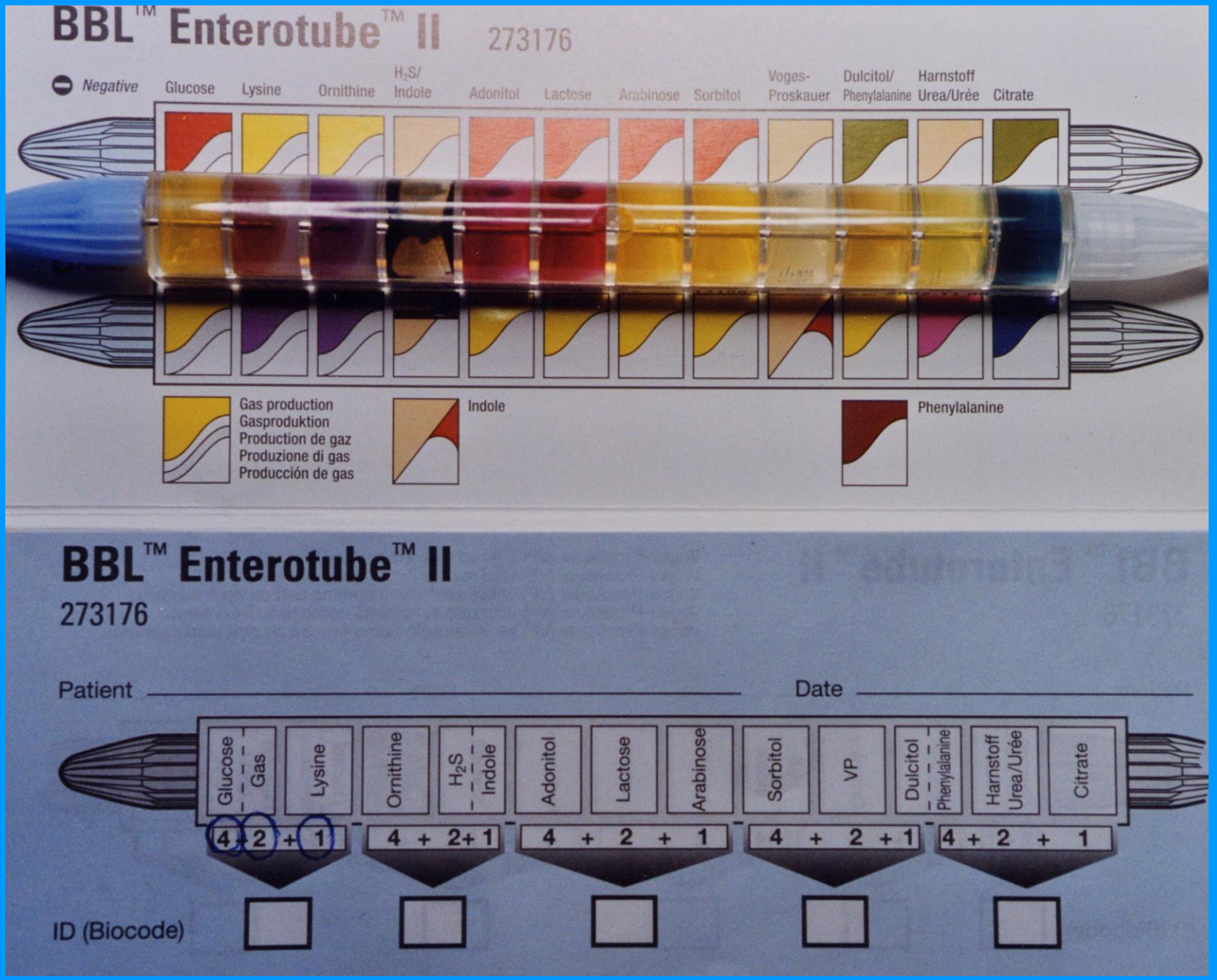
biochemical confirmation — **18 - 24 h 37°C**

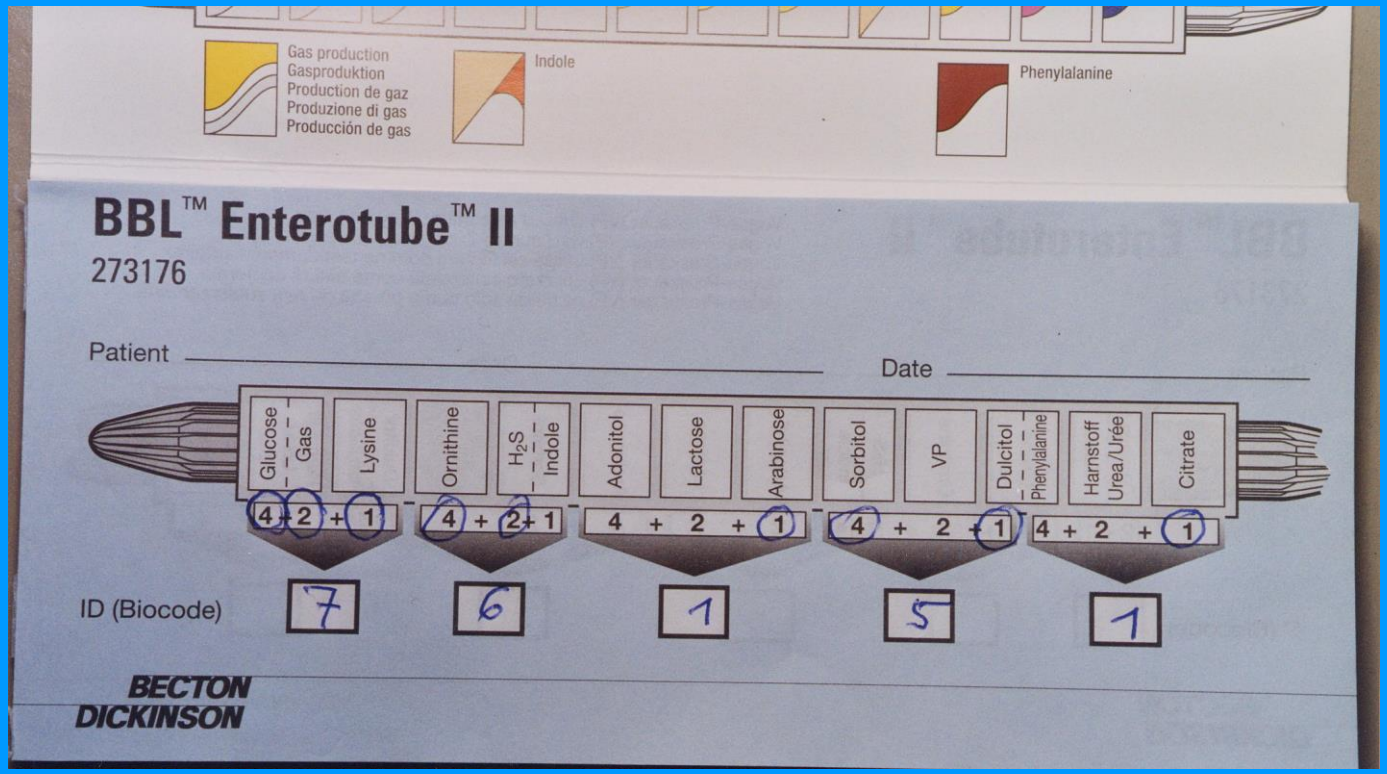
- **Triple Sugar Iron agar**
- **urea agar**
- **L-lysine decarboxylase medium**
- **β-galactosidase test**
- **indole test**
- **VOGES-PROSKAUER test**


BBL™ Enterotube™ II
273176
25
BECTON DICKINSON
Made in Germany
Glucose/Gas
Lysine
Ornithine
H2S/Indole
Adonitol
Lactose
Arabinose
Sorbitol
VP
Dulcitol/PA
Urea
Citrate
Patient:
BBL® Enterotube™ II
Lot
Ch.-B.
1310645
Exp.
Verw. bis
2002-11-06
E 0001




BBL™ Enterotube™ II
273176
Negative
Glucose
Lysine
Ornithine
H2S/ Indole
Adonitol
Lactose
Arabinose
Sorbitol
Voges-Proskauer
Dulcitol/ Phenylalanine
Harnstoff Urea/Urée
Citrate
Gas production
Gasproduktion
Production de gaz
Produzione di gas
Producción de gas
Indole
Phenylalanine
BBL™ Enterotube™ II
273176
Patient
Date
Glucose Gas
Lysine
Ornithine
H2S Indole
Adonitol
Lactose
Arabinose
Sorbitol
VP
Dulcitol Phenylalanine
Harnstoff Urea/Urée
Citrate
4 + 2 + 1
4 + 2 + 1
4 + 2 + 1
4 + 2 + 1
4 + 2 + 1
ID (Biocode)
BECTON DICKINSON


BBL™ Enterotube™ II 273176
Negative
Glucose
Lysine
Ornithine
H2S/ Indole
Adonitol
Lactose
Arabinose
Sorbitol
Voges-Proskauer
Dulcitol/ Phenylalanine
Harnstoff Urea/Urée
Citrate
Gas production
Gasproduktion
Production de gaz
Produzione di gas
Producción de gas
Indole
Phenylalanine
BBL™ Enterotube™ II
273176
Patient
Date
Glucose Gas
Lysine
Ornithine
H2S Indole
Adonitol
Lactose
Arabinose
Sorbitol
VP
Dulcitol Phenylalanine
Harnstoff Urea/Urée
Citrate
4 + 2 + 1
4 + 2+ 1
4 + 2 + 1
4 + 2 + 1
4 + 2 + 1
ID (Biocode)


Gas production
Gasproduktion
Production de gaz
Produzione di gas
Producción de gas
Indole
Phenylalanine
BBL™ Enterotube™ II
273176
Patient
Date
Glucose
Gas
Lysine
Ornithine
H2S
Indole
Adonitol
Lactose
Arabinose
Sorbitol
VP
Dulcitol
Phenylalanine
Harnstoff
Urea/Urée
Citrate
4 + 2 + 1
4 + 2 + 1
4 + 2 + 1
4 + 2 + 1
4 + 2 + 1
ID (Biocode)
7
6
1
5
1
BECTON
DICKINSON

Genus *Listeria*

Listeria monocytogenes

Listeria innocua

Listeria ivanovii

Listeria seeligeri

Listeria welshimeri

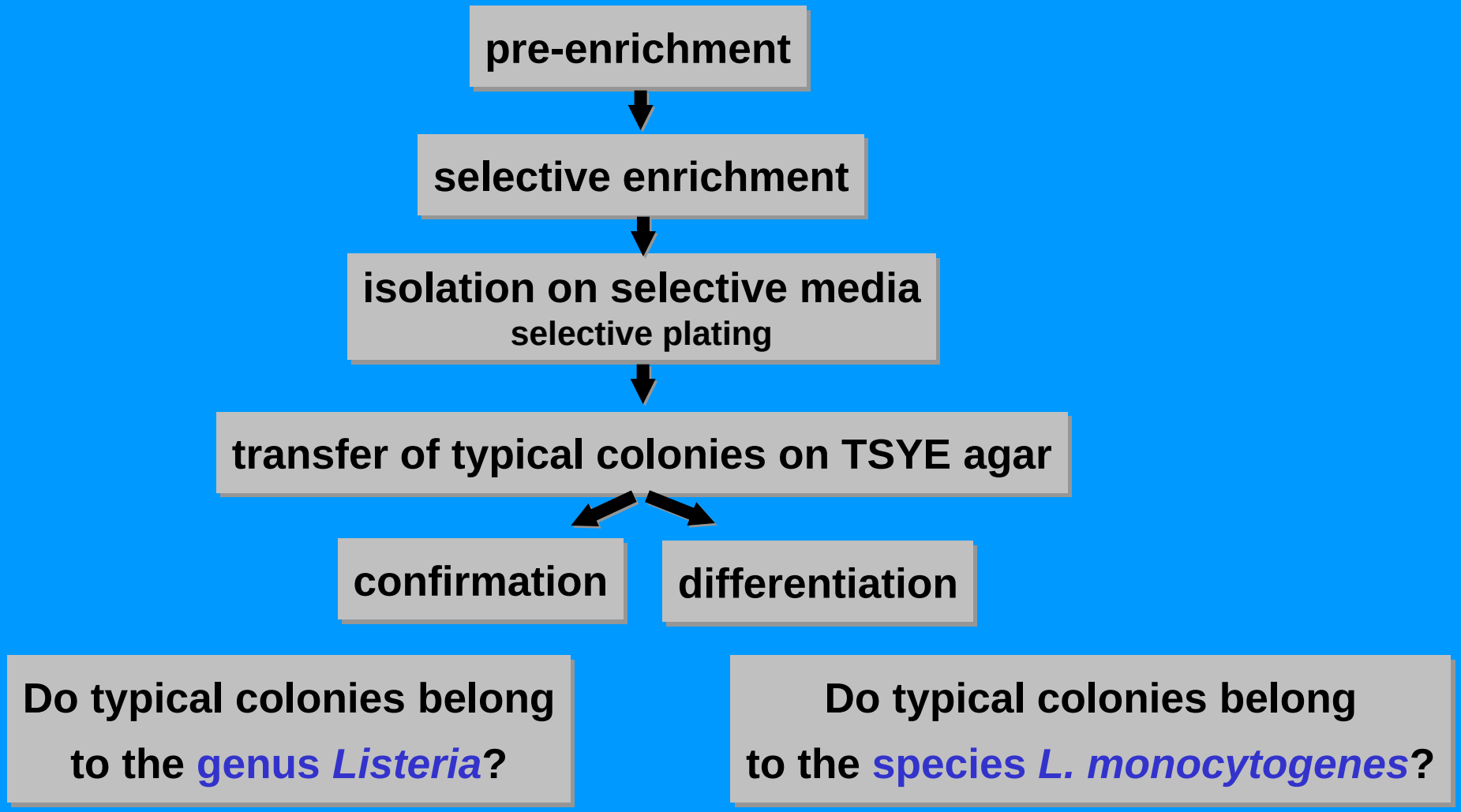
Listeria grayi

Subsp. *grayi*

Subsp. *murrayi*

detection of *Listeria monocytogenes*

ISO method 11290-1 :1997



detection of *Listeria monocytogenes* ISO method 11290-1 :1997

pre-enrichment

resuscitation of sublethally damaged Listeria

25 g sample + 225 ml 1/2 FRAZER broth

Contains only 50 % of selective agents

incubation: 24 ± 2 h at 30°C

⬇

selective enrichment

0.1 ml of PE
+
10 ml FRAZER broth

selective agents:
Lithium chloride
Nalidixic acid
Acriflavine

incubation: 48 ± 2 h at 37°C

⬇

isolation on selective media
selective plating

streaking on **PALCAM** agar

detection of *Listeria monocytogenes* ISO method 11290-1 :1997

Selective agents

Polymyxine
Acriflavine
Lithium chloride
Ceftacidime

isolation on selective media

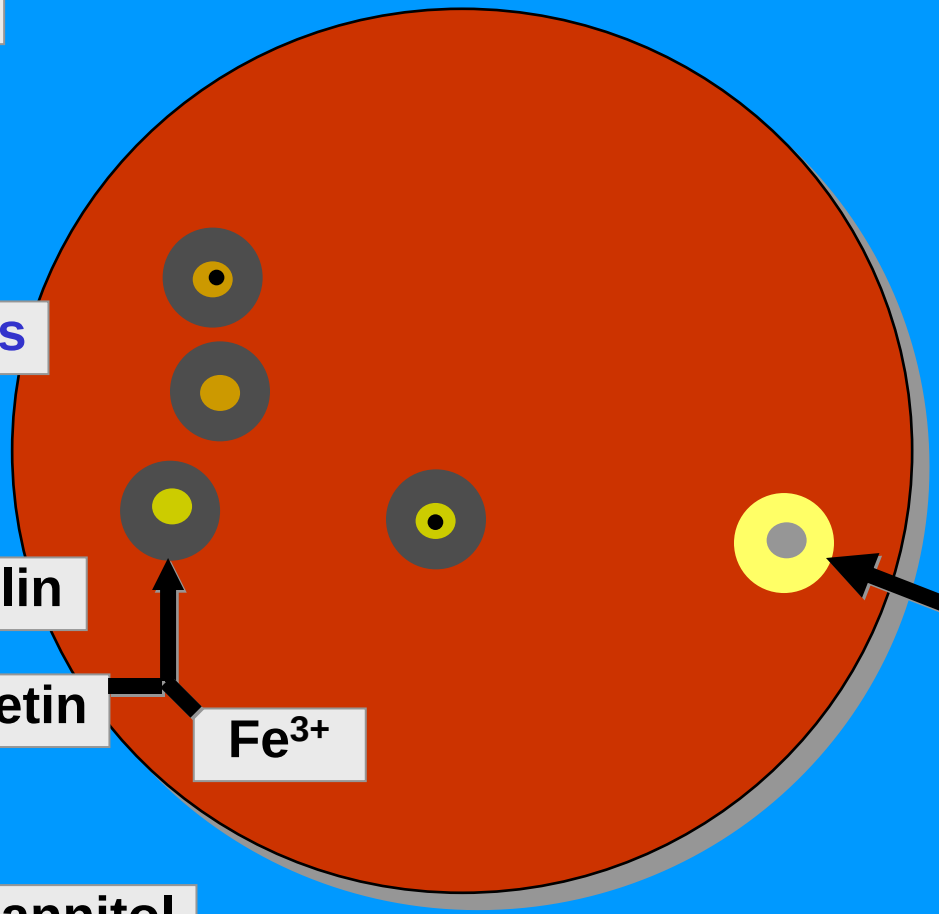
PALCAM agar

incubation: 24 and 48 h at 37°C

Aesculin
Mannitol

Phenol red

differential agents



Listeria spp.

hydrolyse Aesculin

Aesculetin

Fe^{3+}

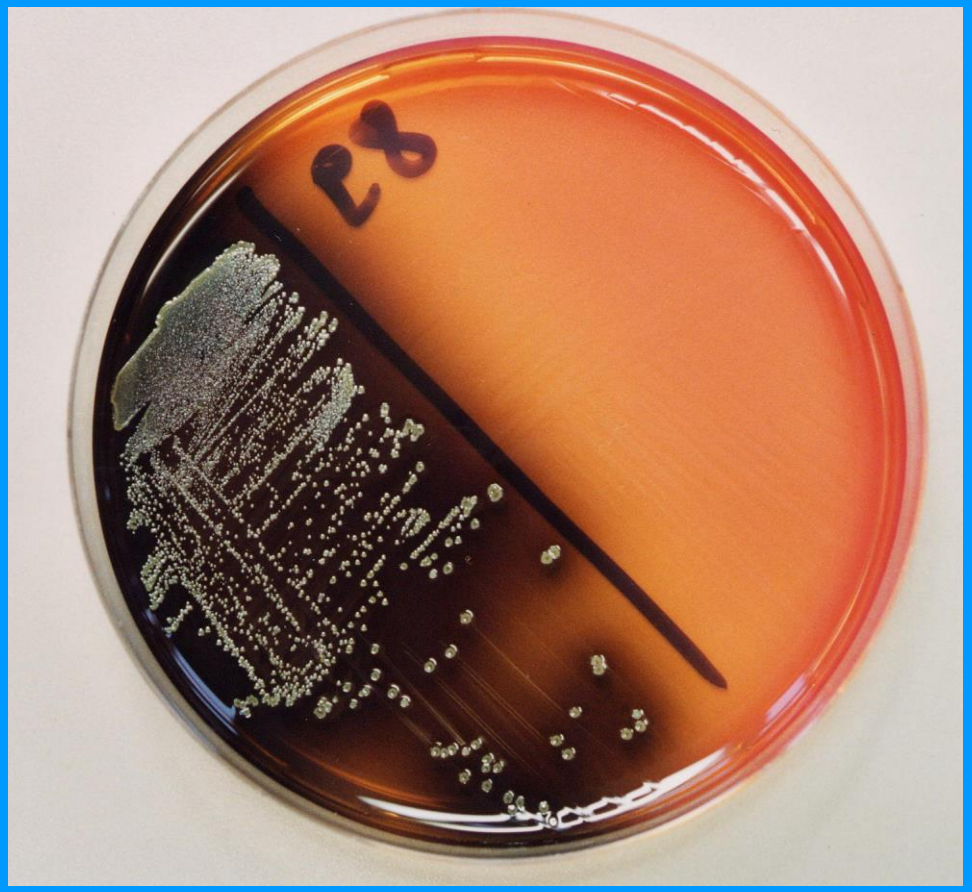
other bacteria

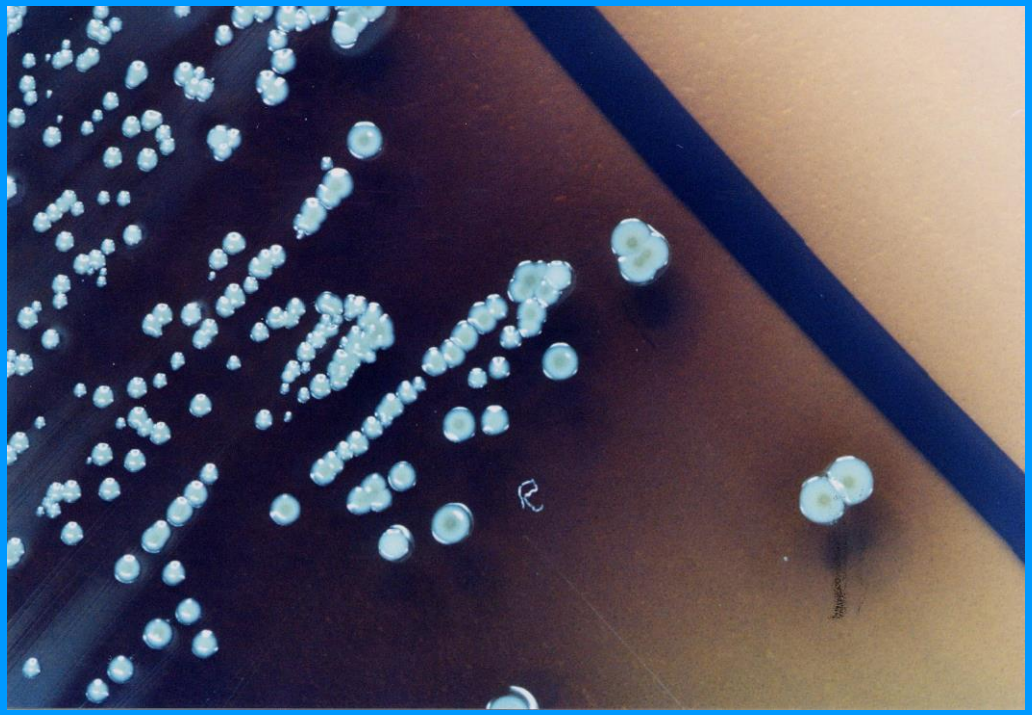
ferment Mannitol

pH drops

pH indicator Phenol red turns to yellow

do not ferment Mannitol





detection of *Listeria monocytogenes*
ISO method 11290-1 :1997

isolation on selective media
selective plating

↓

transfer of typical colonies on TSYE agar

18 - 24 h
37°C

↓

confirmation

Do typical colonies belong to the genus *Listeria*?

GRAM positive short rods

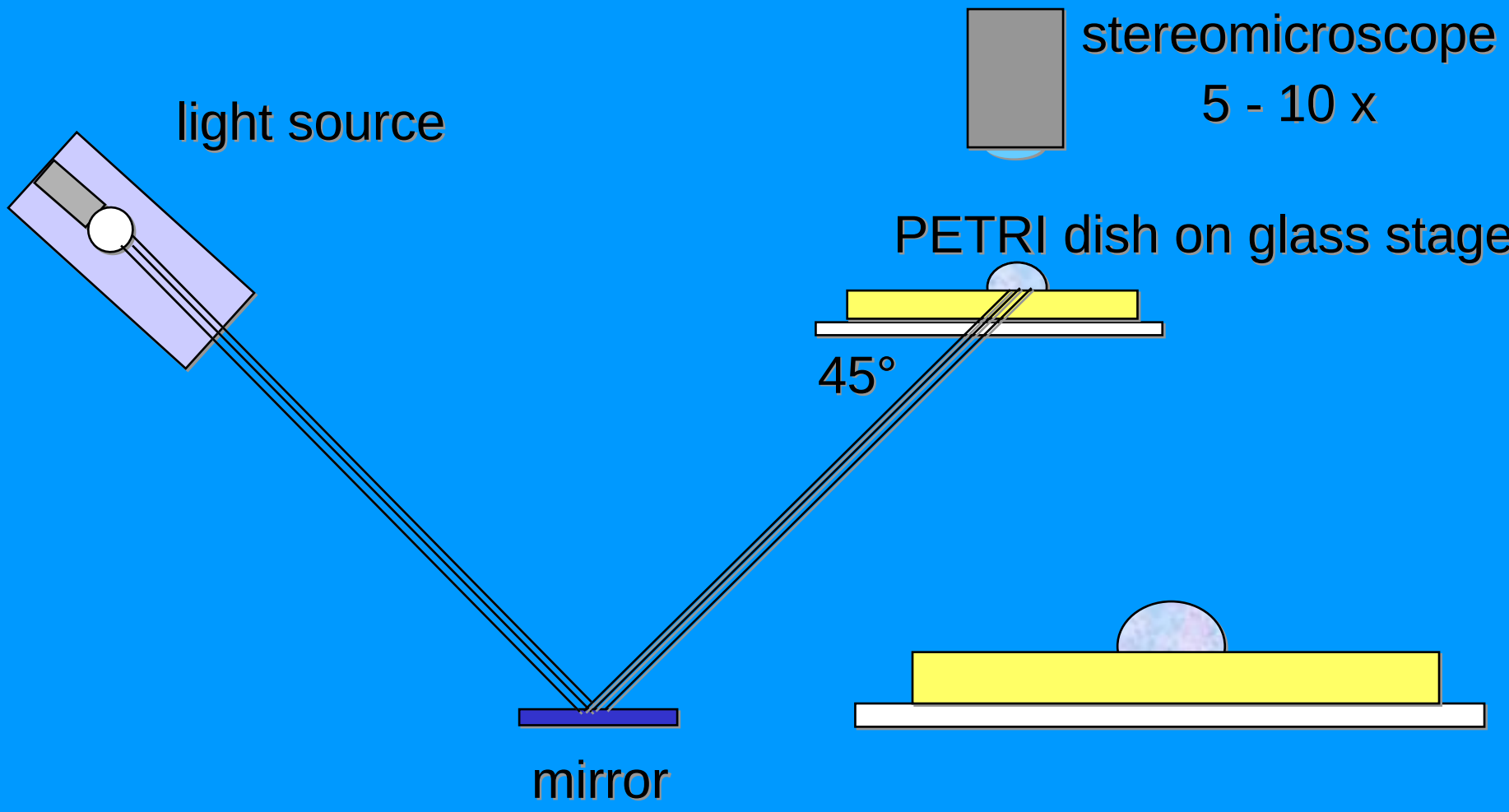
Catalase positive

3% H_2O_2

motility

typical appearance using HENRY illumination

HENRY illumination



detection of *Listeria monocytogenes*
ISO method 11290-1 :1997

isolation on selective media
selective plating

↓

transfer of typical colonies on TSYE agar

↓

confirmation — Do typical colonies belong to the genus *Listeria*?

↓

differentiation — Do they belong to species *L. monocytogenes*?

inoculate

Purple broth containing Rhamnose — acid from Rhamnose?

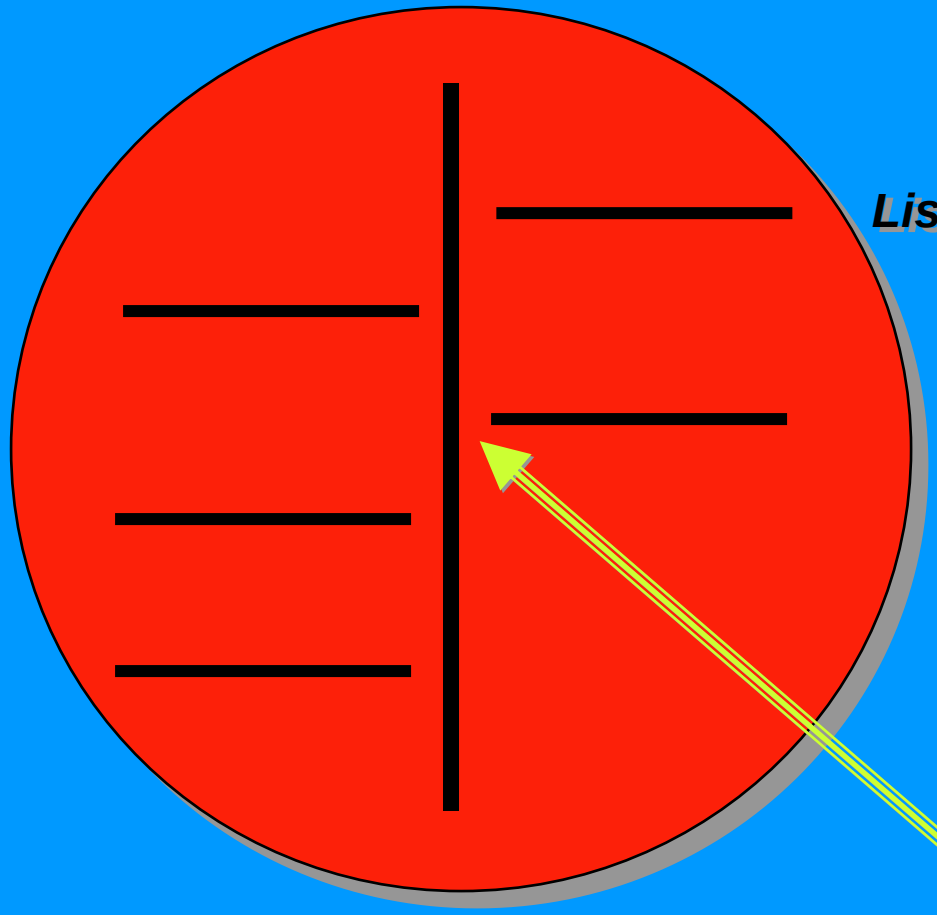
Purple broth containing Xylose — acid from Xylose?

incubate at 37°C for up to 7 d

positive if purple turns to yellow — CAMP test

CAMP test

Christie, Atkins, Munch-Petersen (1944)



material from typical colonies

Listeria monocytogenes as + control

Listeria innocua as - control

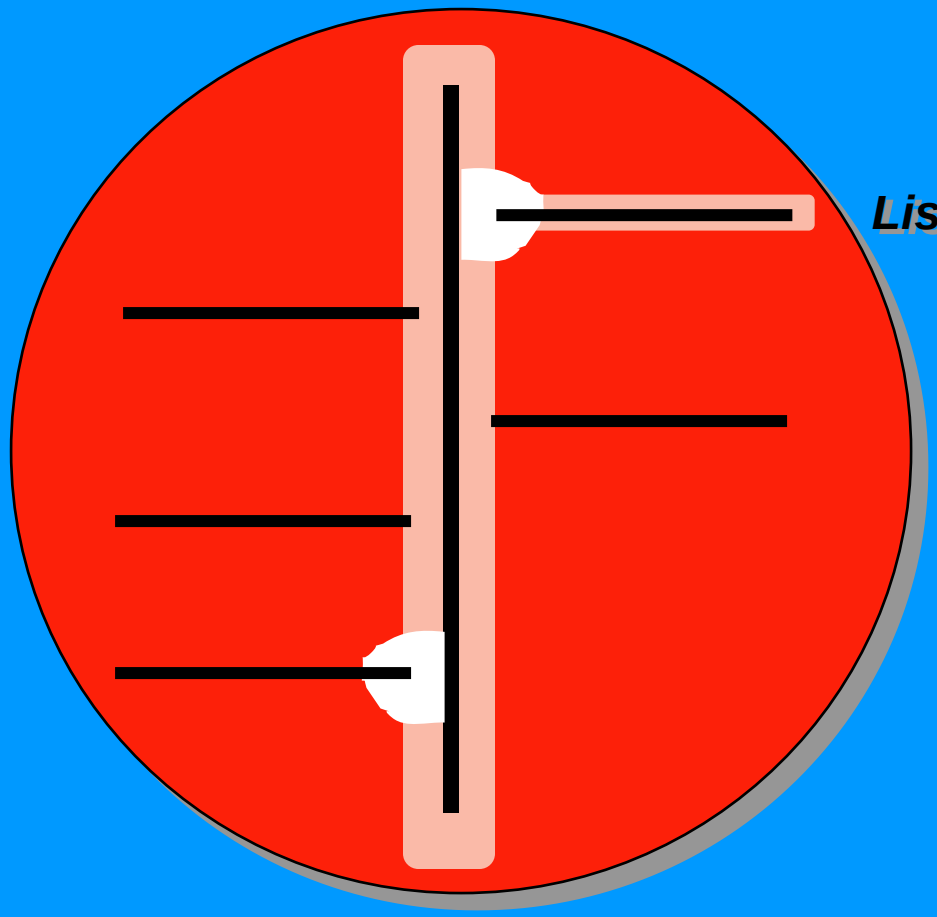
2 - 3 mm distance

Staphylococcus aureus

CAMP test

after 24 h incubation at 37 °C

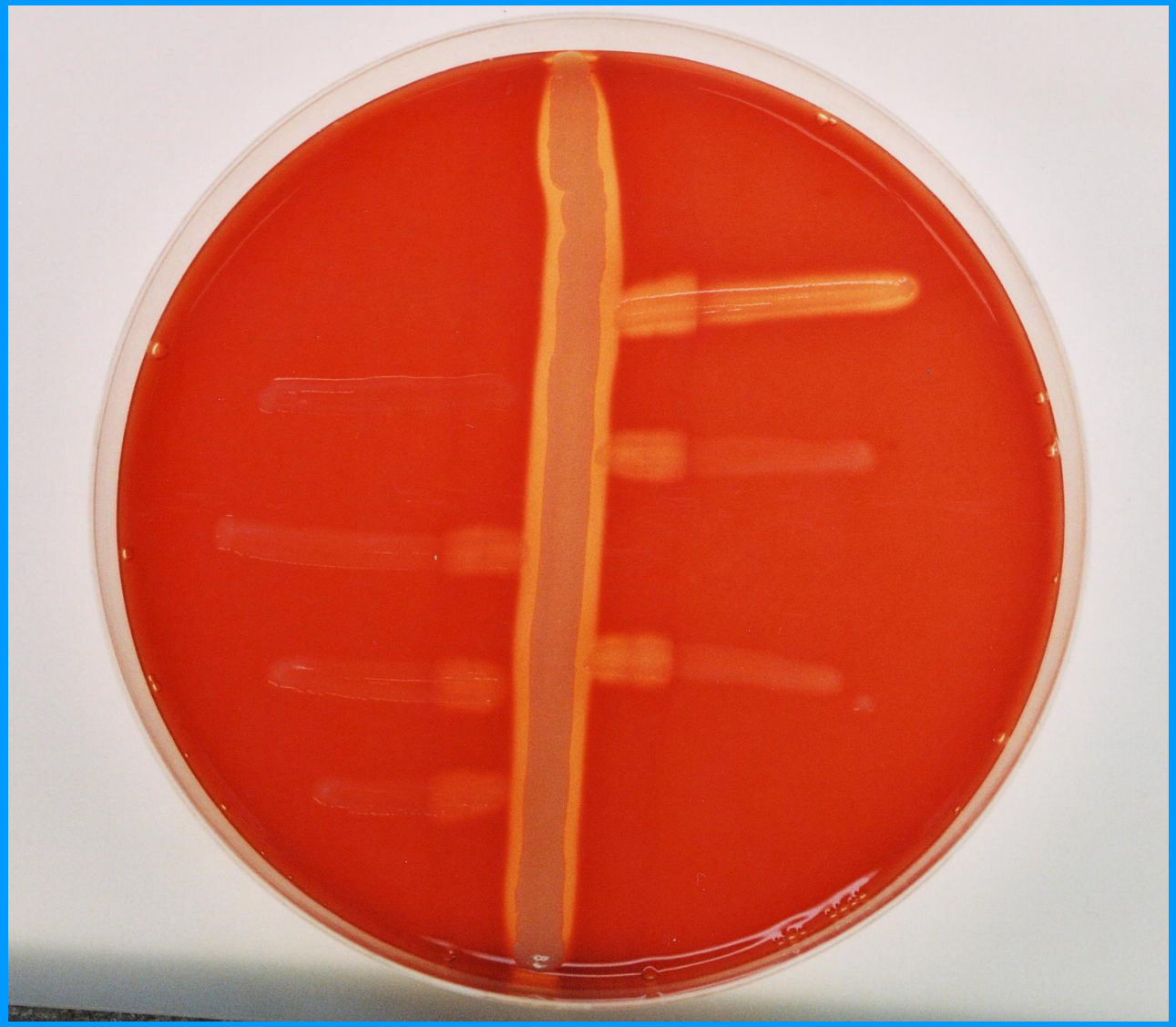
material from typical colonies

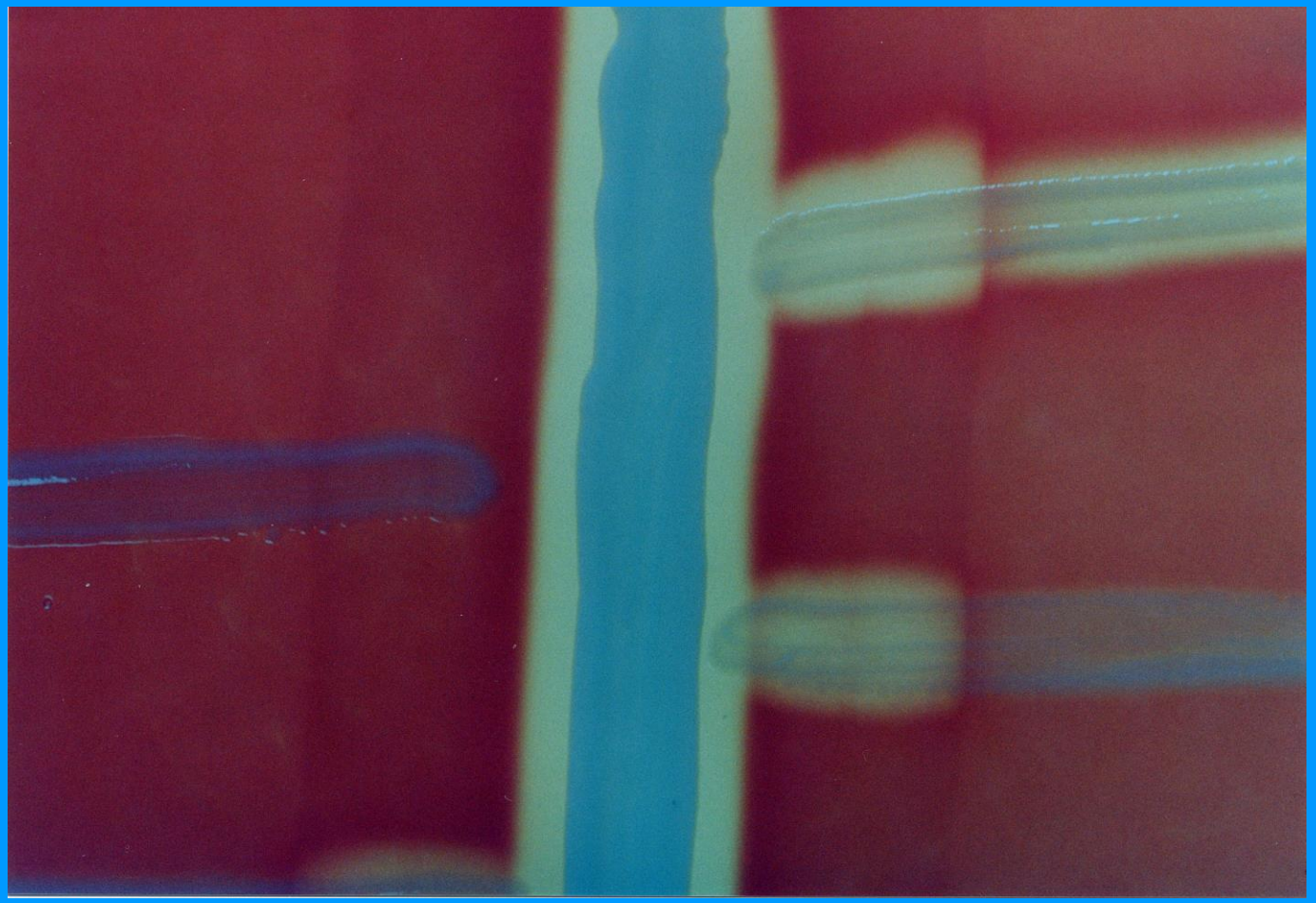


Staphylococcus aureus

Listeria monocytogenes as + control

Listeria innocua as - control





Differentiation of Listeria spp.

species	acid from Rhamnose	acid from Xylose	CAMP test
L. monocytogenes	+	-	+
L. innocua	v	-	-
L. ivanovii	-	+	-
L. seeligeri	-	+	(+)
L. welshimeri	v	+	-
L. grayi subsp. grayi	-	-	-
L. grayi subsp. murrayi	v	-	-

Lactobacilli

MRS agar

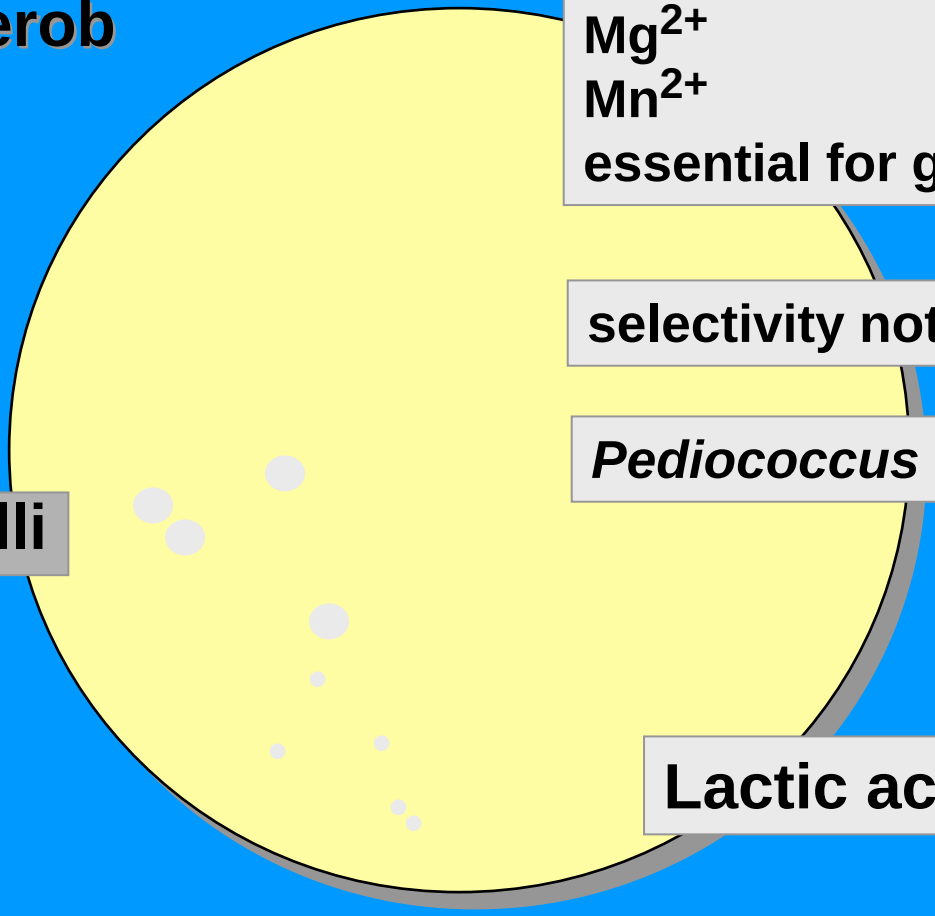
incubation: 48 h at 30°C

anaerob

contains
Acetate
Mg^{2+}
Mn^{2+}
essential for growth of Lactobacilli

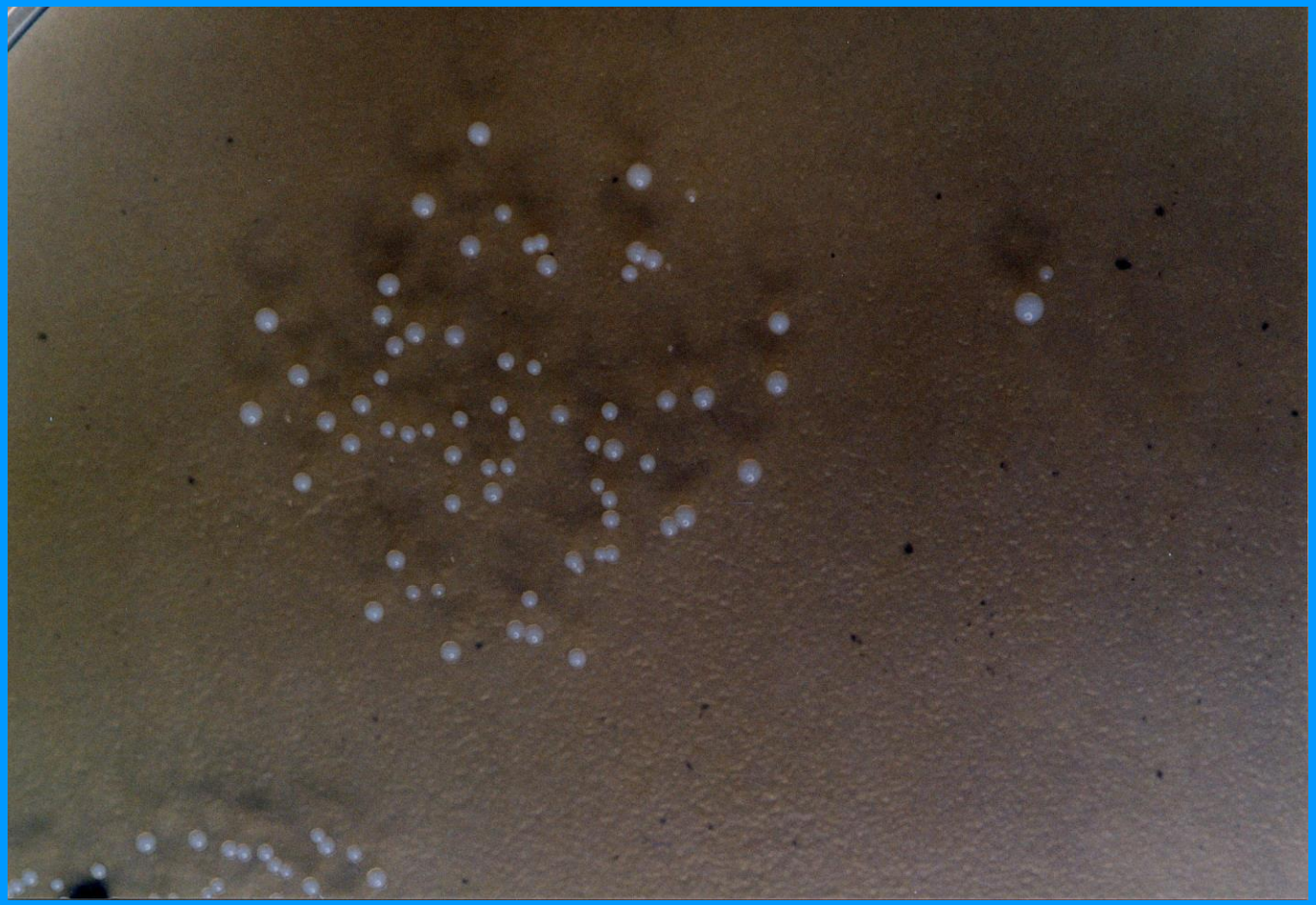
selectivity not high

***Pediococcus* and *Leuconostoc spp*.**



Lactobacilli

Lactic acid bacteria



Enterobacteriaceae

Edwardsiella
Citrobacter
Hafnia
Serratia
Proteus
Morganella
Providencia
Klebsiella
Enterobacter
Escherichia
Yersinia
Salmonella
Shigella

Coliforms **produce gas and acid from lactose**

Escherichia coli
Enterobacter cloacae, E. sakazakii, E. aerogenes
Serratia rubidea
Klebsiella pneumoniae, K. oxytoca, K. ozaenae

Fecal Coliforms **produce gas and acid from lactose at 44°C**

members of Coliforms group
strain dependent

Enterobacteriaceae

VRBD agar

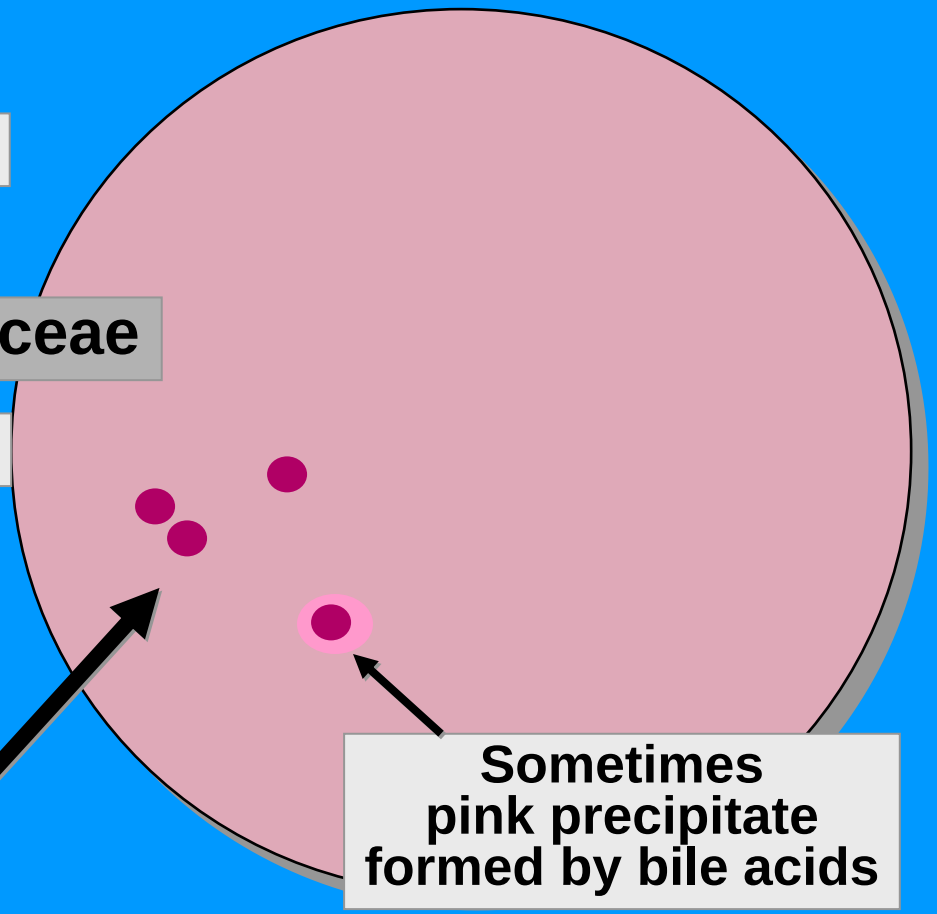
incubation: 48 h at 30°C

selective agents

Crystal Violet
Bile Salts

Neutral Red

differential agent



Enterobacteriaceae

ferment Dextrose

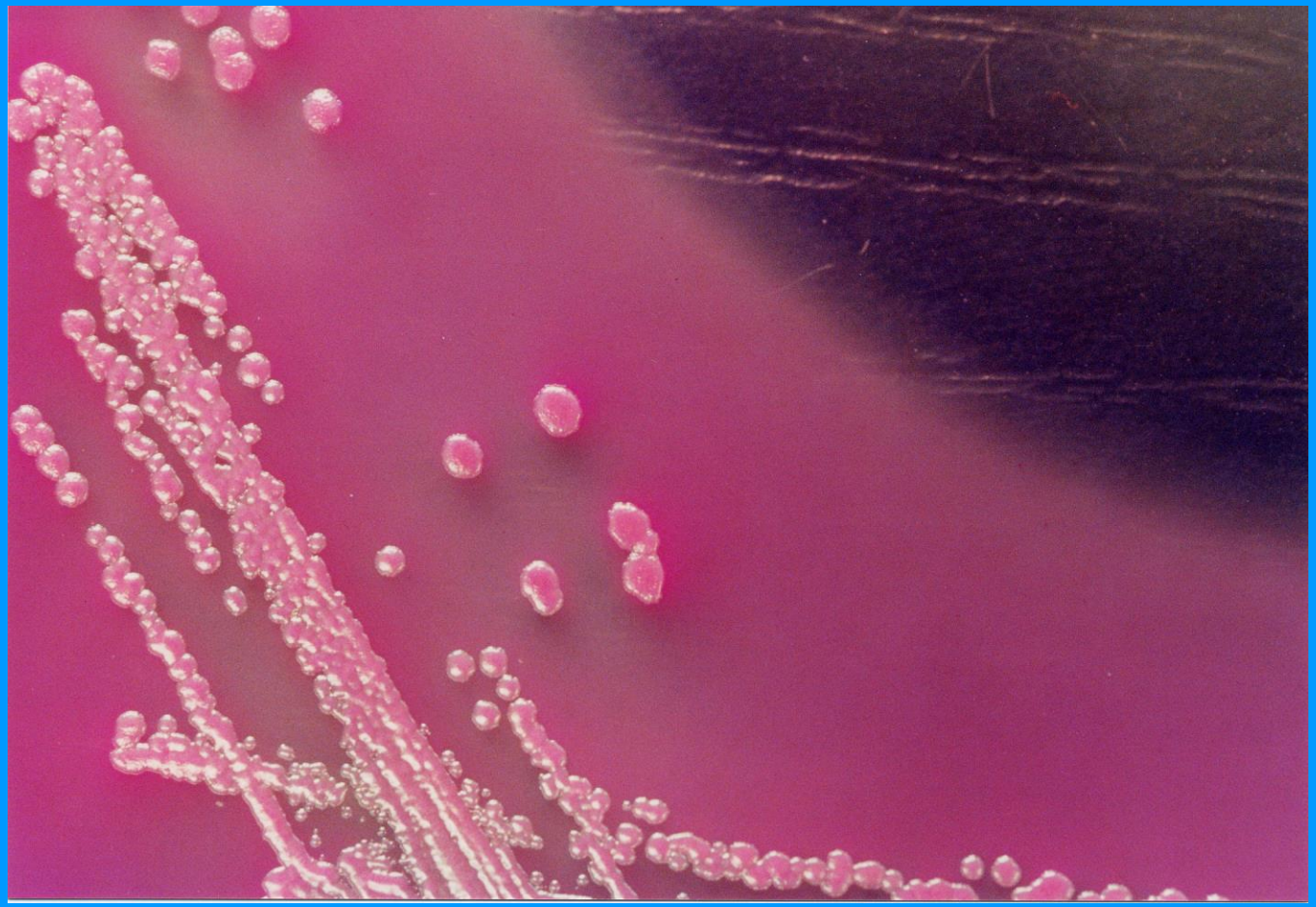
↓

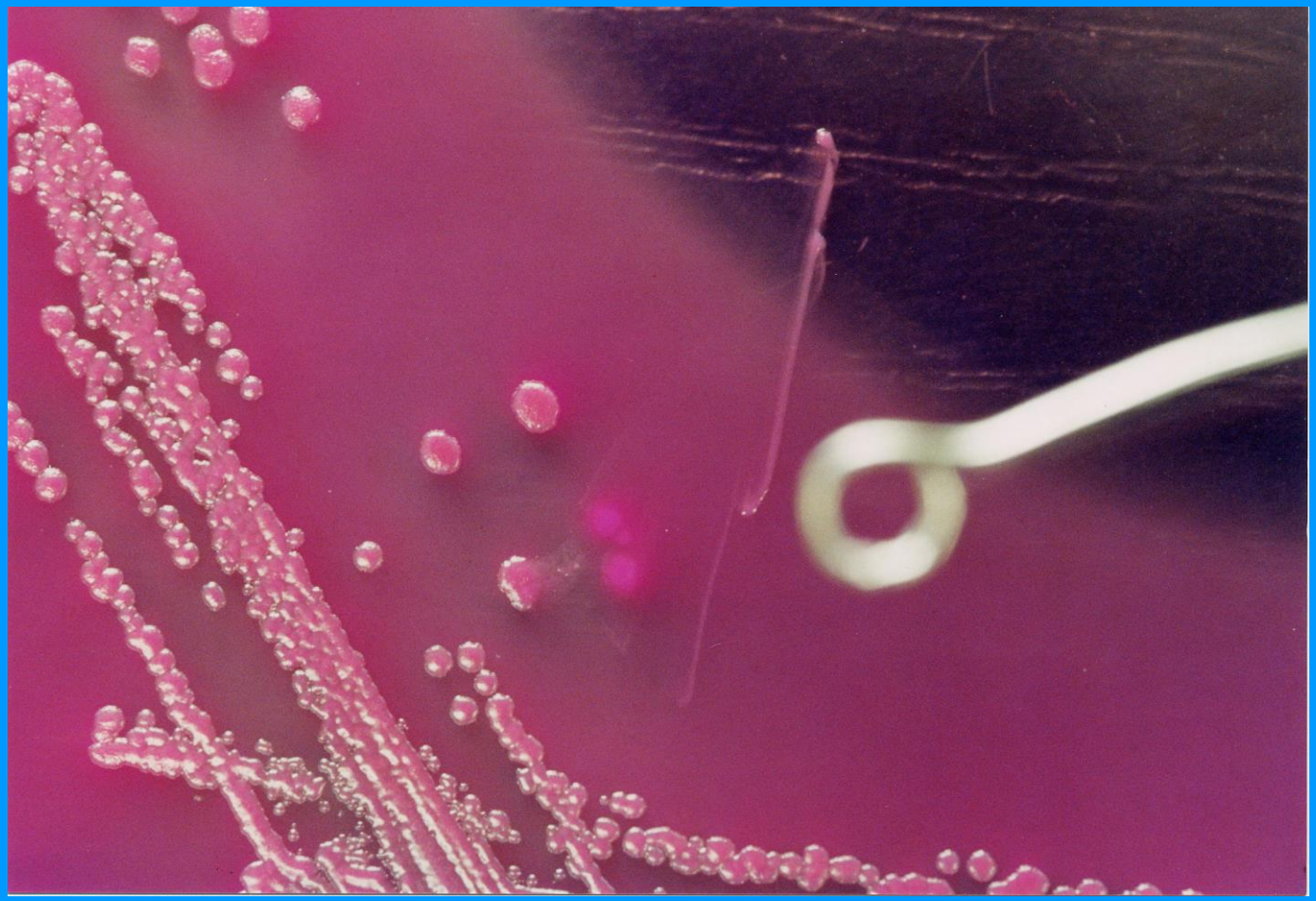
pH drops

↓

pH indicator Neutral red turns to violet

Sometimes pink precipitate formed by bile acids





Coliforms

VRBL agar

incubation: 48 h at 30°C

selective agents

Crystal Violet
Bile Salts

Neutral Red

differential agent

Coliforms

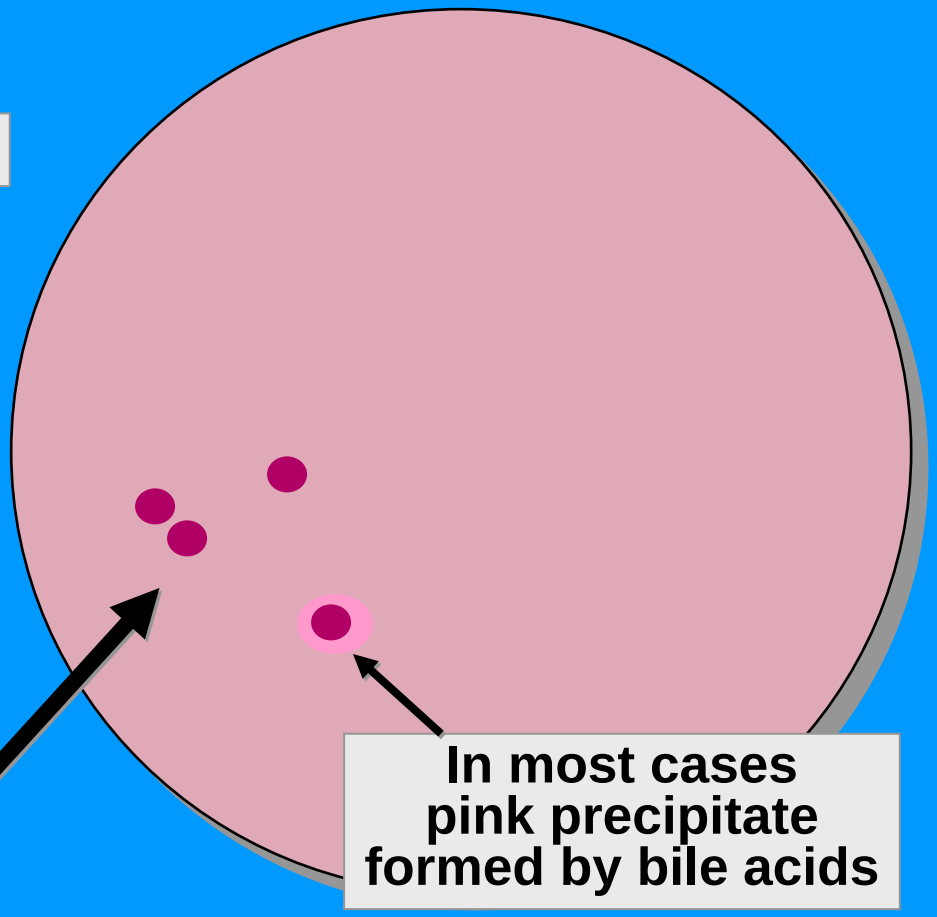
ferment Lactose

↓

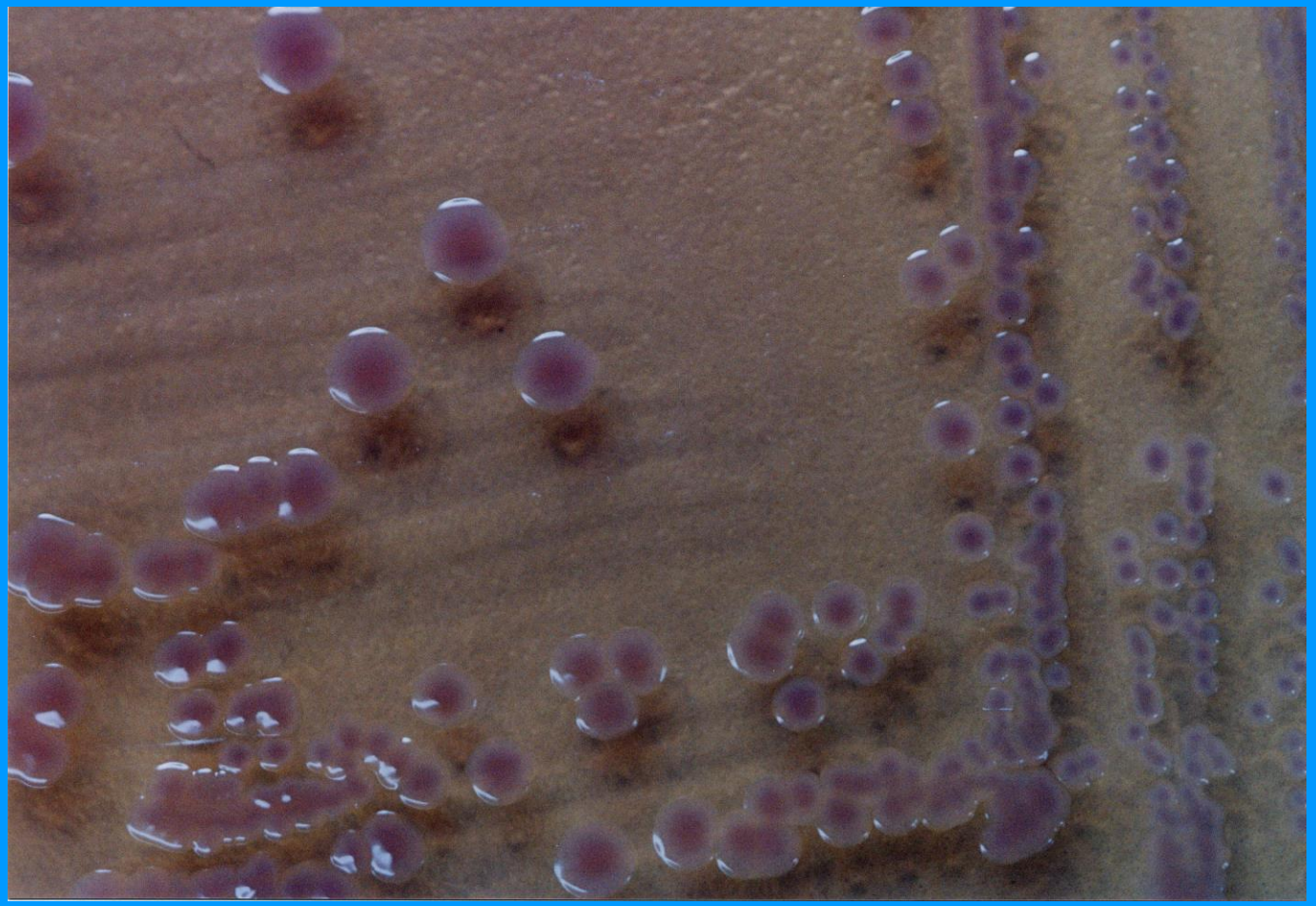
pH drops

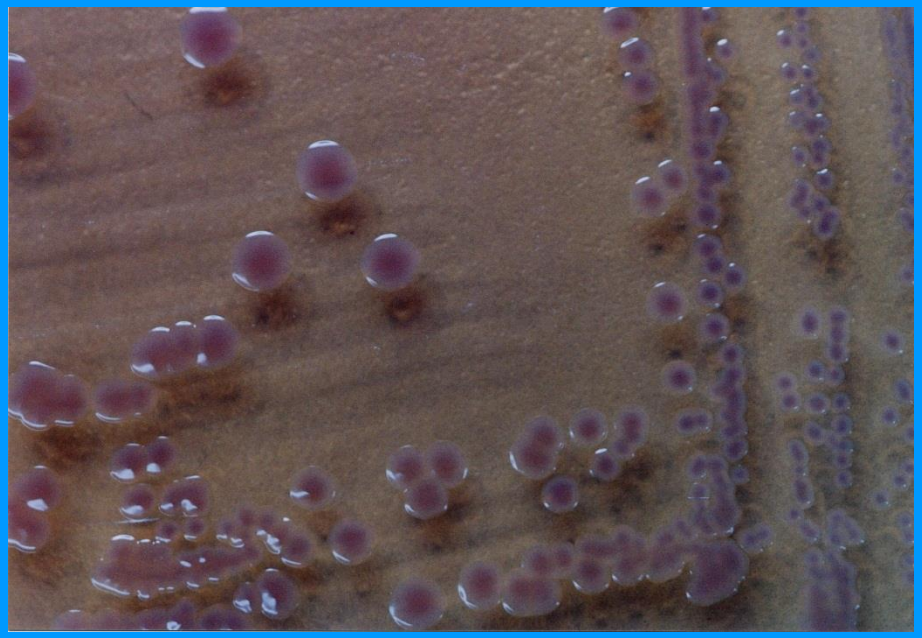
↓

pH indicator Neutral red turns to violet



In most cases pink precipitate formed by bile acids





Pseudomonads Aeromonads

GSP agar

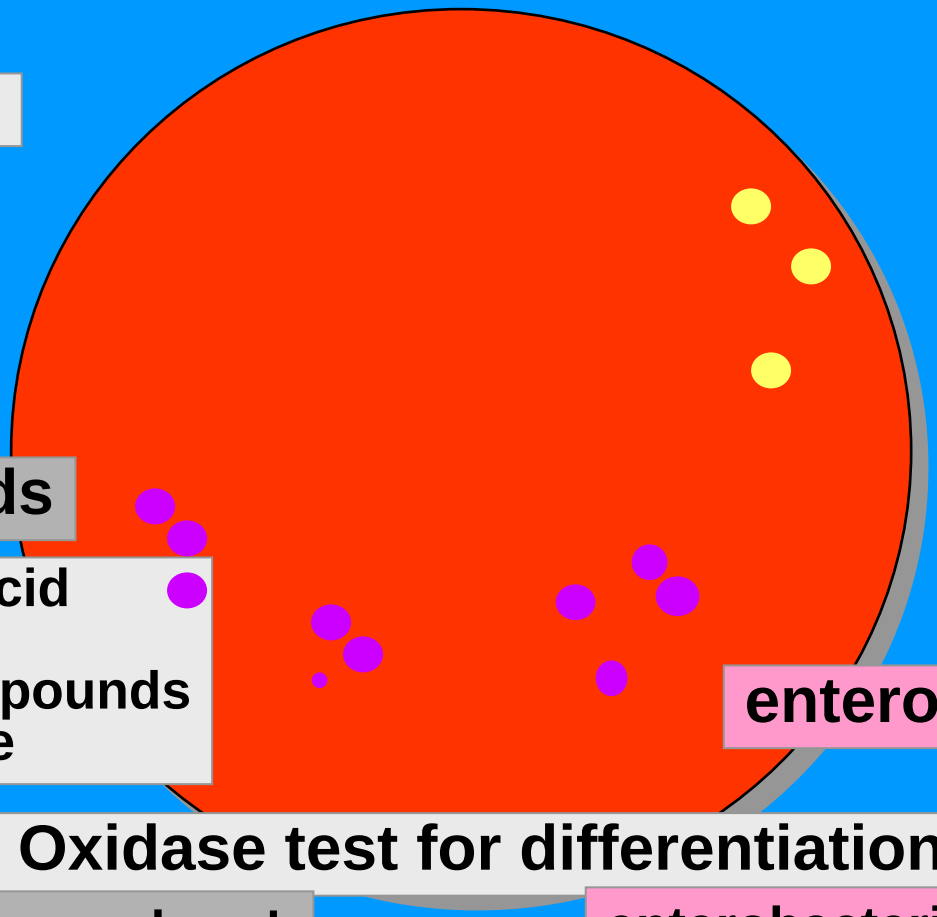
Substrates

Glutamate
Starch

Phenol red
differential agent

selective agents
penicilline

incubation: 48 h at 25°C



aeromonads

produce acid from starch

↓

pH drops

↓

Phenol red turns to yellow

pseudomonads

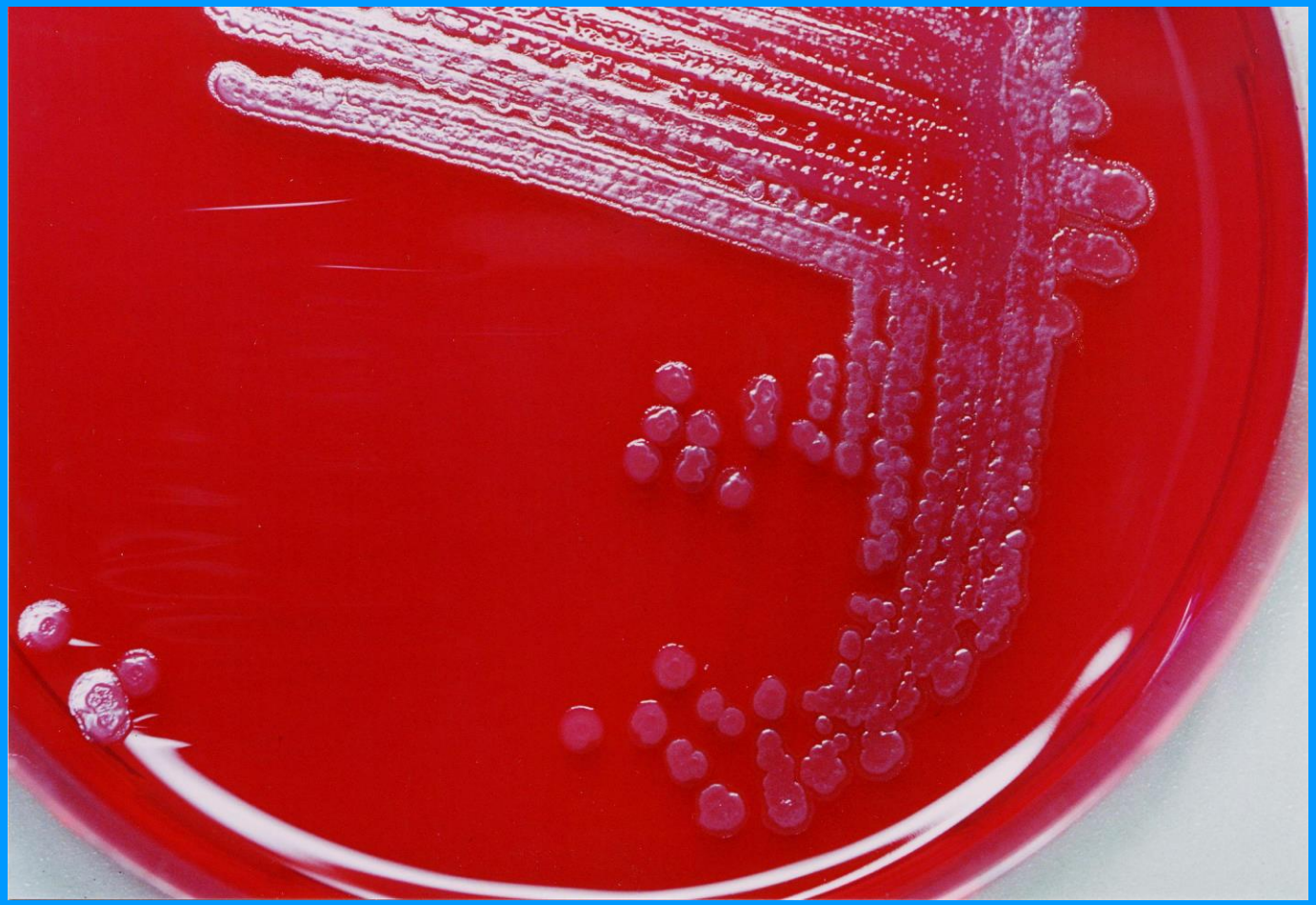
do not produce acid from starch
produce alkaline compounds from glutamate

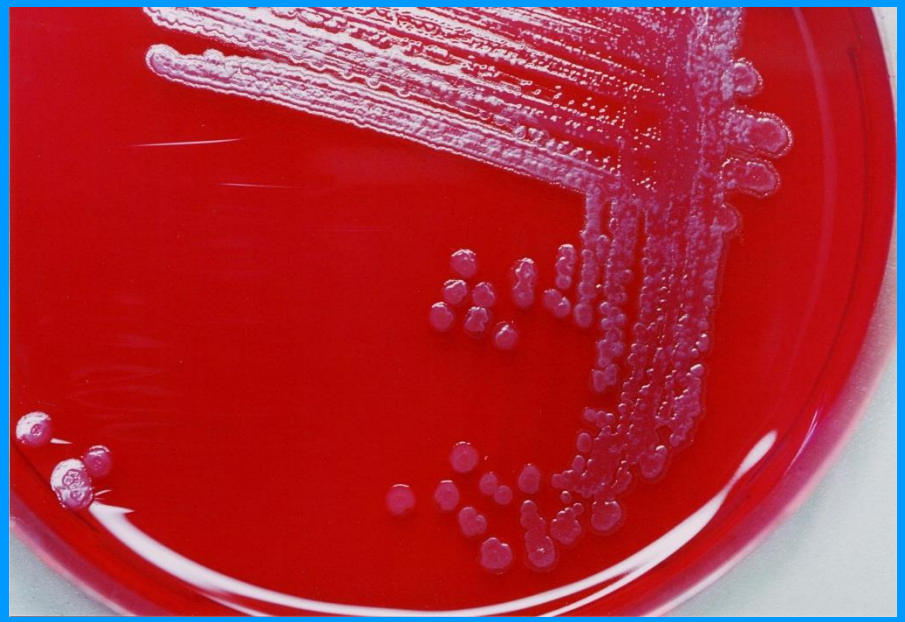
enterobacteriaceae

Oxidase test for differentiation

pseudomonads +

enterobacteriaceae -





fecal streptococci
Enterococcus faecalis
Enterococcus faecium

CATC agar

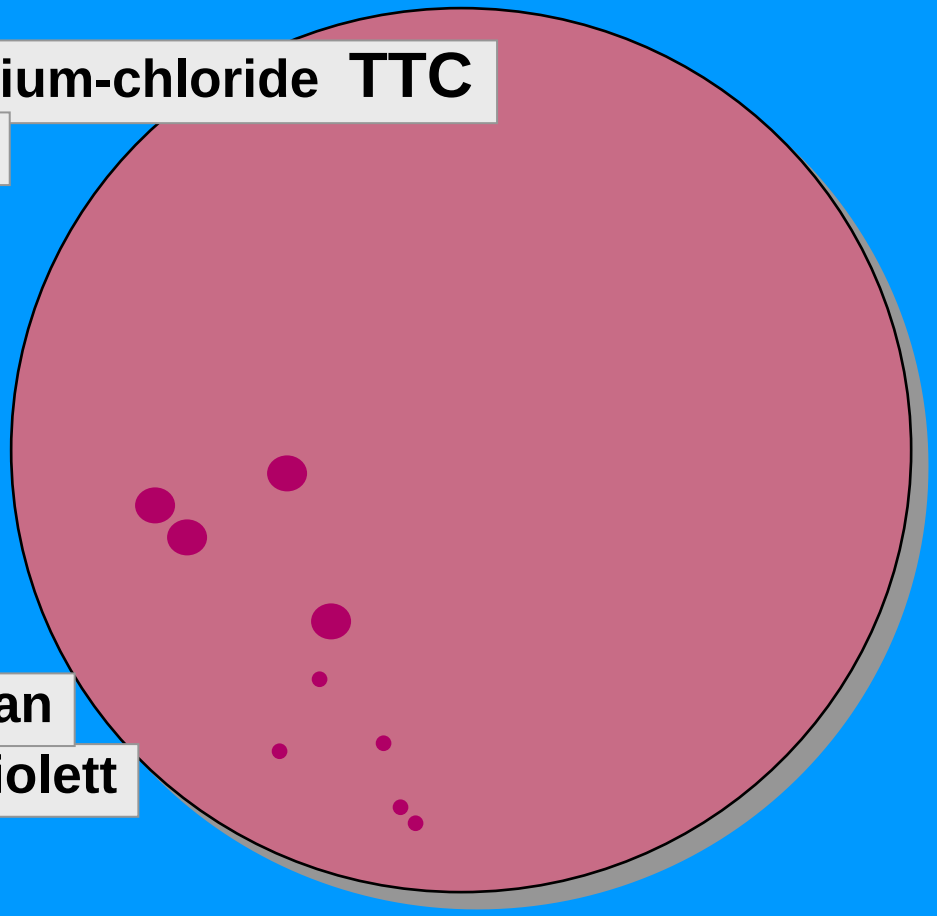
incubation: 24 h at 42°C

selective agents

Citrate
Azide

Triphenyl-tetrazolium-chloride **TTC**

differential agent

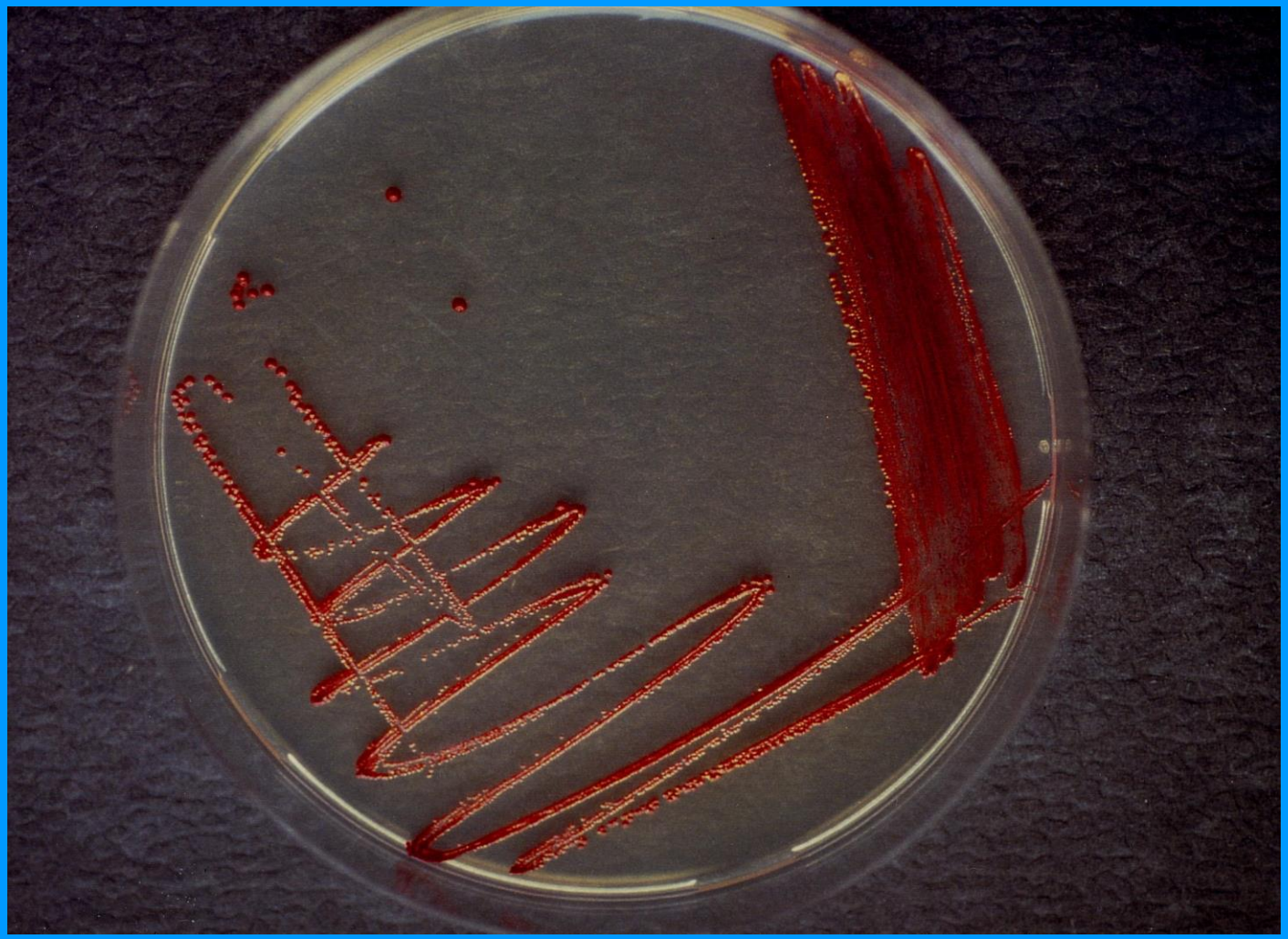


enterococci

reduce TTC

Formazan

deep red violett



Staphylococcus aureus

Baird-Parker agar

incubation: 48 h at 37°C

selective agents

Glycine
Lithium chloride
Tellurite

Tellurite egg yolk

differential agents

pyruvate

enhances growth of (sublethally damaged) *S. aureus*

S. aureus

reduce Tellurite

Tellur

black

Staphylococcus epidermidis

Staphylococcus saprophyticus

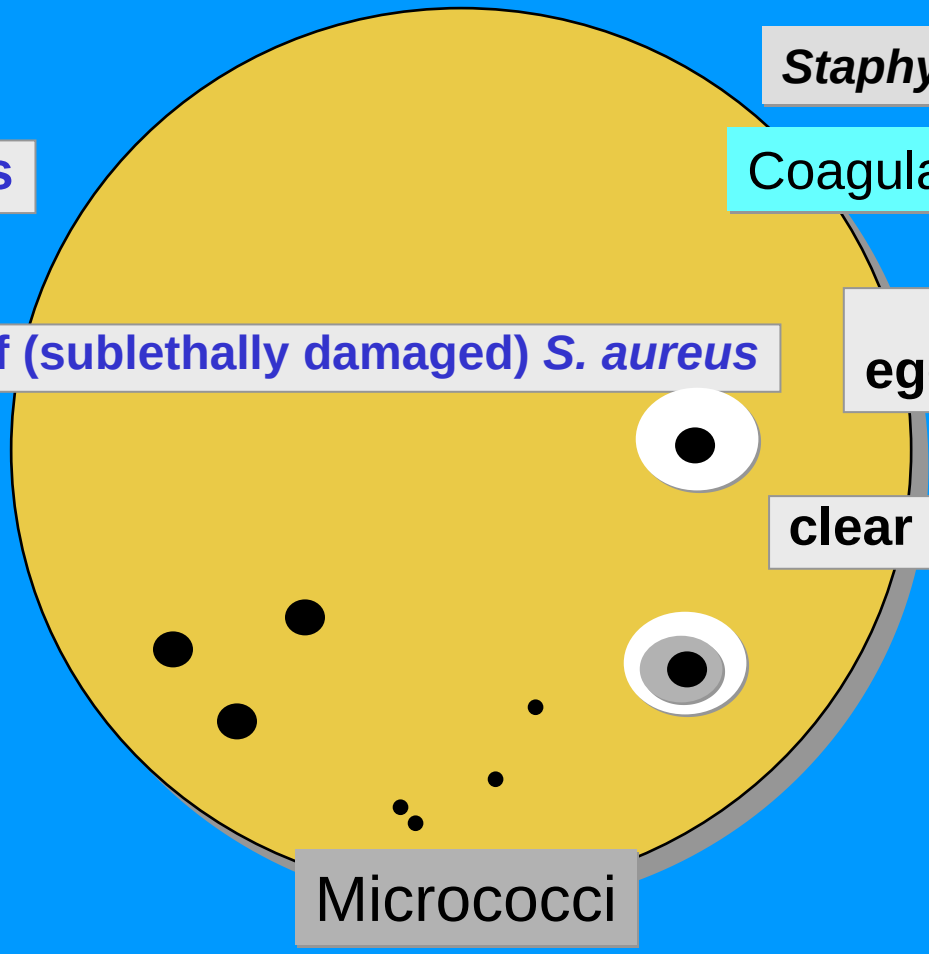
Coagulase test for differentiation

Proteolysis of egg yolk compounds

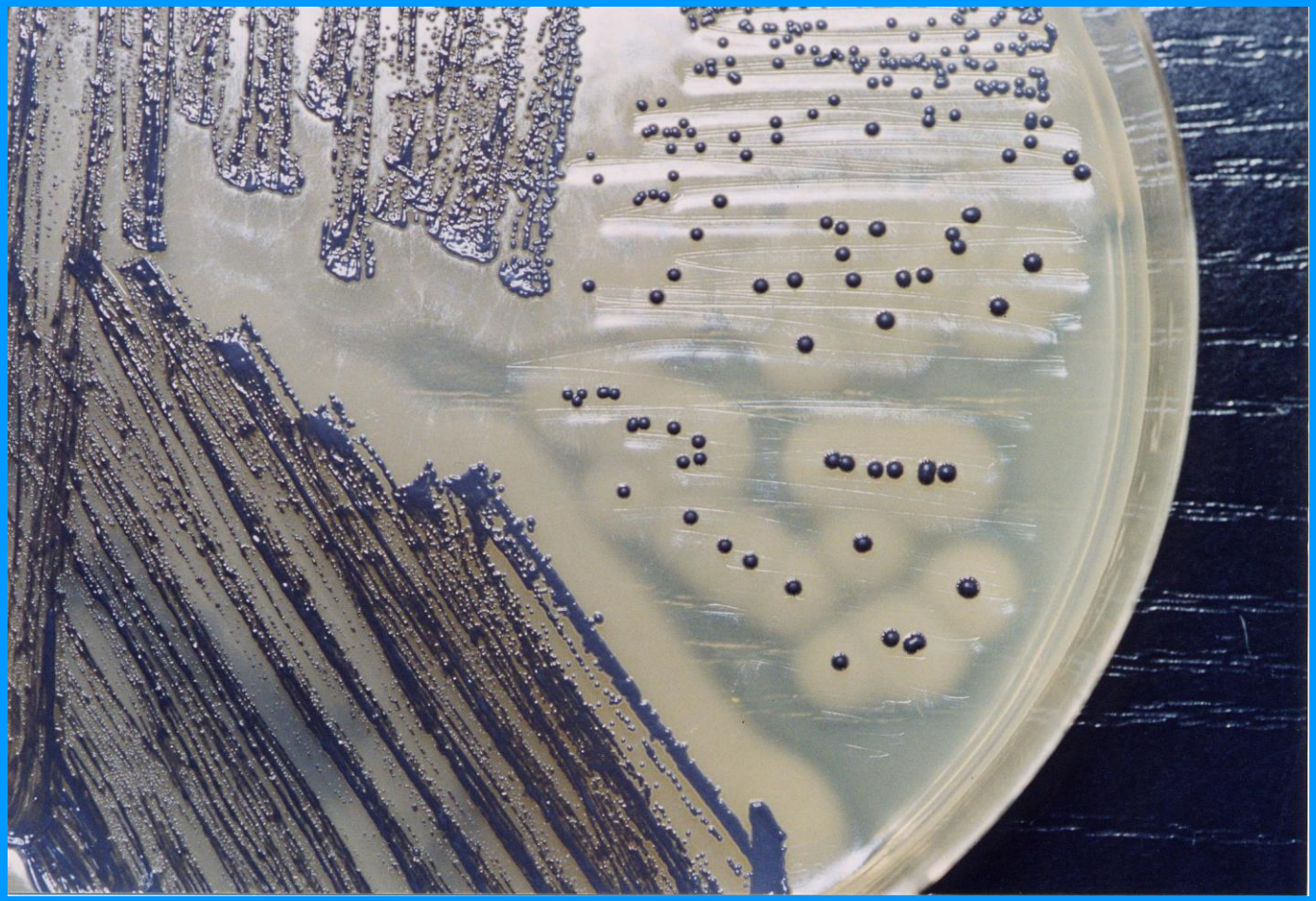
clear zone around colonies

Later lipolysyis

Opaque halo



Micrococci



Staphylococcus aureus

Confirmation
by coagulase test

inoculate suspected colonies separately in BHI broth

incubate at 37°C for 18 - 24 h

place 0.5 ml of diluted rabbit plasma in small test tubes

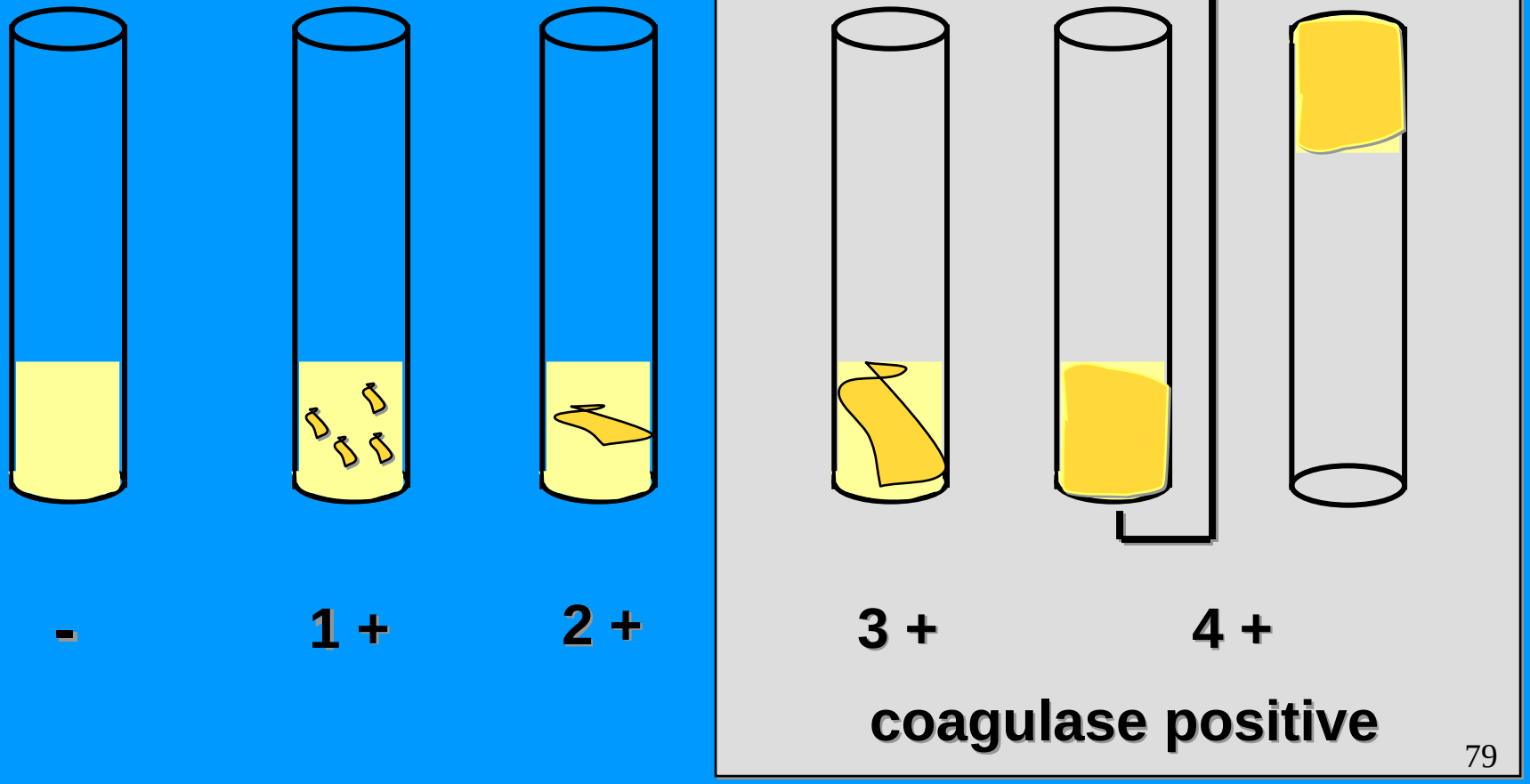
add 0.5 ml of BHI culture to each tube

incubate at 37°C

examine after 1 h and at intervals for up to 24 h

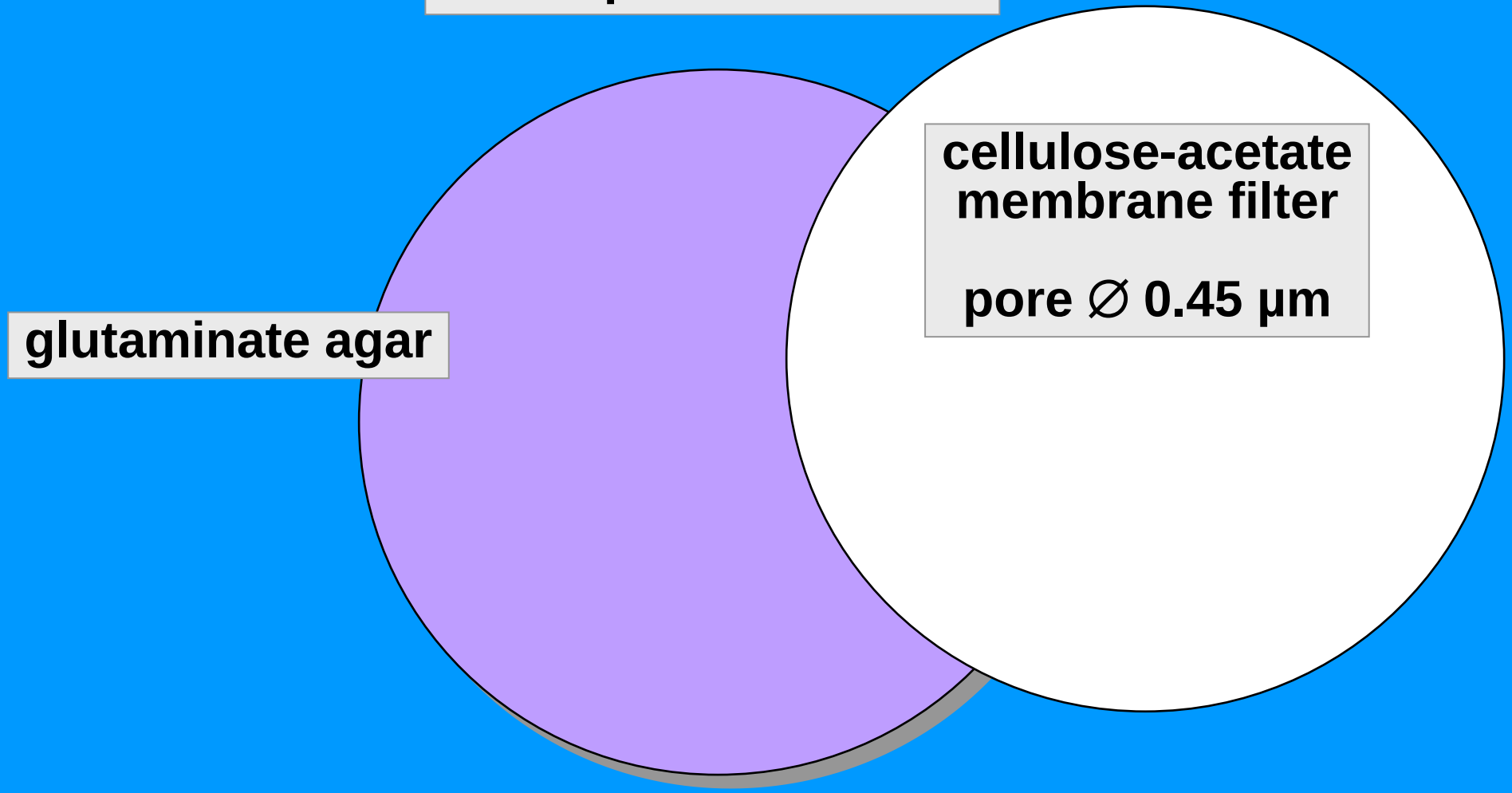
Staphylococcus aureus

Confirmation
by coagulase test



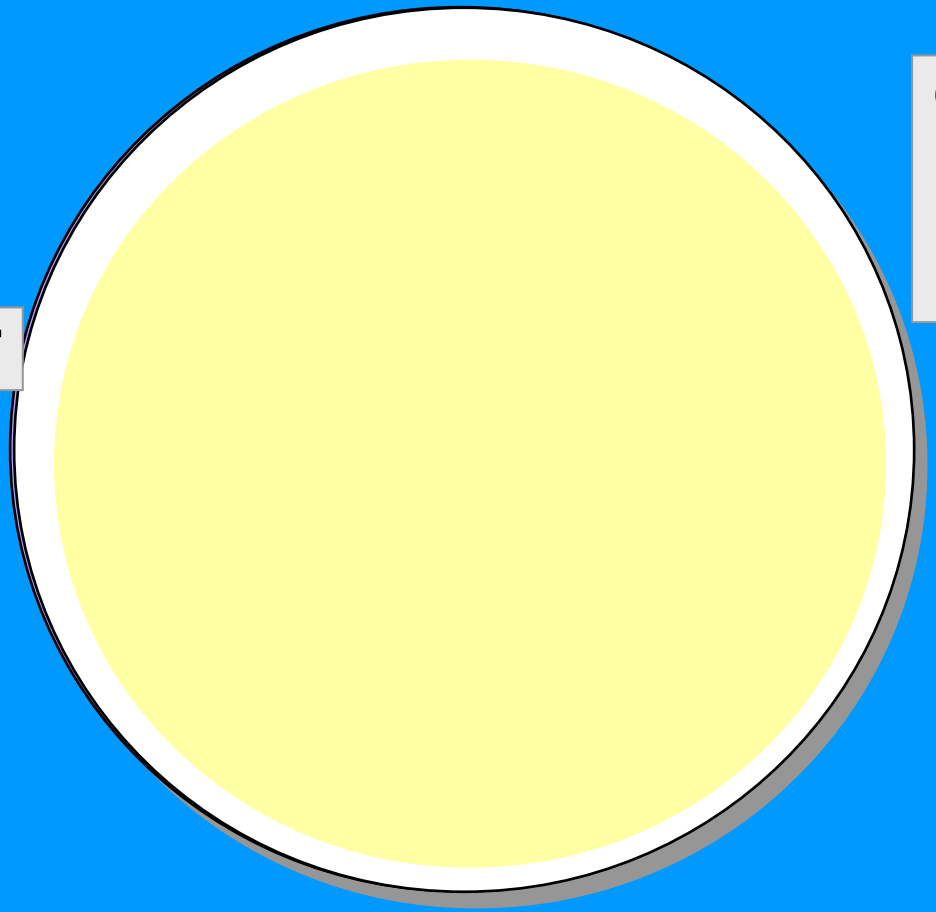
E. coli
fluorescent optic method

1st step: resuscitation



E. coli
fluorescent optic method

1st step: resuscitation



glutaminate agar

cellulose-acetate membrane filter

pore ∅ 0.45 µm

0.1 ml

incubation: 4 h at 37°C

E. coli
fluorescent optic method

1st step: resuscitation

incubation: 4 h at 37°C

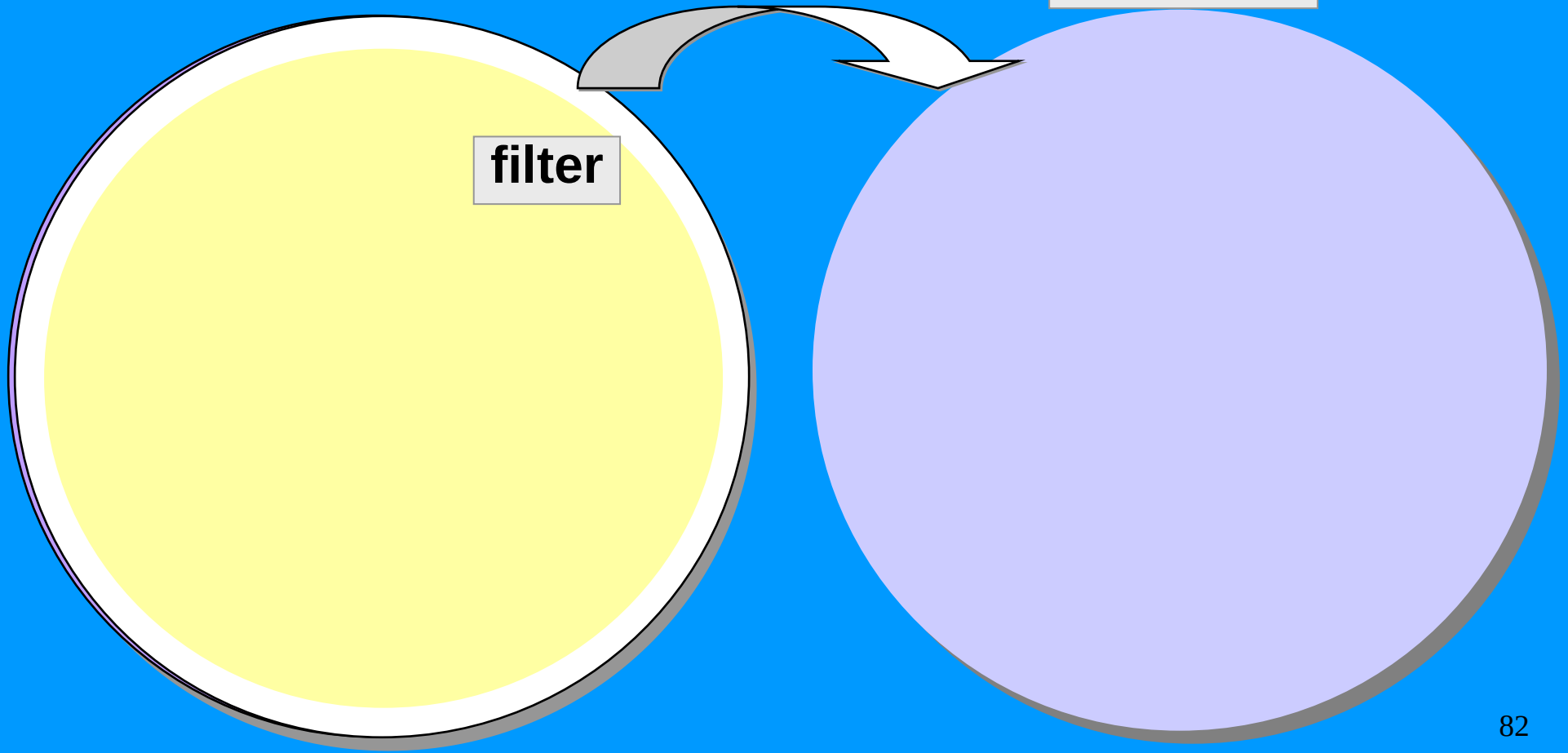
glutaminate agar

filter

2nd step: selective agar

incubation: 18 - 24 h at 44°C

ECD agar



E. coli
fluorescent optic method

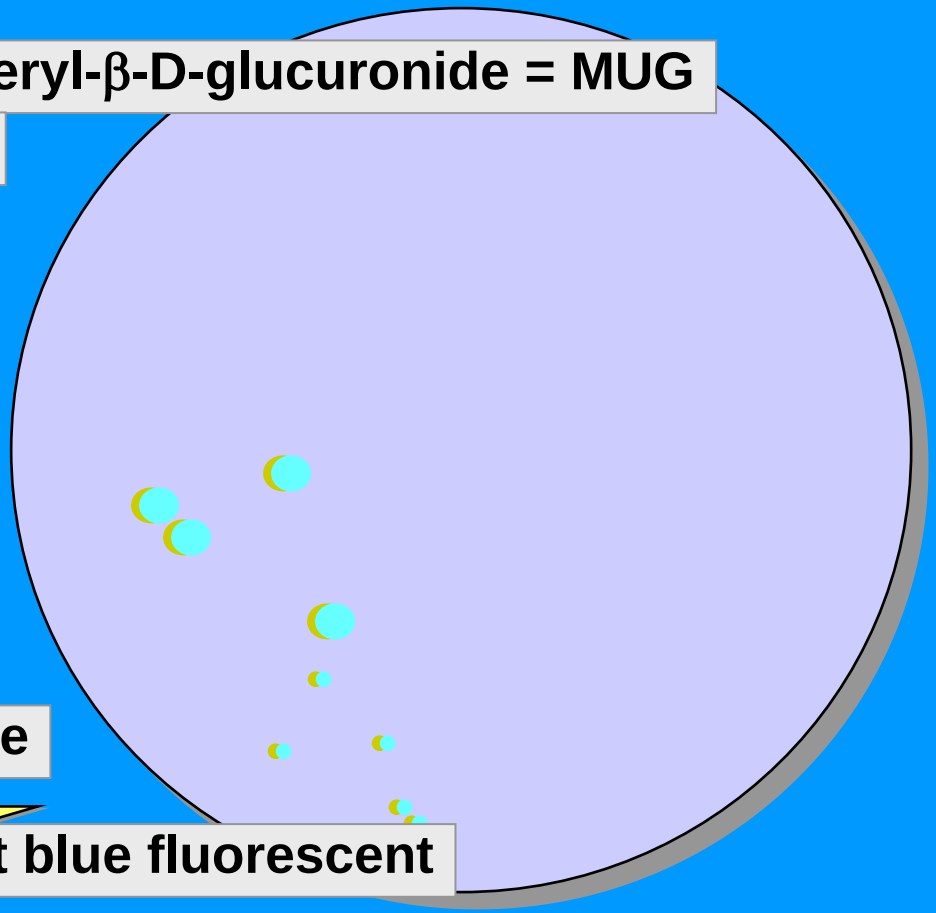
EHEC
do not produce
β-D-Glucuronidase

selective agents
Bile salts

ECD agar

incubation: 18 - 24 h at 44°C

4-Methyl-umbelliferyl-β-D-glucuronide = MUG
differential agent



some other Enterobacteriaceae may also produce β-D-Glucuronidase

indole test for differentiation

E. coli **+**

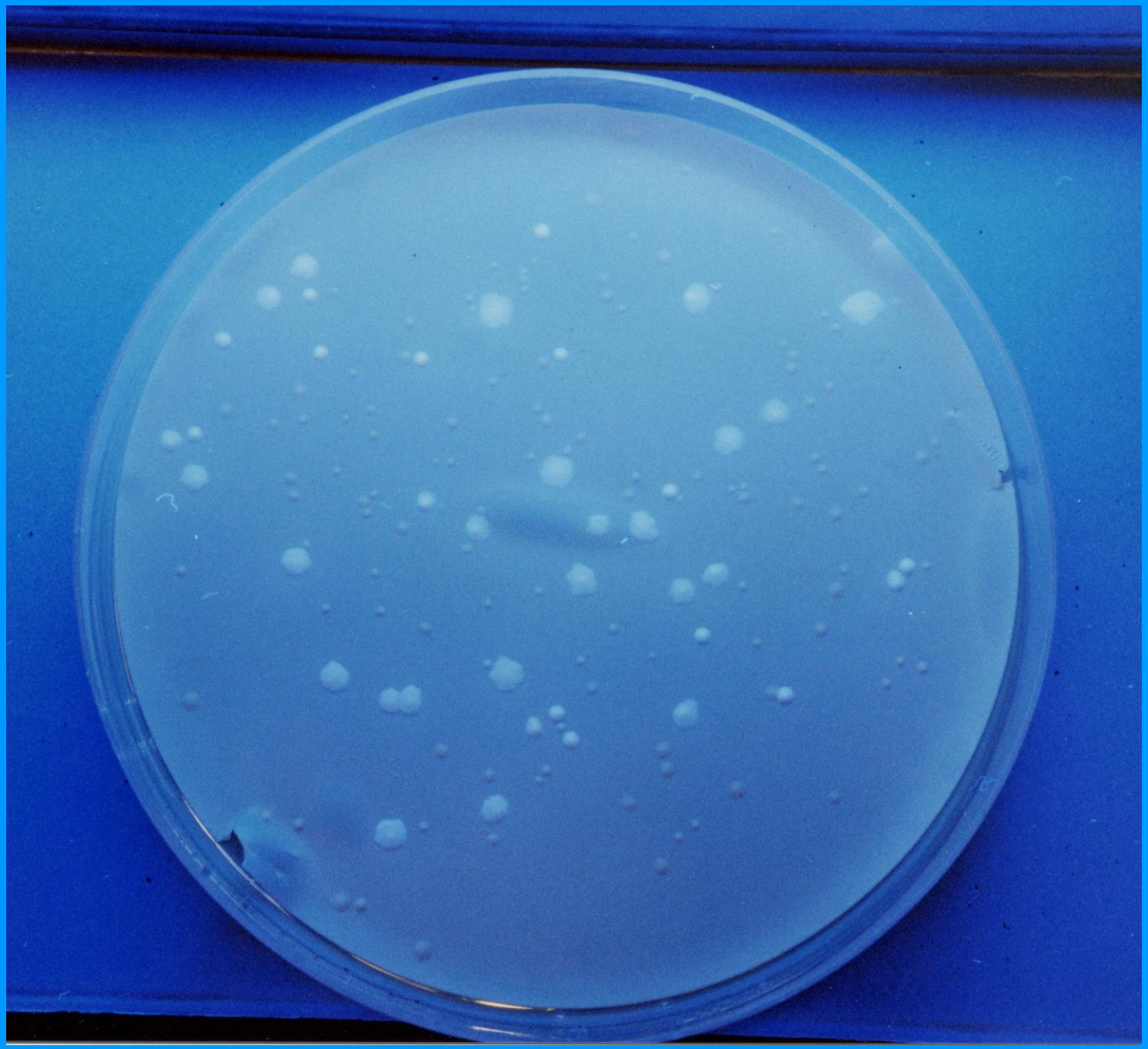
E. coli

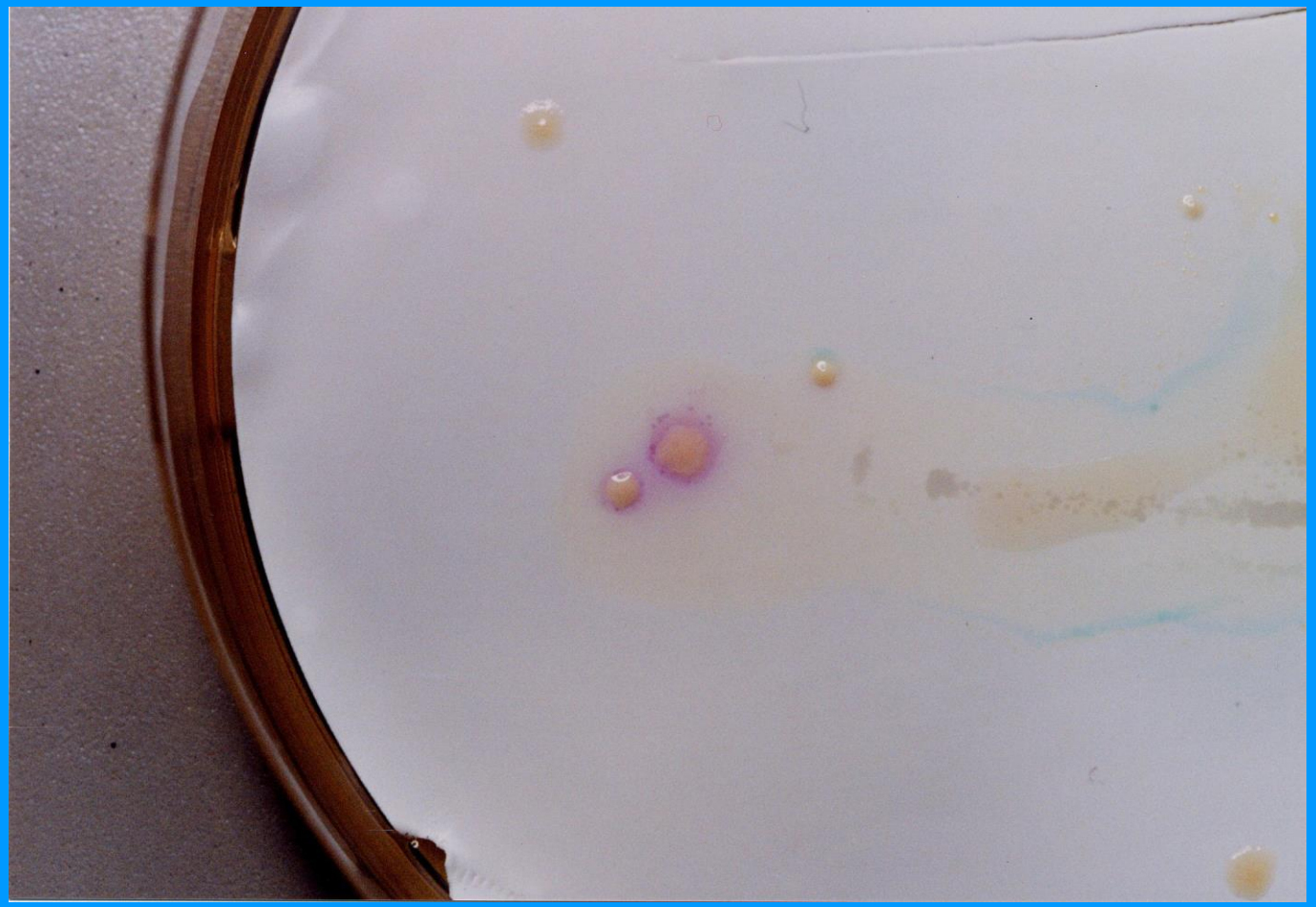
hydrolyse MUG

Methyl-umbelliferone

UV light 366 nm → **light blue fluorescent**

pH dependent





cleaning and disinfection

of processing environment and food contact surfaces

essential part of food processing operations

surfaces may appear to be clean

but can still be heavily contaminated

assessing levels of contamination

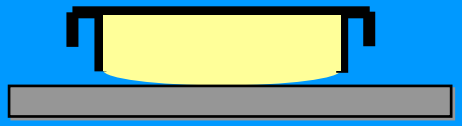
essential to monitoring or verifying

the efficiency of cleaning and disinfection

Monitoring the efficiency of

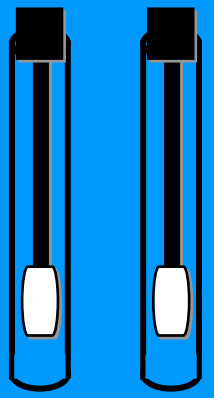
cleaning and disinfection

of processing environment



Contact plates

Swabbing



both techniques work best with smooth surfaces

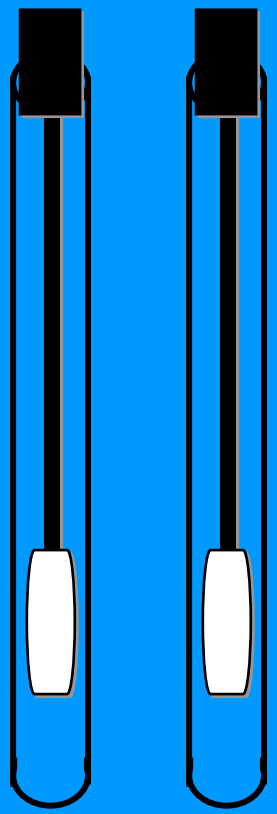
for plane surfaces only

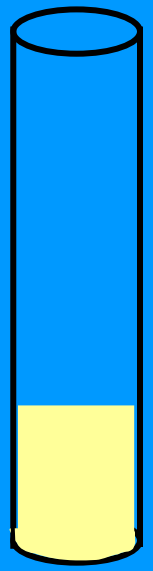
usefull when contamination levels

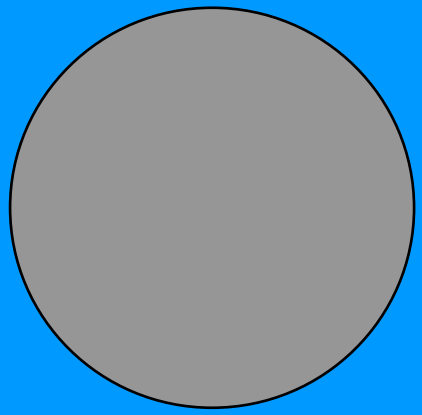
are low

are relatively high

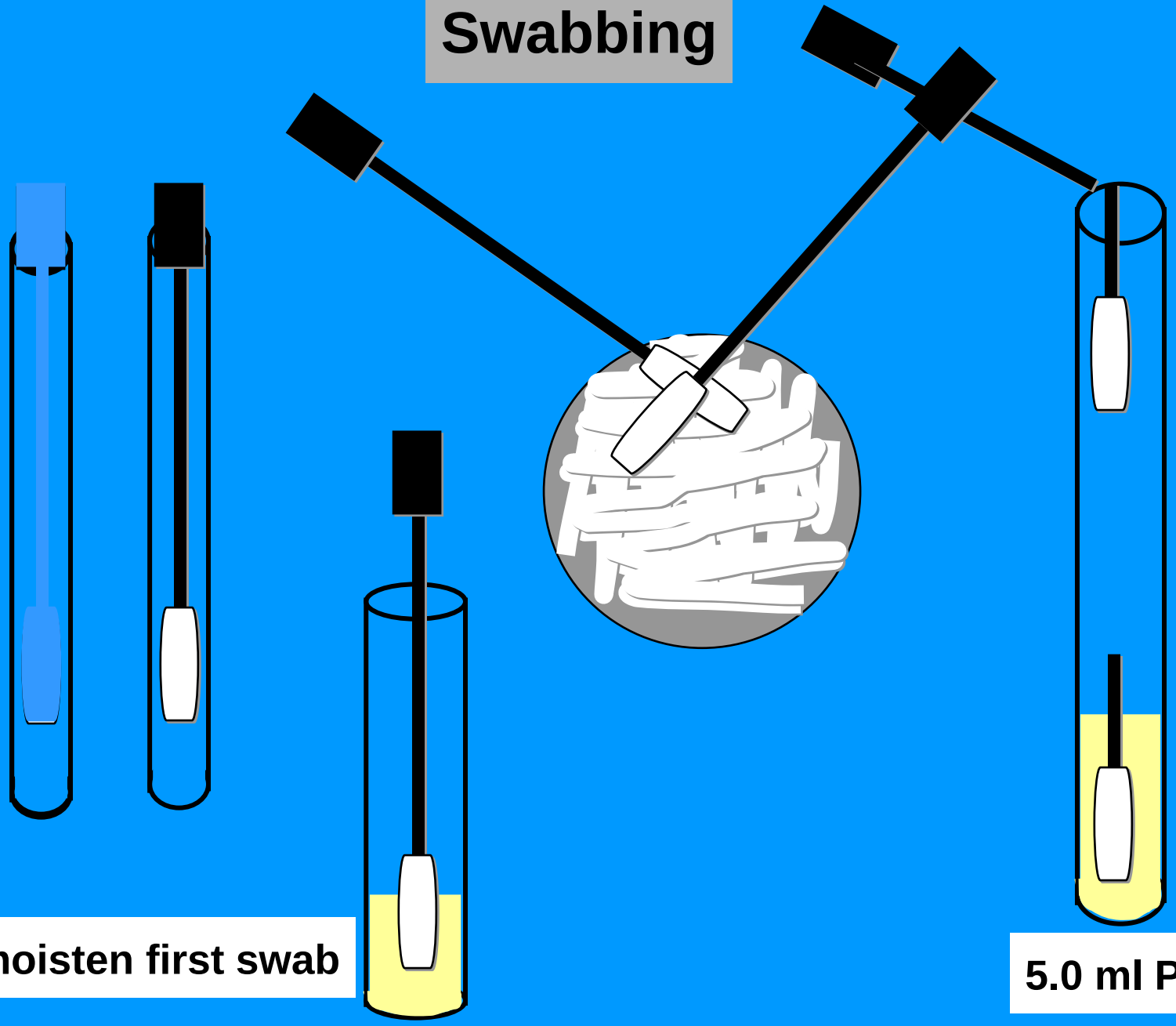
Swabbing







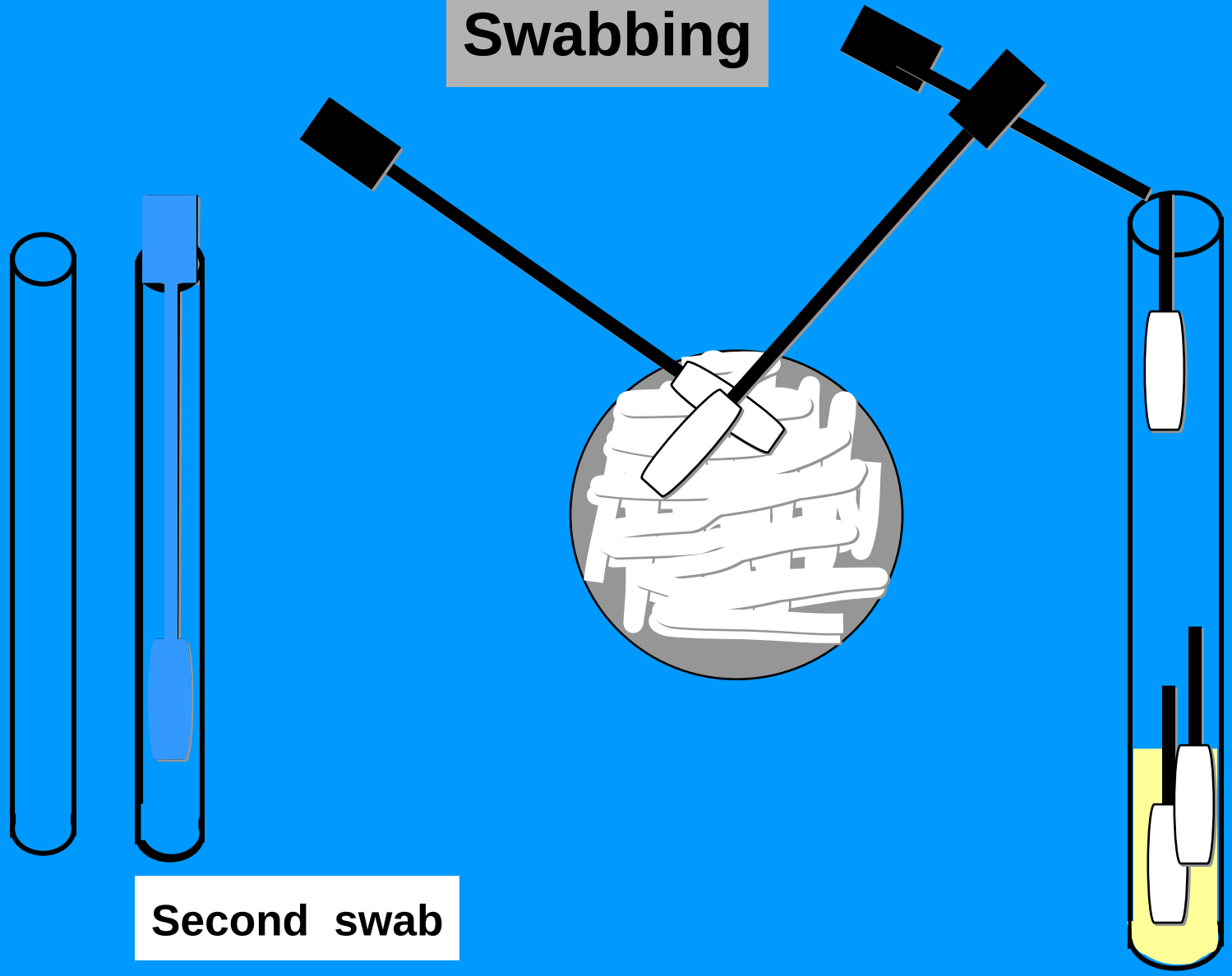
Swabbing



PW to moisten first swab

5.0 ml PW

Swabbing

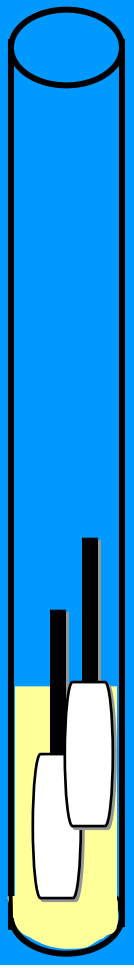


Second swab

5.0 ml PW

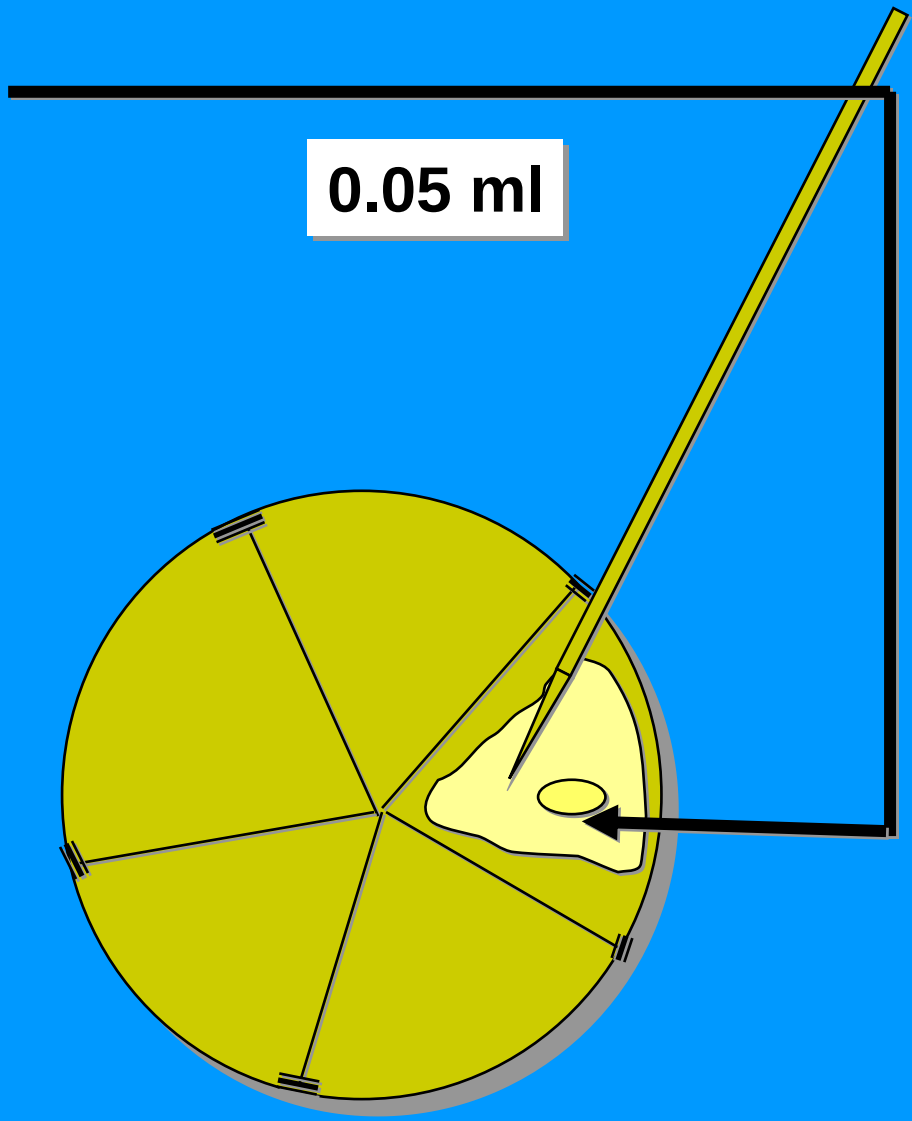
Swabbing

Mix thoroughly



5.0 ml PW

0.05 ml

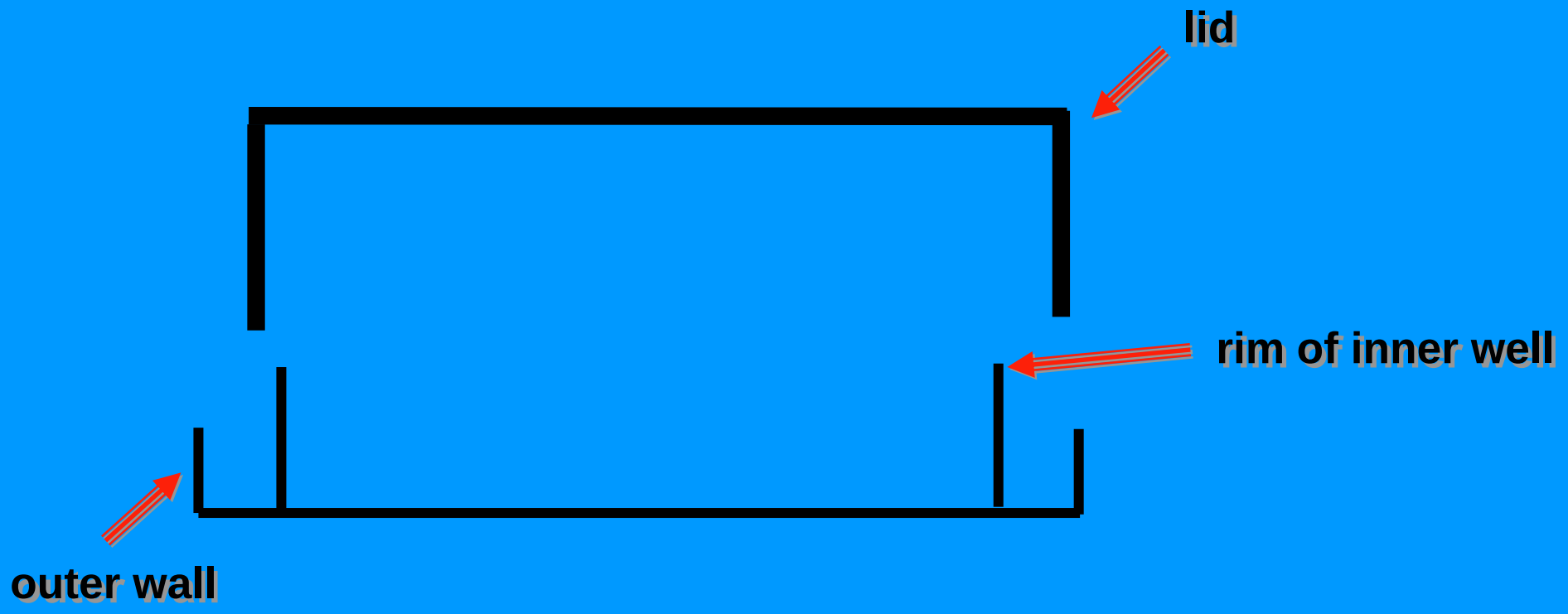


RODAC plate

Replicate Organism Direct Agar Contact

impression plate

contact plate

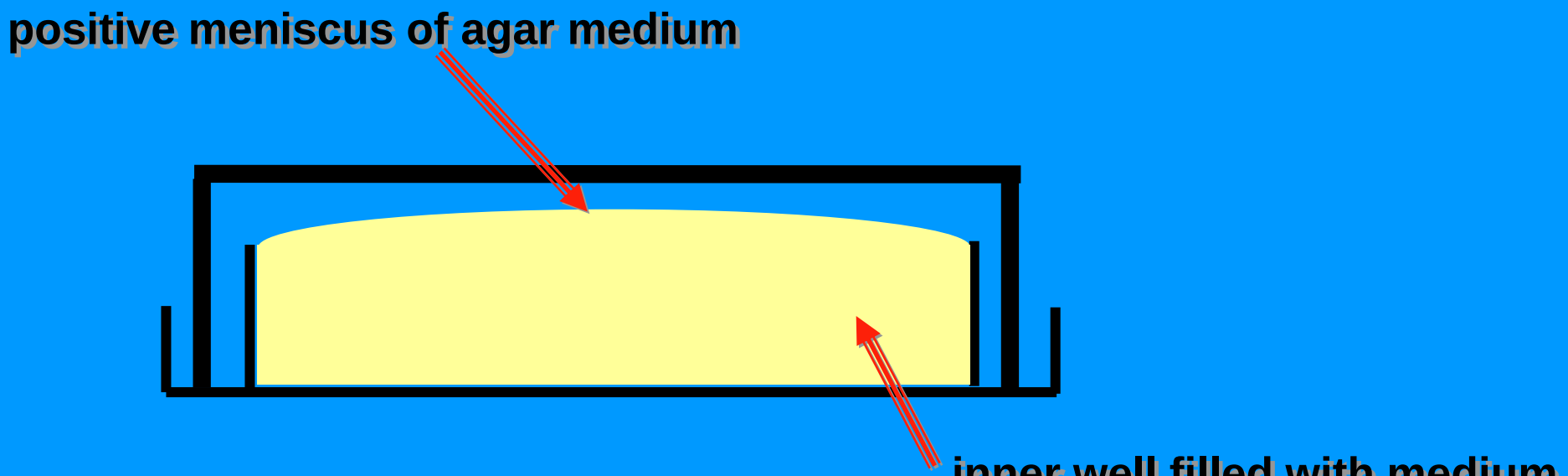


RODAC plate

Replicate Organism Direct Agar Contact

impression plate

contact plate

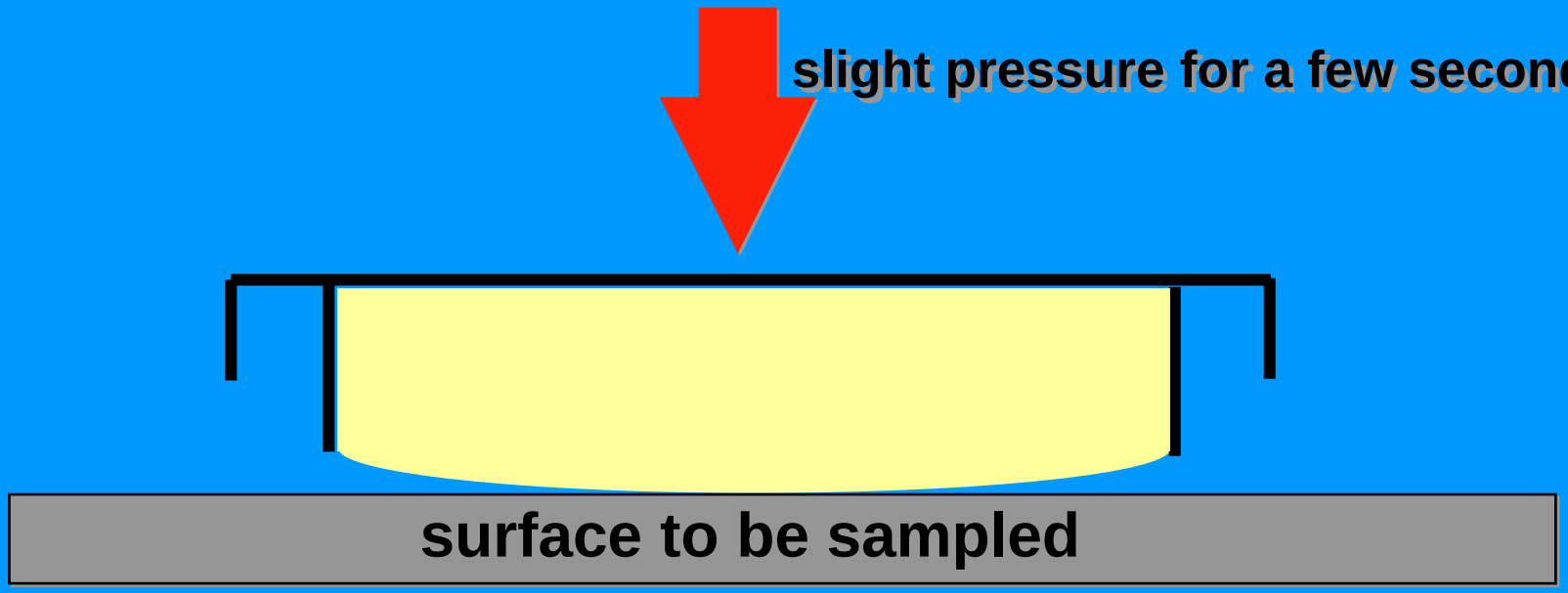


RODAC plate

Replicate Organism Direct Agar Contact

impression plate

contact plate



Microbiological sampling and testing

- **a) Contact method**

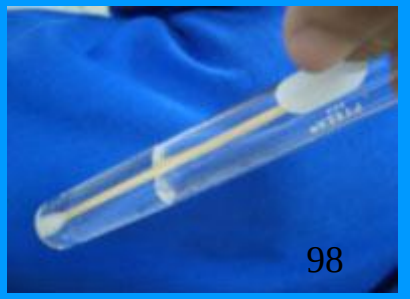
Advantage	**Simple procedure, can be carried out without laboratory.**
Disadvantage	In case of heavy contamination, colonies may overgrow/overlap and individual colonies are difficult or impossible to distinguish. Result in this latter case would be “heavy contamination”, but conclusion on the exact degree is not possible.

Impression on test surface (purple colour).

Direct transfer of bacteria from test surface to culture medium (in petridish

- **Testing of surface of meat processing equipment (above).**
- **Below left: Contact plate before impression. Below right: Contact plate after impression and incubation**

- **b) Swab method**
- **Contaminating bacteria are removed from the surface to be tested by using a sterile swab. Standardization by using a reference square area is needed (e.g. by sterile metal frame). Microorganisms collected by the swab technique are rinsed off with sterile water . The microbial content of the liquid is tested.**
- **Advantage : Even in case of heavy contamination, the number of microorganisms can be determined by applying dilution techniques .**
- **Disadvantage : Part of the contaminating flora may not be recovered, in particular in case of uneven rugged surfaces, e.g. meat.**



c) Destructive methods
(for use on meat/meat products)

- **The testing includes all microorganisms present in the sample. Samples can be exactly standardized according to surface area (cm^2) or weight (g). The sample comprises not only superficial contamination, but also microorganisms from the interior of meat/meat products.**