ΠΡΟΓΡΑΜΜΑ ΔΙΑ ΒΙΟΥ ΜΑΘΗΣΗΣ ΑΕΙ ΓΙΑ ΤΗΝ ΕΠΙΚΑΙΡΟΠΟΙΗΣΗ ΓΝΩΣΕΩΝ ΑΠΟΦΟΙΤΩΝ ΑΕΙ (ΠΕΓΑ)

«Οι σύγχρονες τεχνικές βιο-ανάλυσης στην υγεία, τη γεωργία, το περιβάλλον και τη διατροφή»

METHOD FOR THE MICROBIOLOGICAL EXAMINATION OF FOODS

METHOD

Traditional method

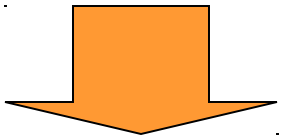
- Plate counts
- Membrane filtration
- Most probable number
- Direct microscopic count
- Dye reduction tests
- Indicator

Rapid Method

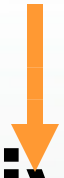
- Direct epifluorescent filter technique (DEFT)
- Electrical impedance
- Enzyme-linked immunosorbent assay(ELISA)

Plate count method

Standard plate count (SPC)
Aerobic plate count (APC)



Total bacteria count (TBC)
Total viable count (TVC)



"Live"

Plate count method

- Pour plate
- Spread plate
- Drop plate

- Diluent
  - 0.85%NaCl
  - 0.1% peptone
  - Phosphate buffer
- Medium
  - Elective medium
  - Selective medium
  - General
- Petri dish plate
- Replication

PLATE COUNT DEPENDS ON

- Diluent
- Food homogenate
- Dilution series
- Medium
- Plating method
- Incubate conditions

BAIRD-PARKER AGAR

- Selective agent
  - Sodium tellulite
  - Lithium chloride
- Elective agent
  - Sodium pyruvate
  - Glycine
- Diagnostic agent
  - Egg yolk

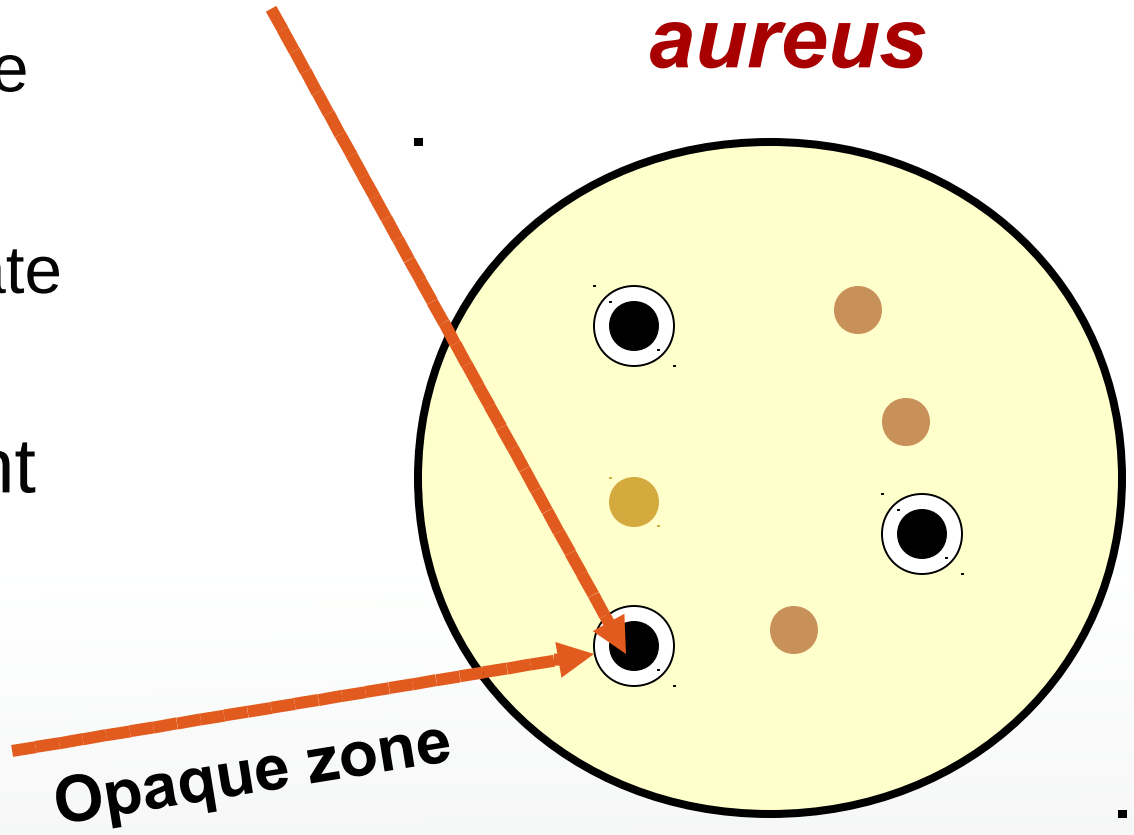
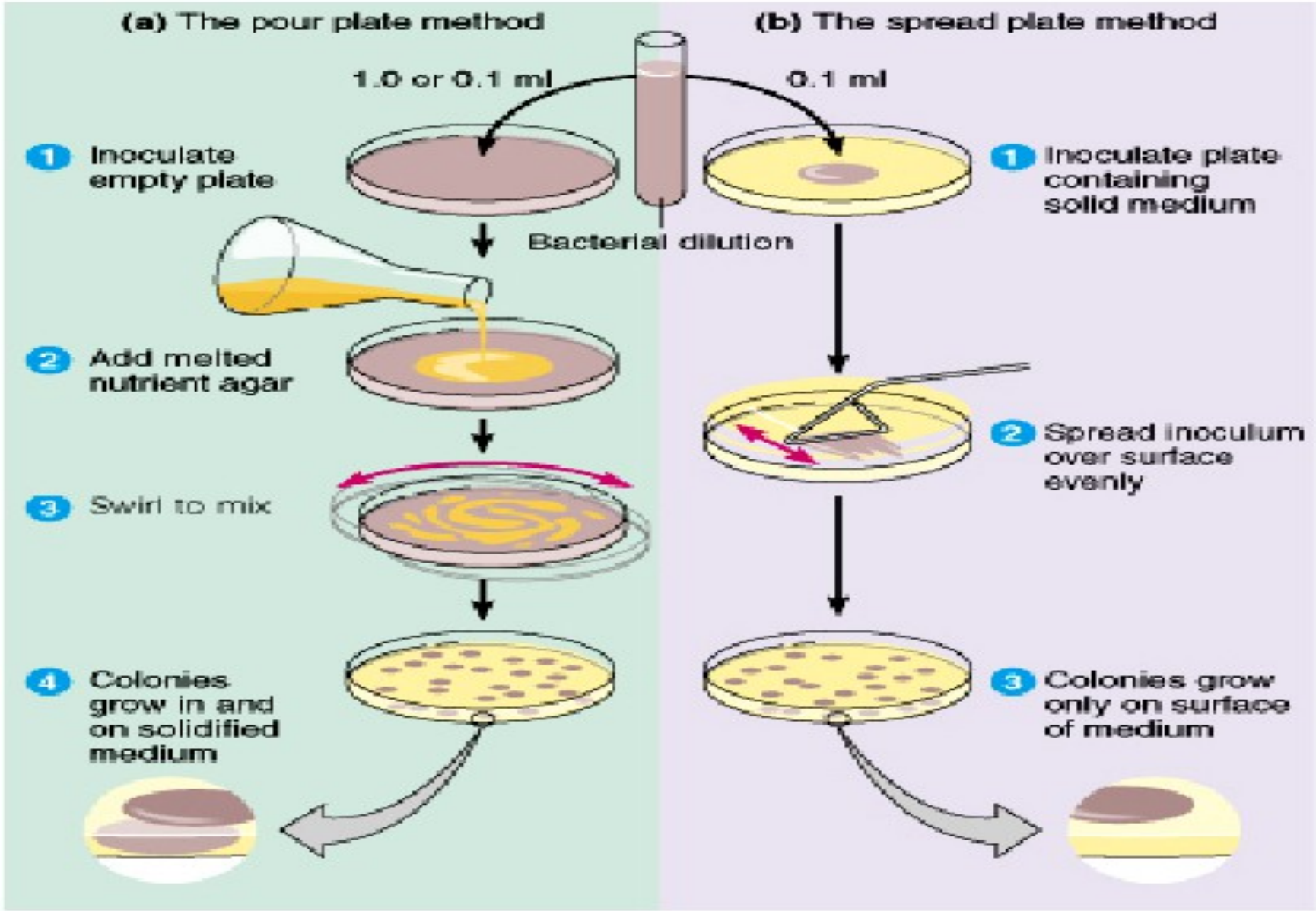
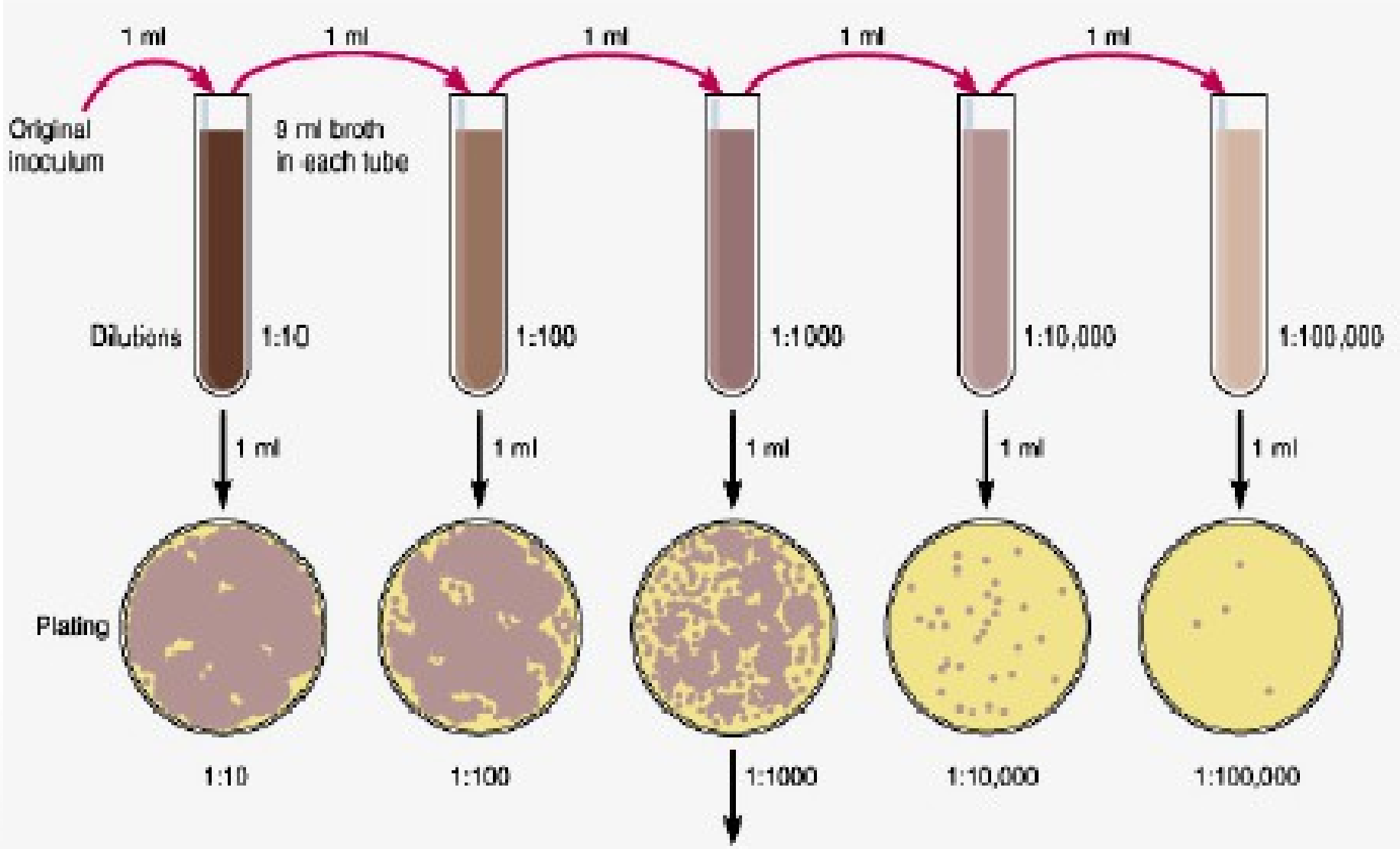


Plate count method

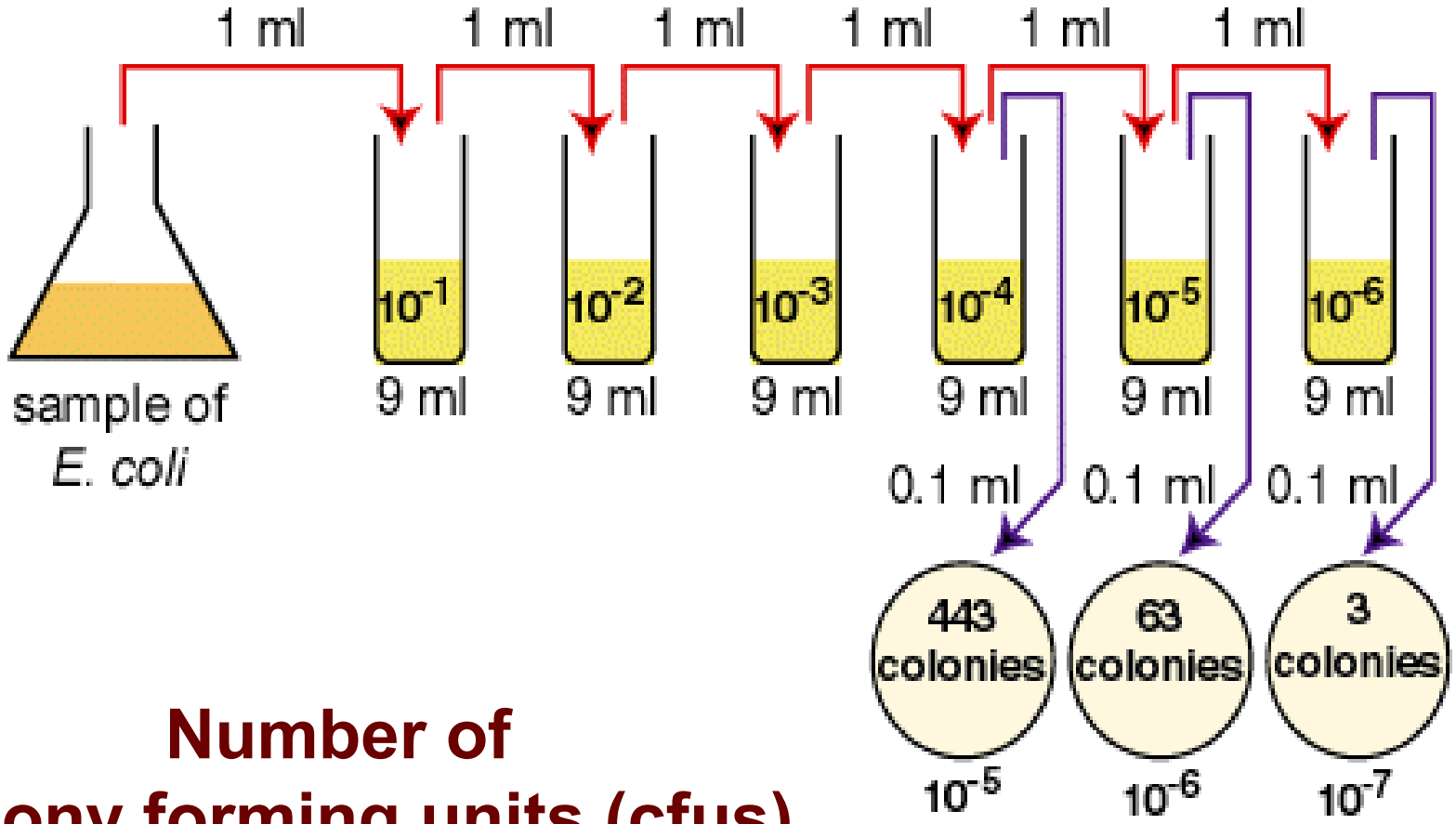


Pour plate



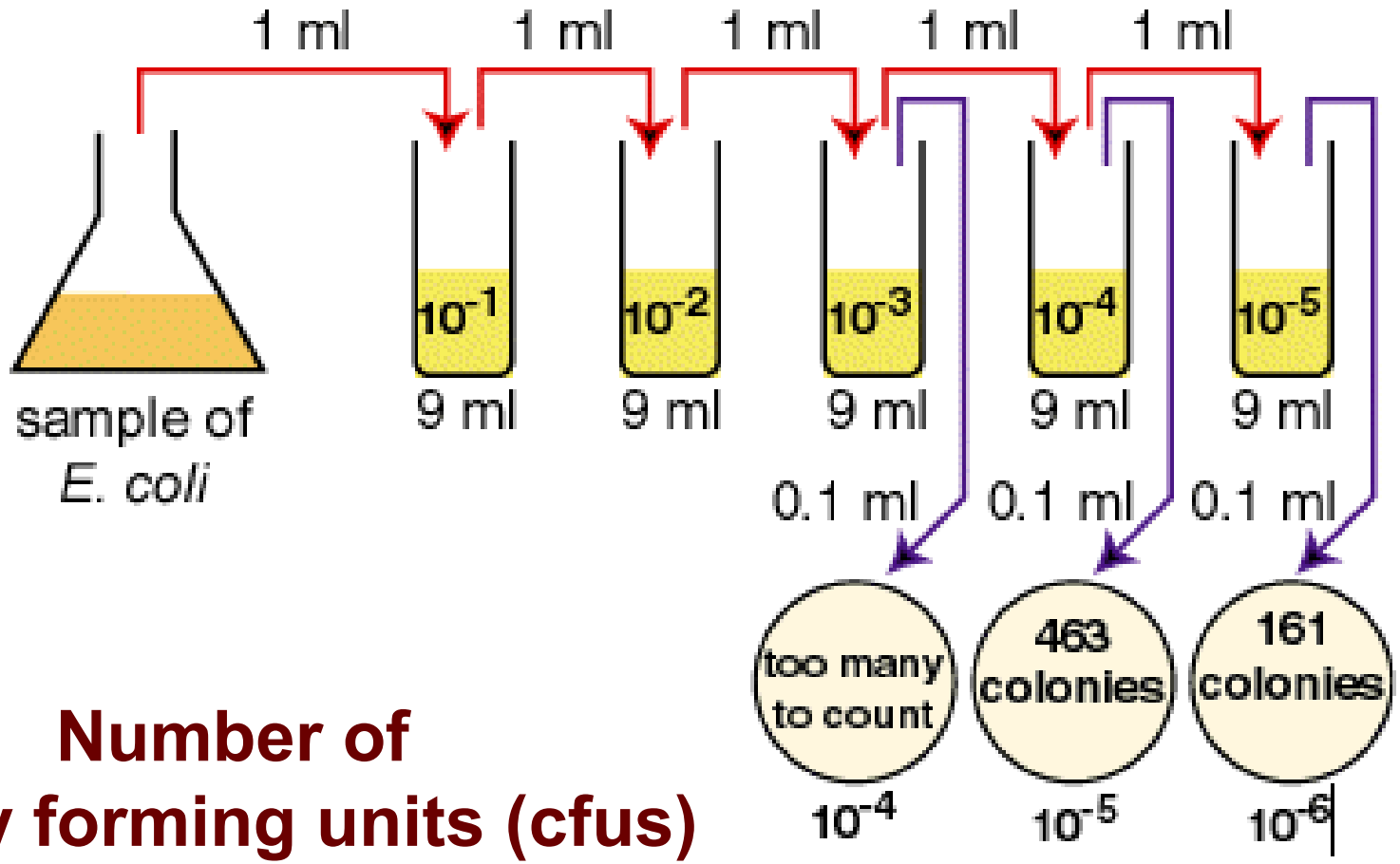
Calculation: Number of colonies on plate × reciprocal of dilution of sample = number of bacteria/ml
(For example, if 32 colonies are on a plate of $^{1}/_{10,000}$ dilution, then the count is 32 × 10,000 = 320,000/ml in sample.)

Spread plate



Number of colony forming units (cfus) ?????

Spread plate



Number of colony forming units (cfus) ?????

DISADVANTAGE OF PLATE COUNT ???

???????

Drop plate

- **Sample:**
  - **Small vol. ≈ 20 µL**
- **Cost**

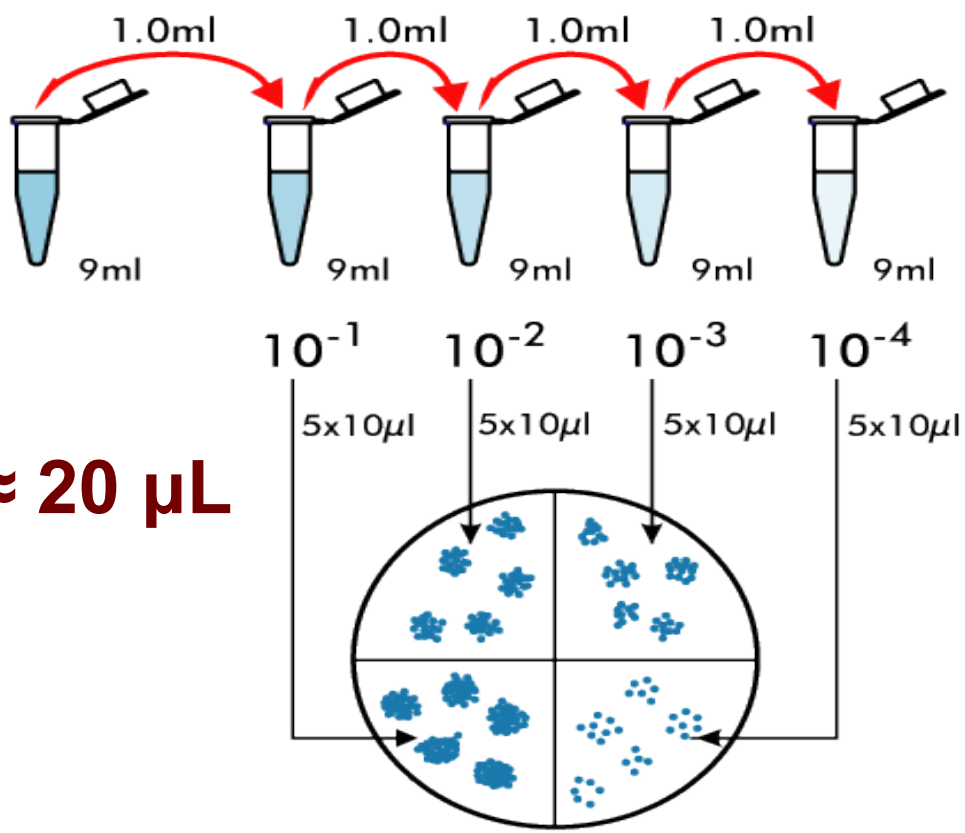


Figure 1. Dilution series followed by drop-plating techniques

Drop plate

APPLICATION OF PLATE COUNT

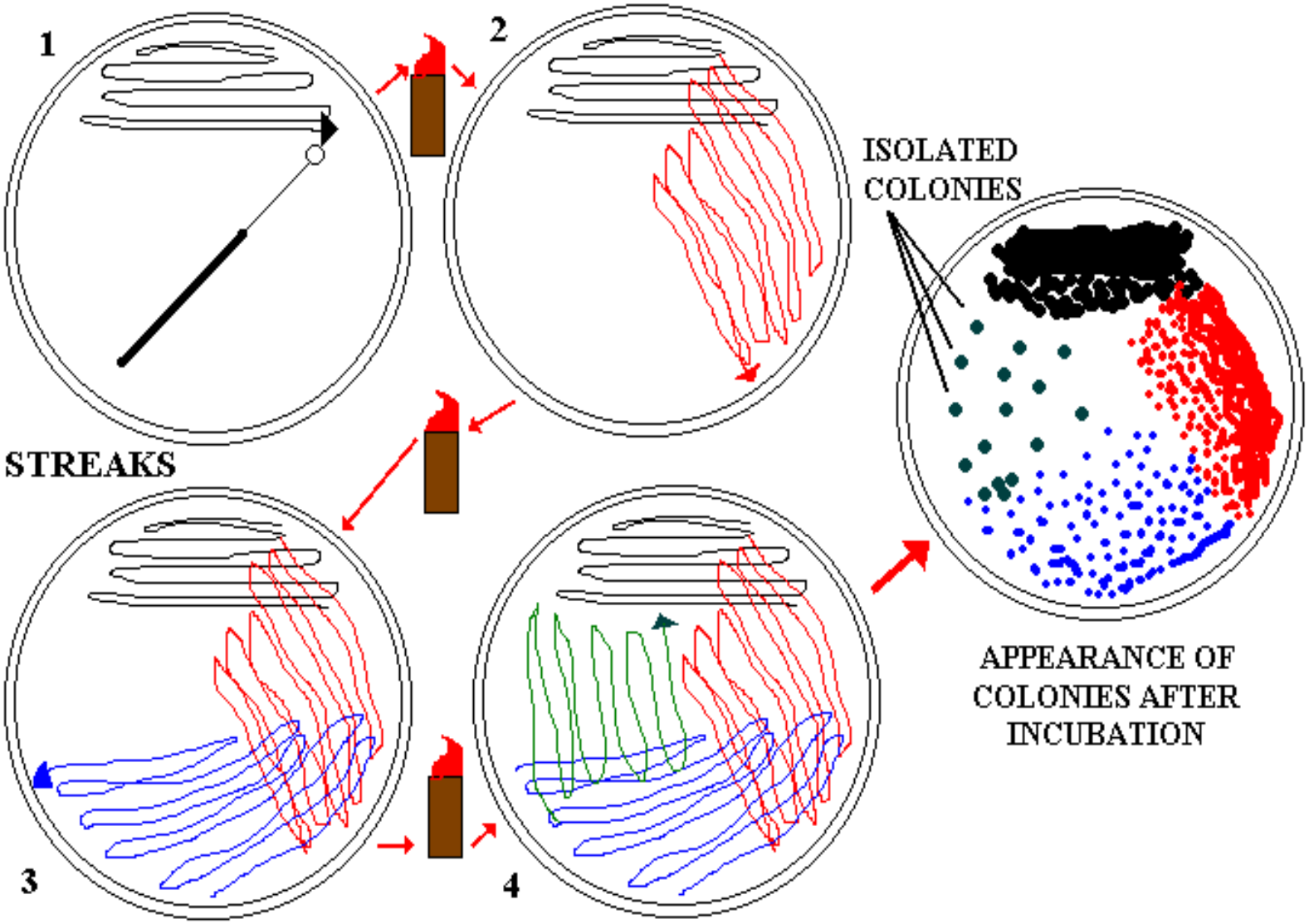
- Check quality of RM & final products
- Check condition hygiene
- Estimate storage life of products
- Determine
  - Production
  - Transport
  - Storage
- Determine pathogens

SELECTION OF MEDIA IN FOOD MICROBIOLOGY

Medium	Use
Plate count agar	Aerobic mesophilic count
MacConkey broth	MPN of coliforms in water
Brilliant green/Lactose/Bile broth	MPN of coliforms in food
Braid Parker agar	*Staphylococcus aureus*
Thiosulfate/Bile/Citrate/agar	*Vibrio* sp.

Adam and Moss (2003)

STREAK TECHNIQUE



http://www.towson.edu/~cberkowe/medmicro/images/streak.gif

Filtration

0.45 µm

Liquid food

- **Low number of MO.**
- **Large volume of food**

- **Count**
- **Sterilize**

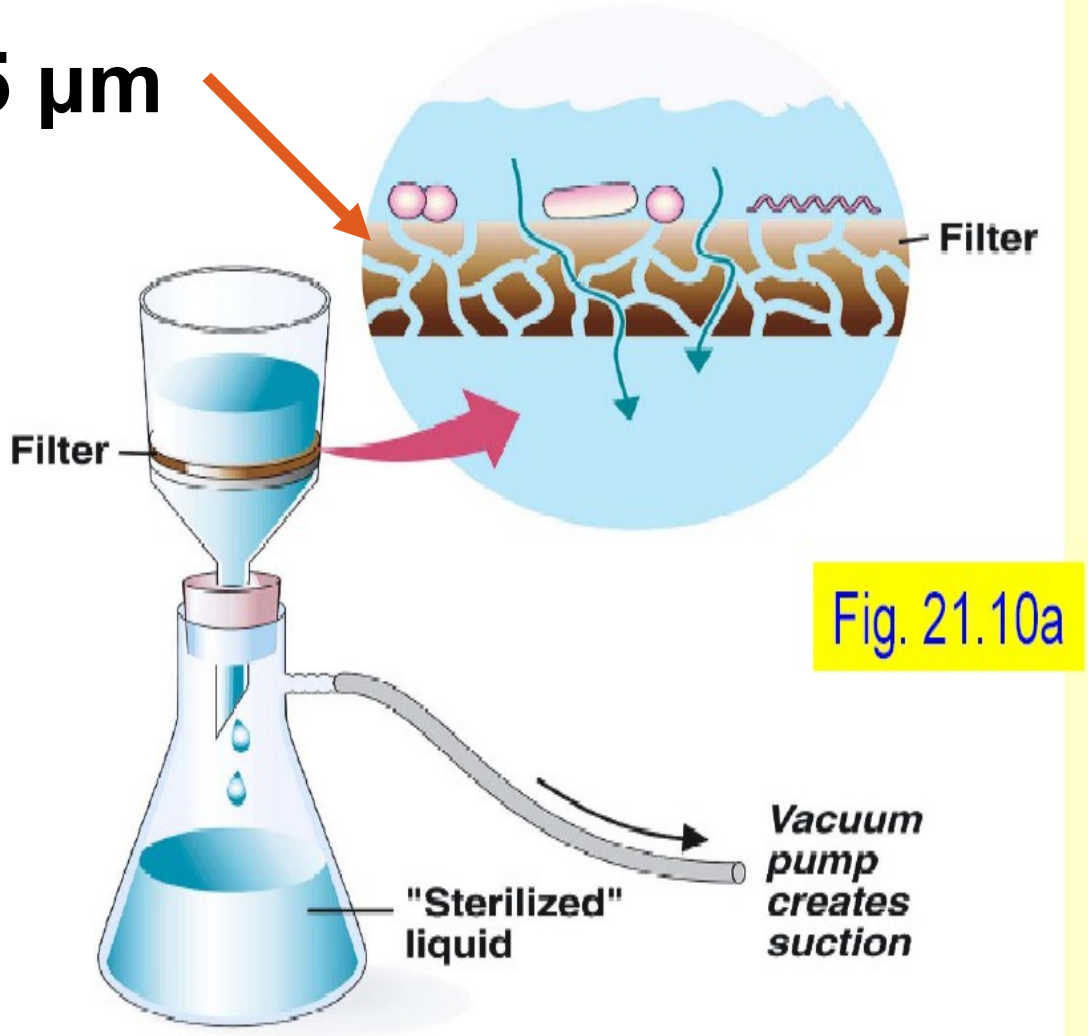


Fig. 21.10a

http://www.biology.lsu.edu/webfac/rgayda/biol1011/Lecture_notesF2004/lecture8.pdf

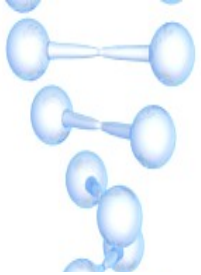
MOST PROBABLE NUMBER

Most probable number (MPN)

Multiple tube techniques

- **Pathogen**
  - **Number too low**

- **Coliform**
- ***Escherichia coli***
- ***Staphylococcus aureus***
- **Feacal streptococci**



MOST PROBABLE NUMBER

Medium	Organisms assessed
Lauryl sulfate tryptose broth	Coliforms
MacConkey purple broth	Coliforms
EC broth	Faecal coliform
Glucose azide	Faecal streptococci
Minerals modified glutamate medium	Coliforms
Baird-Parker broth	*Staphylococcus aureus*

MICROSCOPIC COUNT

Direct microscopic count (DMCs)

Small sample (0.01 ml) & rapid

Optical light microscope

Total cell

- living & dead cells

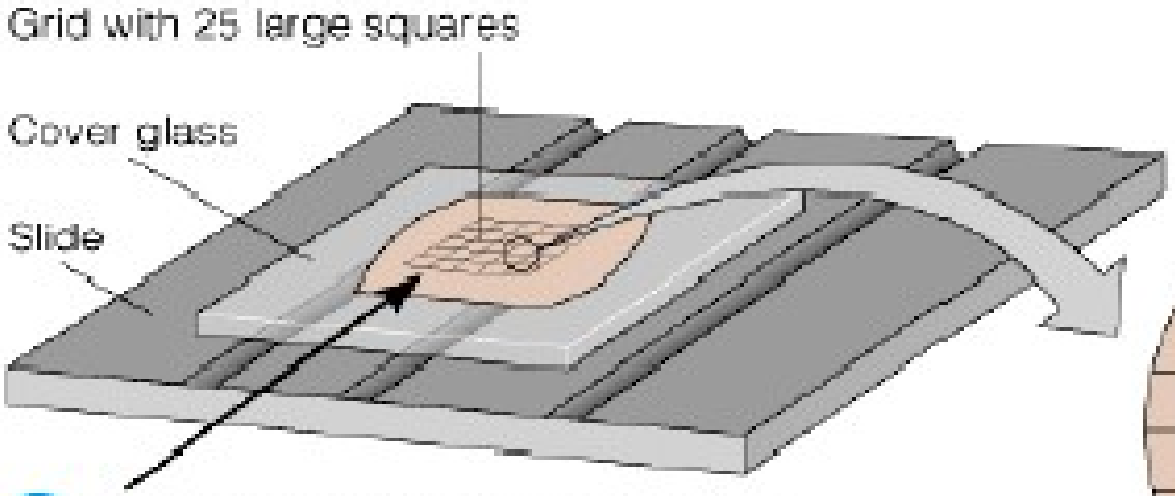
Foods

- Liquid
- Semi-solid

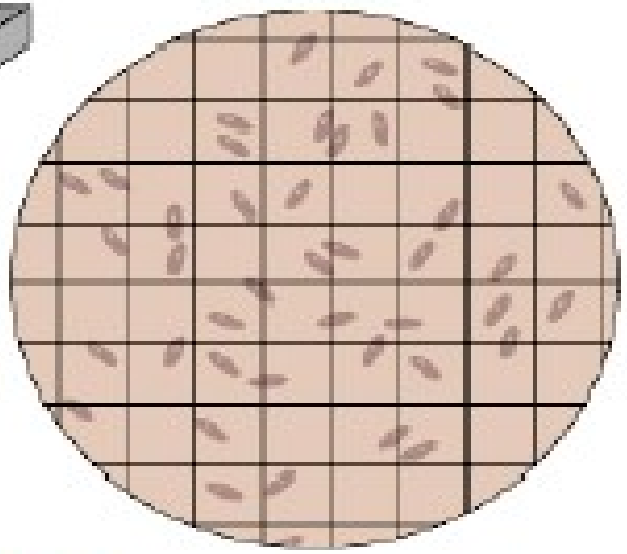
Ex.

- **Milk**
- **Wine**
- **Yogurt starter**
- **Tomato sauce**
  - **Howard mold**

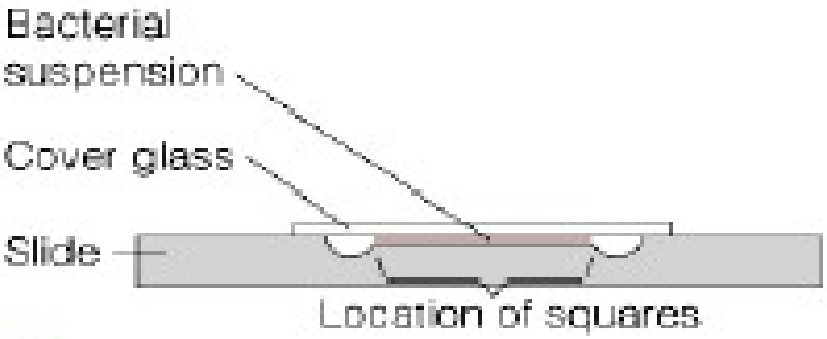
MICROSCOPIC COUNT



1 Bacterial suspension is added here and fills the shallow volume over the squares by capillary action.



3 Microscopic count.



2 Cross section of a cell counter.

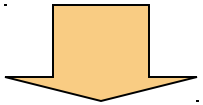
4 The volume of fluid over the large square is 1/1,250,000 of a milliliter.

COMPARISON OF SENSITIVITY OF METHOD

Method	Vol. of sample (ml)	Count (cfu/g)
Direct microscopy	5×10^{-6}	2×10^{6}
Drop plate (Miles and Misra)	0.02	5×10^{2}
Spread plate	0.1	10^{2}
Pour plate	1	10
MPN	3 x 10 + 3 x 1 + 3 x 0.1	0.36

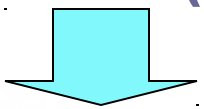
DYE-REDUCTION TEST

Methylene blue



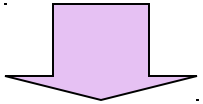
Leuco-methylene blue

Triphenyltetrazolium chloride (leuco)

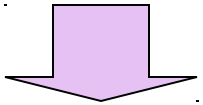


Formazan (red)

Resazurin (blue)



Resorufin (pink)



Dihydroresofin (leuco)

INDICATORS

- Hygiene indicator
- Cross contamination

- Fresh meat
- Raw milk
- Pasteurized milk

ATP PHOTOMETRY

ATP : Adenosine triphosphate

- Synthesis of new cell
- Active transport (uptake of materials from environment)
- Movement
- Light production

ATP PHOTOMETRY

Luciferin + luciferase + ATP + O_2

$\downarrow Mg^{2+}$

Oxyluciferin + luciferase + AMP + light

1 ATP ⟶ light 1 photon

ATP PHOTOMETRY

Bacteria cell

1 fg of ATP

Yeast cell

100 fg of ATP

- **Limit of ATP photometry 10^2-10^3 fg ATP/ml**

fg = femto gram = 10^{-15} g

ATP PHOTOMETRY

Break down the non-microbial cells in food

Remove non-microbial ATP using ATPase

Release ATP from bacteria cell

Addition of luciferin & luciferasee

Record light emission (ATP photometry)

ATP PHOTOMETRY

Application

Fresh meat

Milk

Starter culture

Test UHT milk

Surface contamination

ATP PHOTOMETRY

Disadvantage

Mixed bacteria & yeast cell

Dilution

Remove cell before ATP measured

- Filtration
- Centrifugation

DIRECT EPIFLUORESCENT FILTER TECHNIQUE (DEFT)

Liquid food

Filter through membrane

Acridine orange : fluorescent dye (fluorochrome) pour through filter

Epifluorescent microscopy

Count: manual or automatic

- **Direct microscopy**
- **Membrane filtration**

- **Vol. of sample**
- **Filter area**
- **Area of microscope field**
- **Number of field**

DIRECT EPIFLUORESCENT FILTER TECHNIQUE (DEFT)

Acridine orange bineds to:

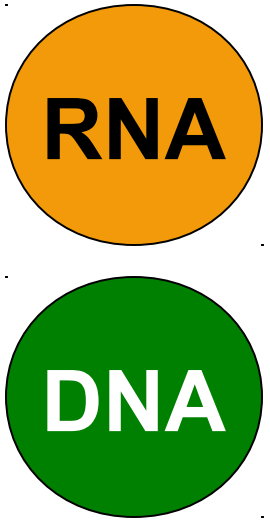
- RNA --- fluorescent orange
- DNA --- fluorescent green

Viable cell

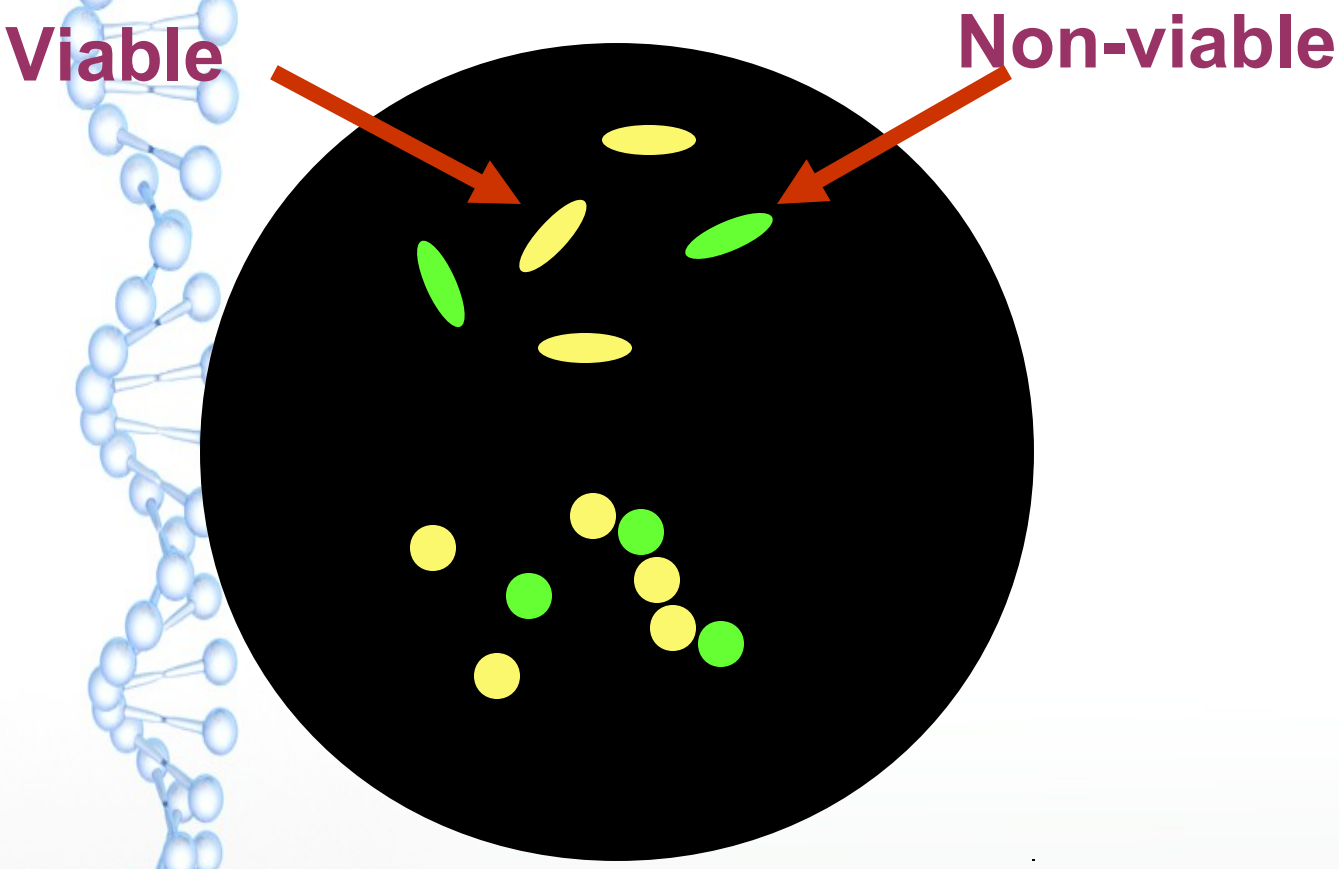
- RNA > DNA --- orange

Non-viable cell

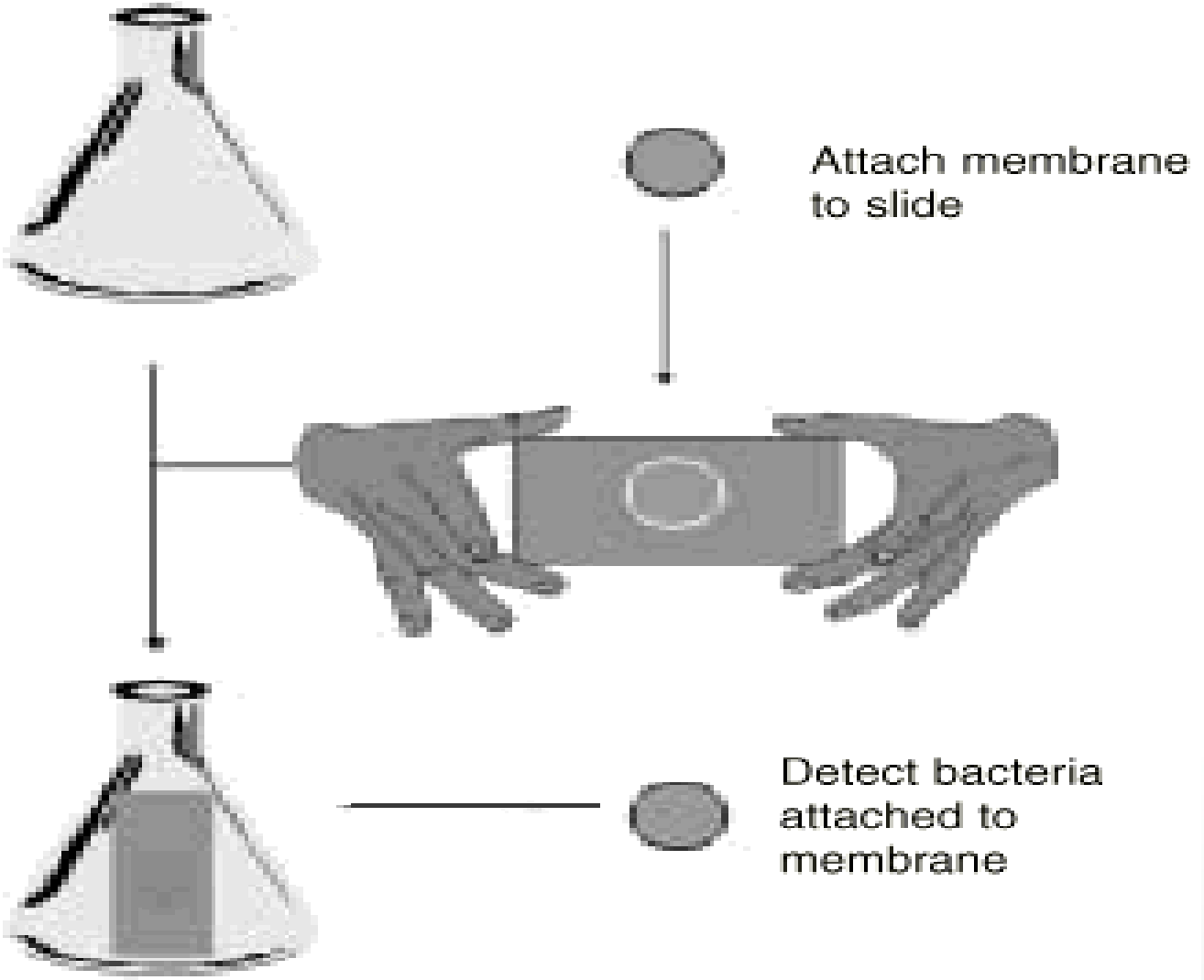
- DNA > RNA ---green



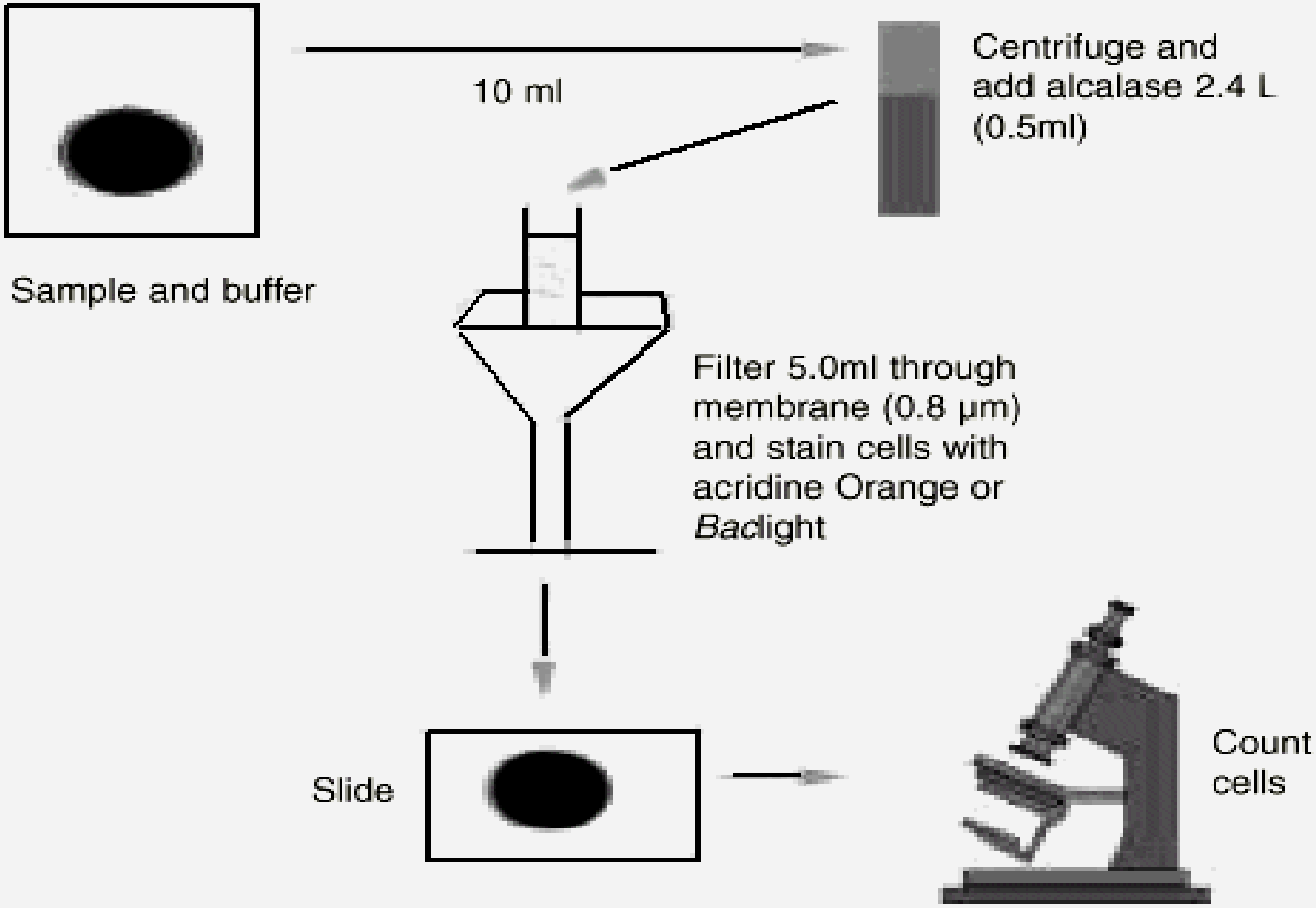
DIRECT EPIFLUORESCENT FILTER TECHNIQUE (DEFT)



Membrane epiflurescent



Membrane epifluorescent



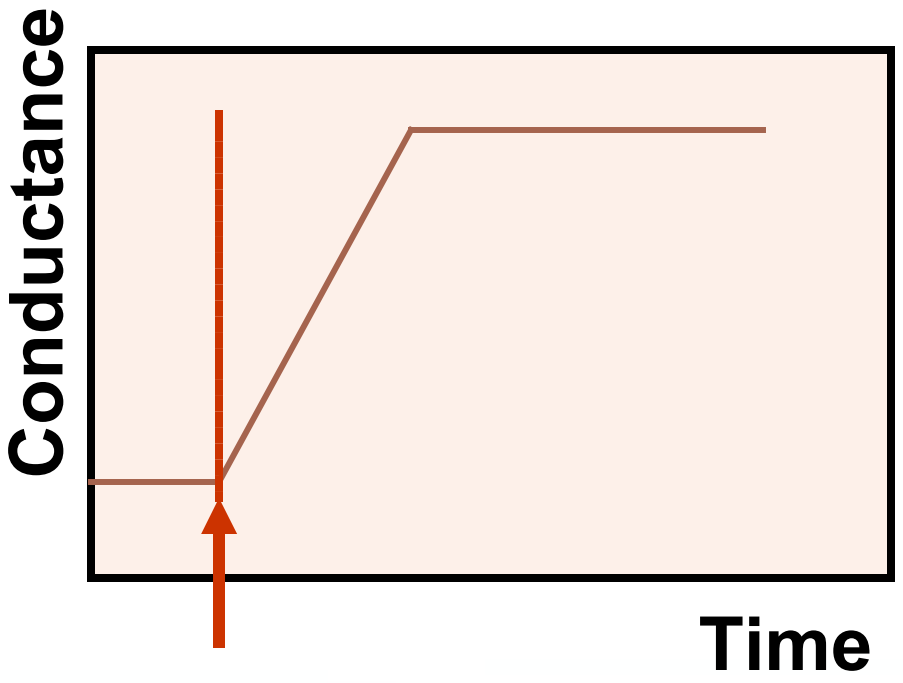
ELECTRICAL IMPEDANCE METHOD

Impedance : resistance

Bacteria growth

----decrease impedance

----increase conductivity



DT
Detection time
10^6-10^7 cells/ml

ELECTRICAL IMPEDANCE METHOD

Bactometer

Vary temp

Small volume

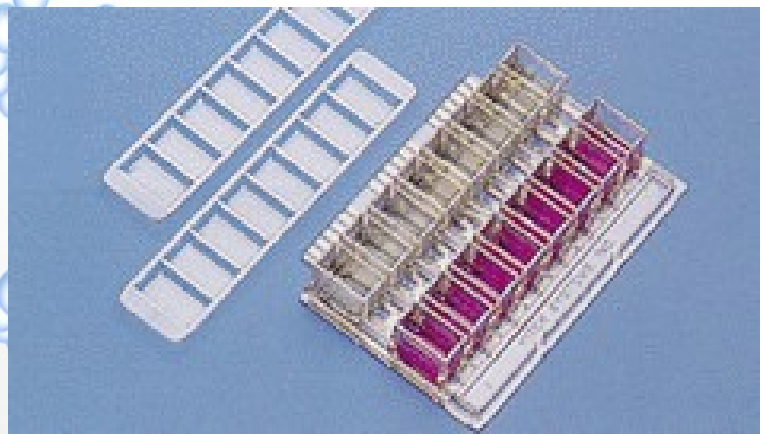
Many wells

Many samples

Automatic

- Count
- Growth

Bactometer



ELISA

Antigen – conjugate enzyme

Antibody – conjugate enzyme

Pathogen

- ***Salmonella***
- ***Listeria***
- ***S. aureus***

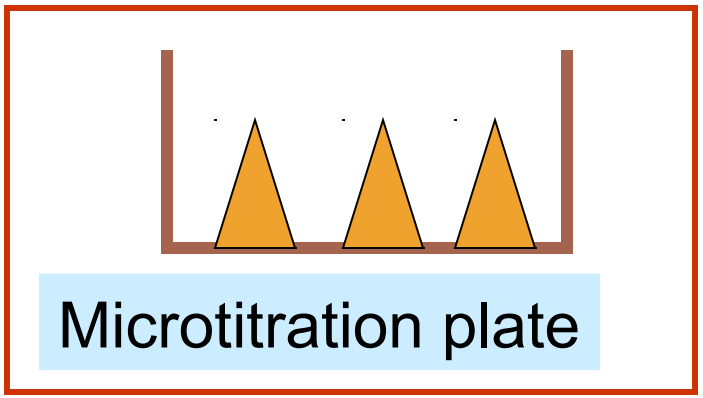
Toxin

- **Staphylocaccal**
- **Botulinum toxin**
- **Mycotoxin**

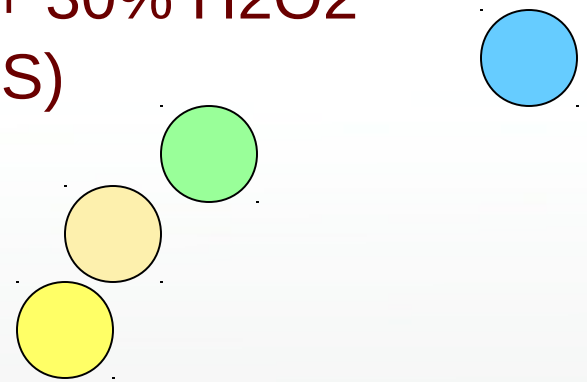
ENZYME-LINKED IMMUNOSORBENT ASSAY

Free toxin

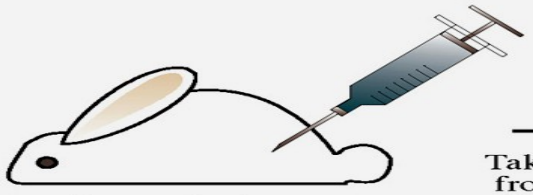
Labeled toxin



- Antibody
- Antigen(toxin)
- Enzyme
  - Alkaline phosphatase (ALP)
  - Horse Radish Peroxidase (HRP)
- Substrate
  - Tetramethylbenzidine (TMB) + 30% H2O2
  - Azinobis sulphonic acid (ABTS)
  - o-phenylinediamine (OPD)
  - p-nitrophenyl phosphate



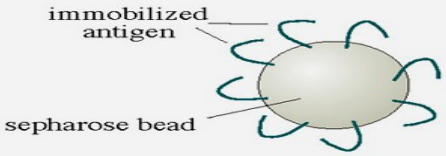
Production and Purification of Polyclonal Antibodies



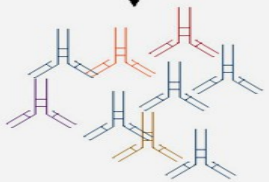
Immunization of rabbit followed by booster shots

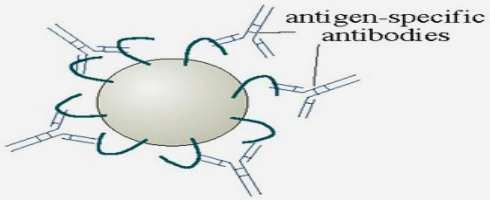
Take whole blood from immunized rabbit and let it clot

Separate serum from blod clot

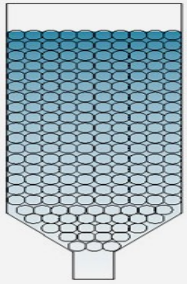


Add whole serum containing all antibodies to antigen-bound sepharose column



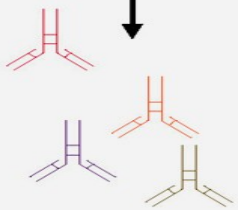


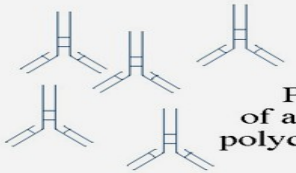
Antibodies that recognize the antigen bind to the immobilized antigen and are retained in the column



Antibodies can be eluted from antigen in high salt

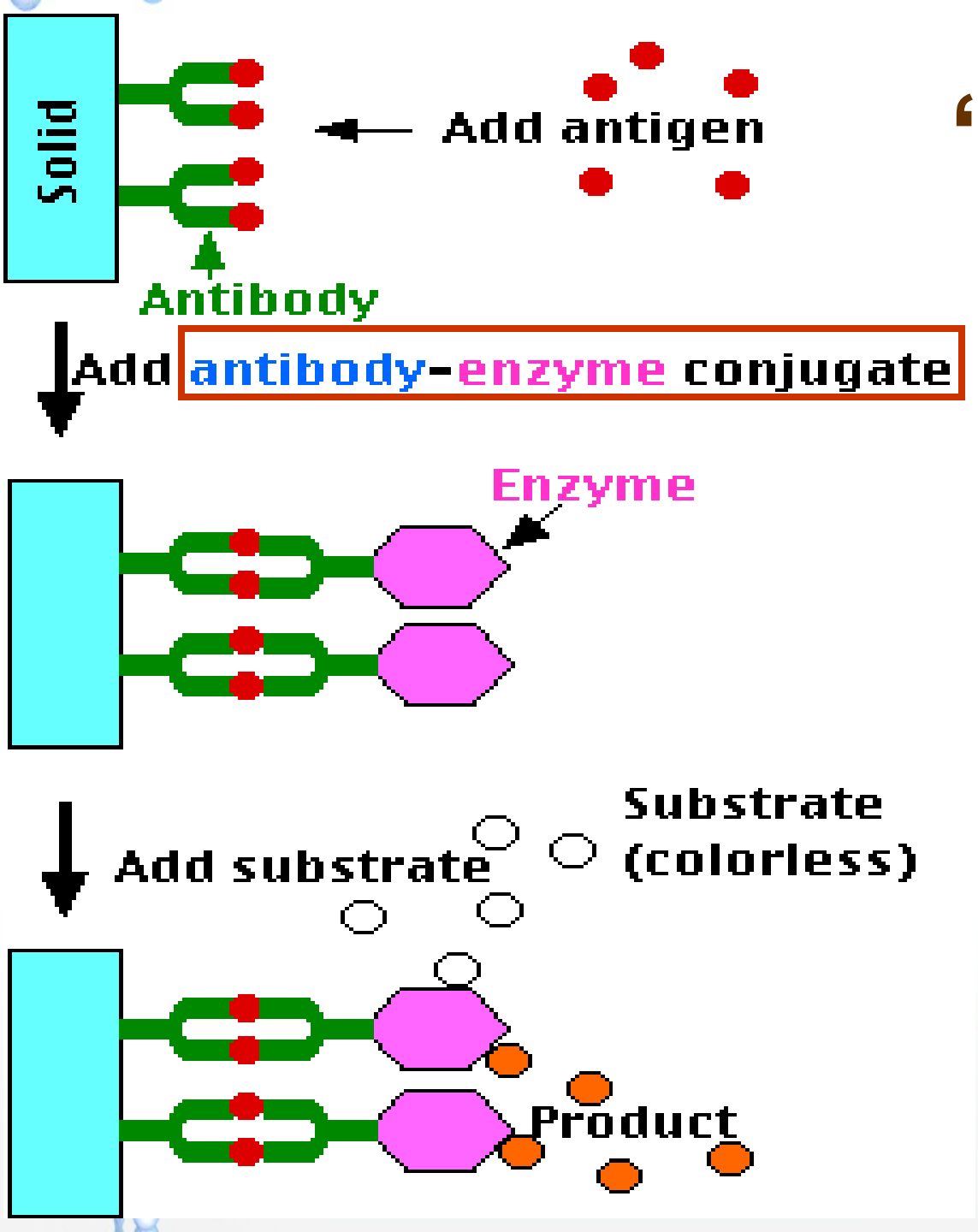
Antibodies that do not recognize the antigen flow through the column



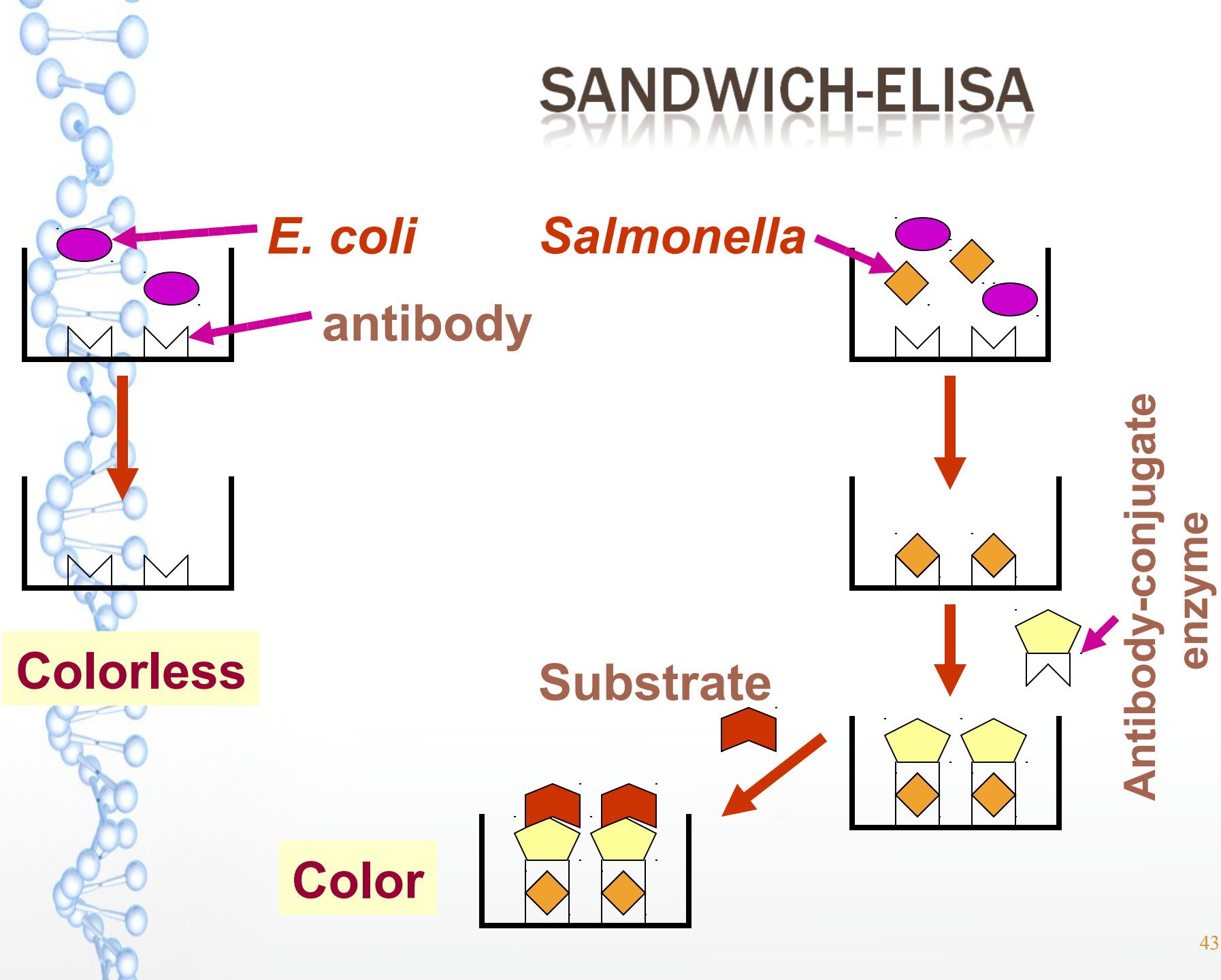


Purified pool of antigen-specific polyclonal antibodies

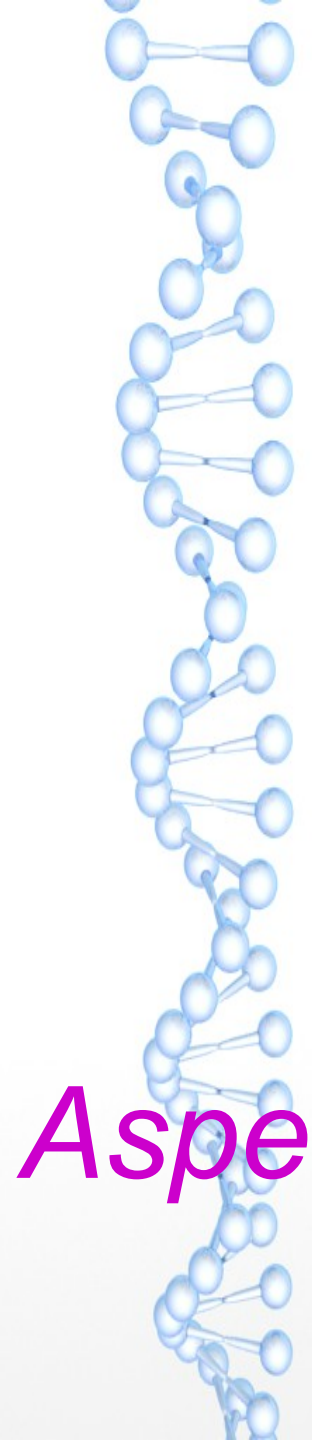
“ELISA”


Solid
Add antigen
Antibody
Add antibody-enzyme conjugate
Enzyme
Add substrate
Substrate
(colorless)
Product

SANDWICH-ELISA



Aflatoxin



A. flavus
A. nomius
A. tamarri
A. parasiticus

flavus

"Aflatoxin"

Aspergillus

toxin

Aspergillus flavus conidiophore

Walnut infected with Aspergillus flavus

Atoxigenic A. flavus biocontrol strain growing on kernels of wheat

http://msa.ars.usda.gov/la/srrc/aflatoxin/afcrsp.htm

ENZYME-LINKED IMMUNOSORBENT ASSAY

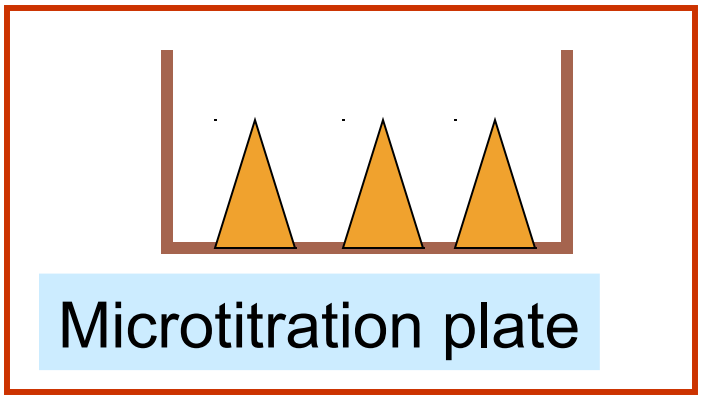
Free toxin

Antibody

Antigen(toxin)

Enzyme

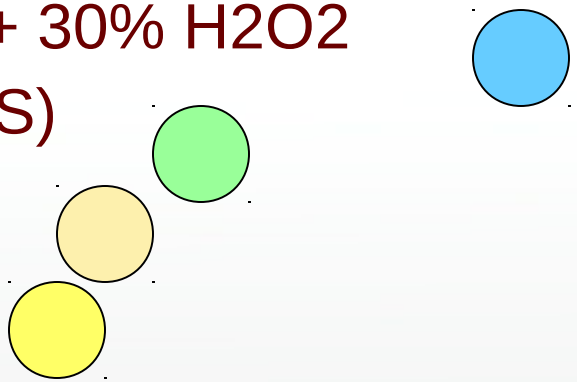
Labeled toxin

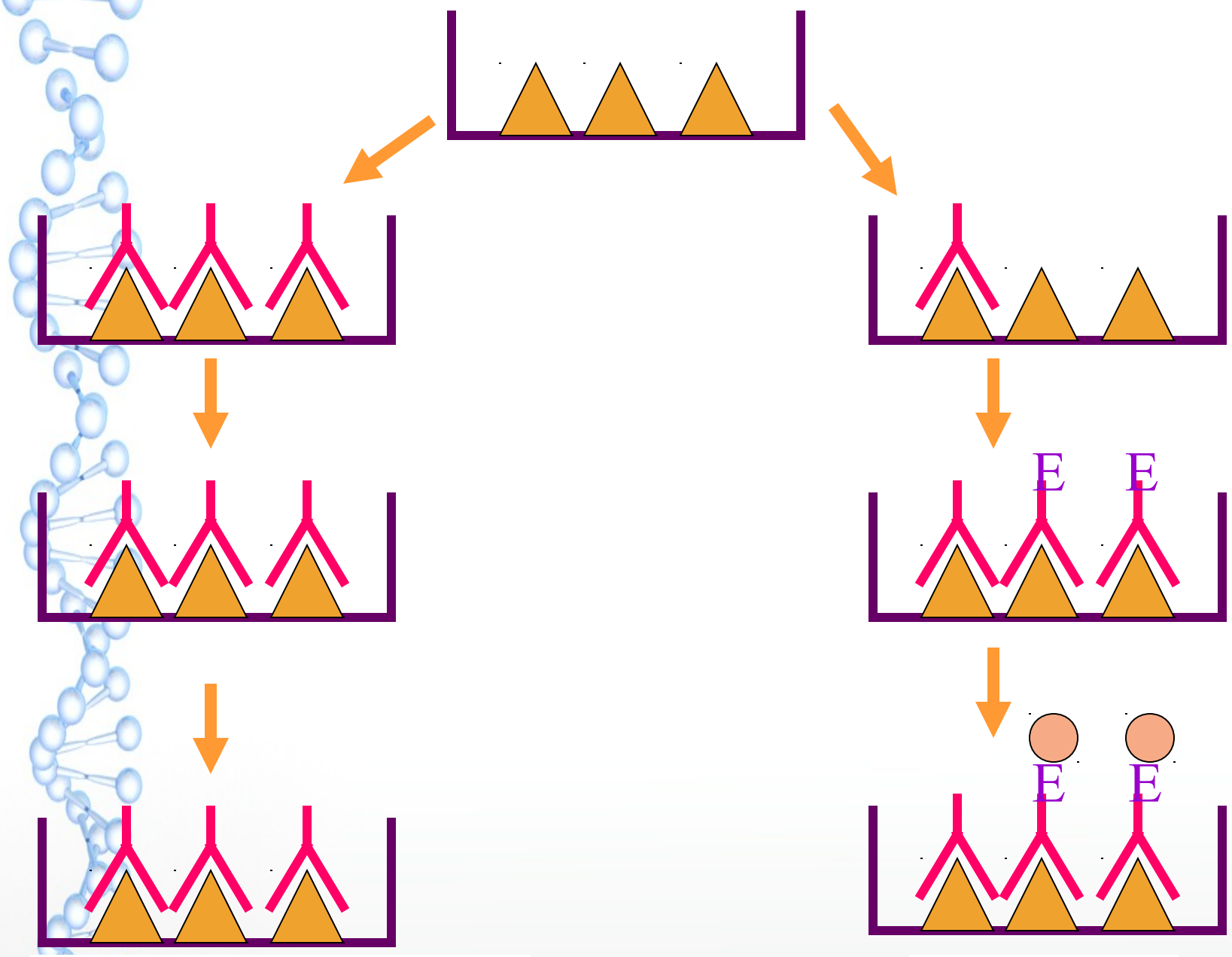


- Alkaline phosphatase (ALP)
- Horse Radish Peroxidase (HRP)

Substrate

- Tetramethylbenzidine (TMB) + 30% H2O2
- Azinobis sulphonic acid (ABTS)
- o-phenylinediamine (OPD)
- p-nitrophenyl phosphate

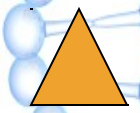




Aflatoxin: Colorless

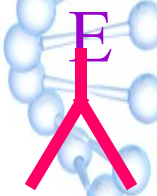
Coloration

“ELISA”



Antibody

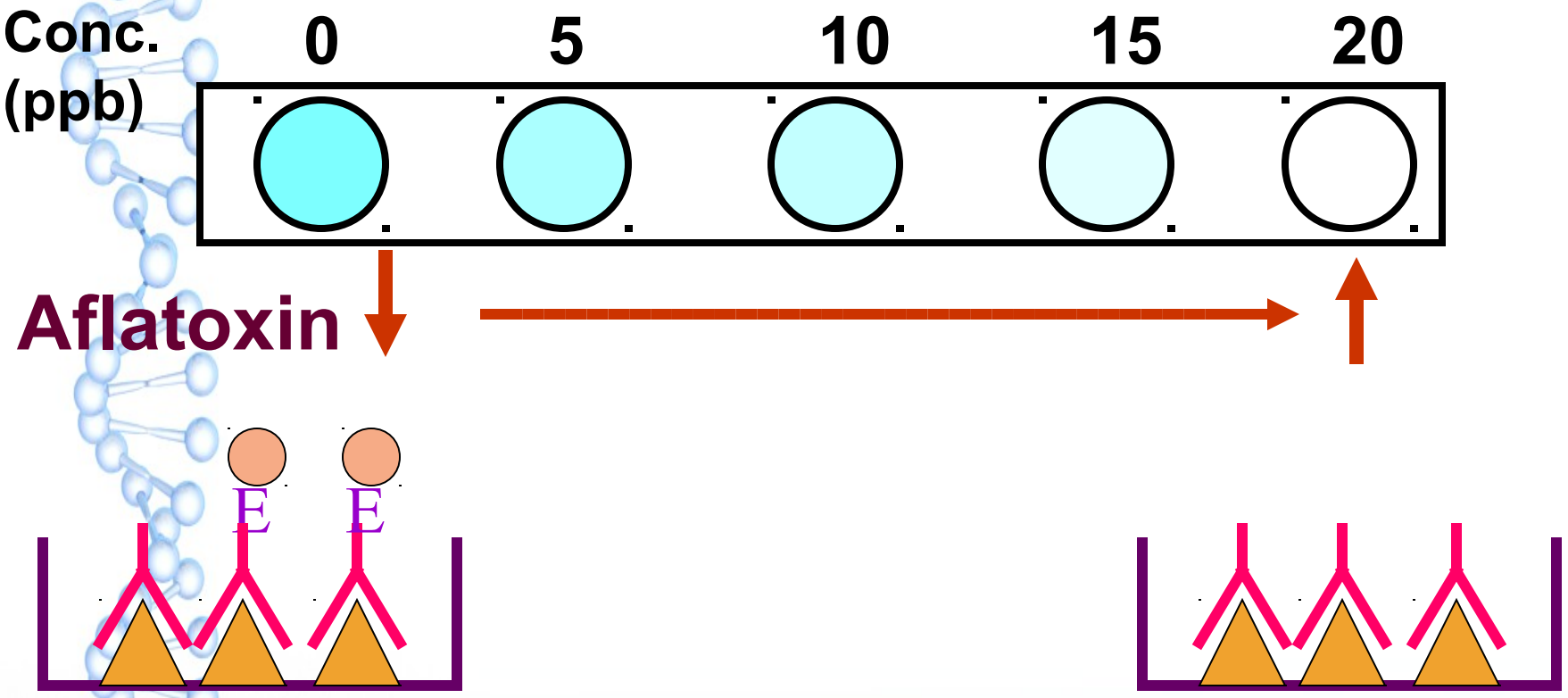
Aflatoxin (free toxin)



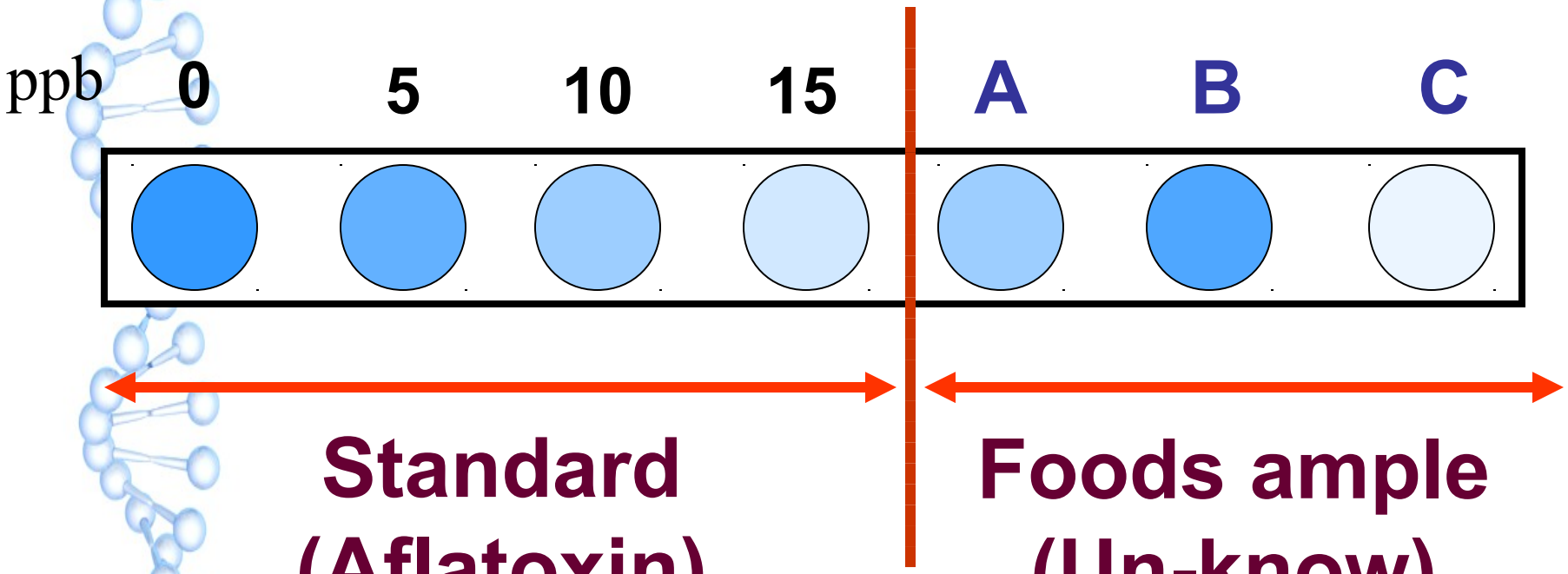
Aflatoxin-enzyme labeled (labeled toxin)

Substrate

Direct Competitive ELISA



Direct Competitive ELISA



A= ?????? ppb

B= ??????

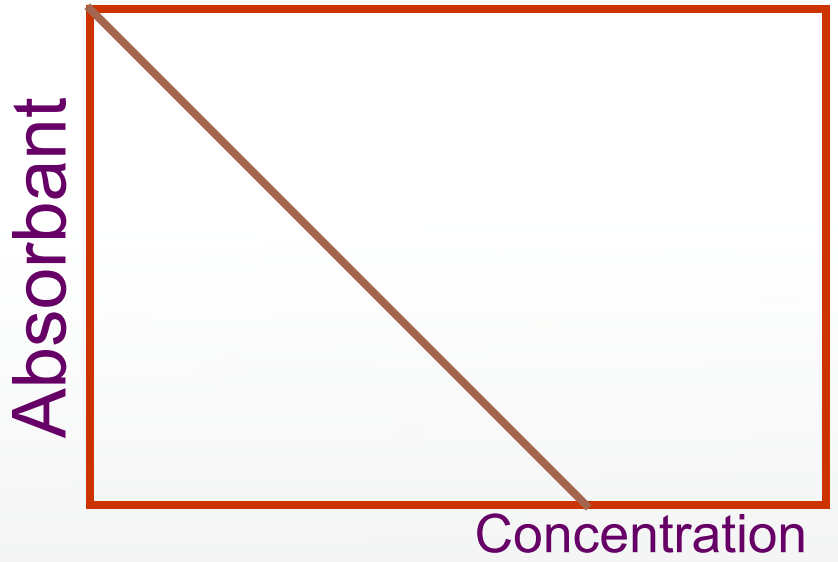
C= ?????? ppb

ELISA

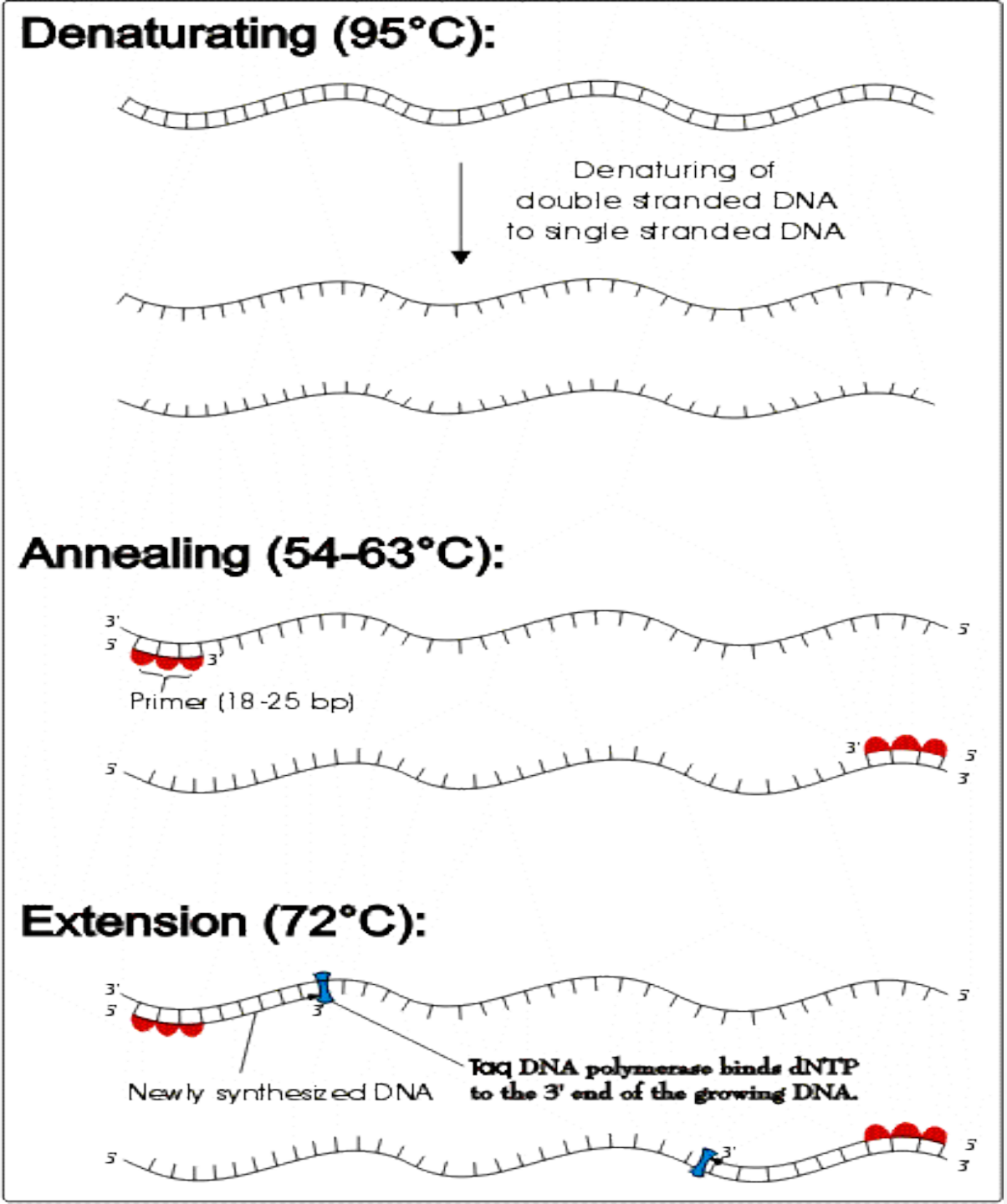
Qualitative result → color

Quantitative result

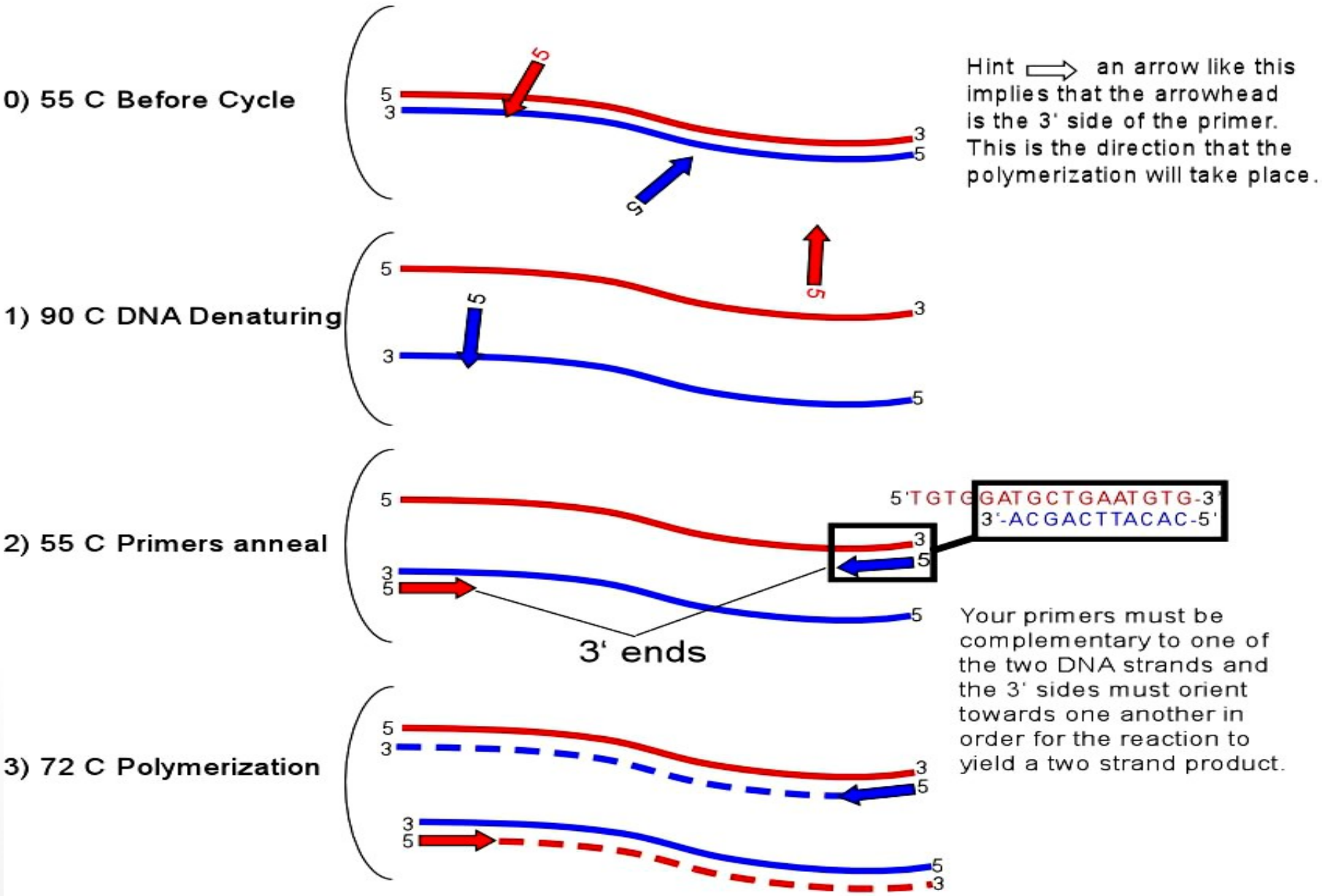
- Micro ELISA reader
- Spectrophotometer
- Standard curve

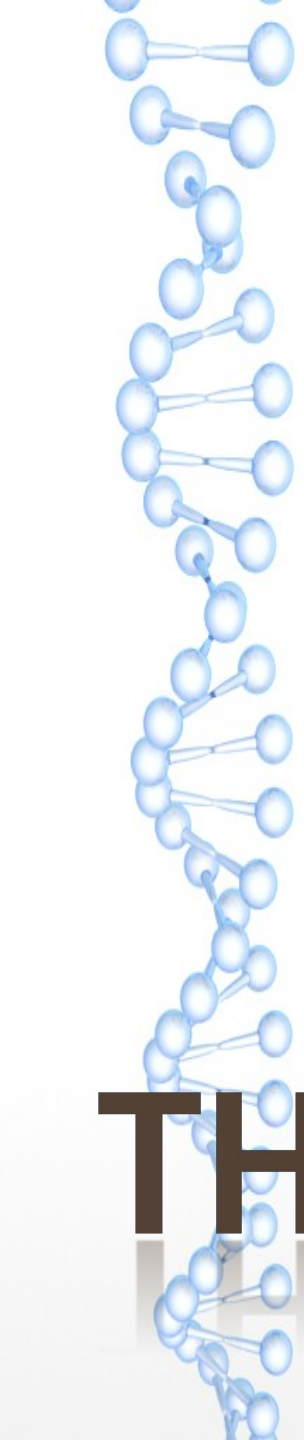


PCR


Denaturating (95°C):
Denaturing of
double stranded DNA
to single stranded DNA
Annealing (54-63°C):
3'
5'
3'
5'
Primer (18-25 bp)
5'
3'
5'
3'
Extension (72°C):
3'
5'
3'
5'
Newly synthesized DNA
Taq DNA polymerase binds dNTP
to the 3' end of the growing DNA.
5'
3'
5'
3'

Inside the PCR reaction tube…





THE END