Traditional and rapid methods for microbiological analysis

FOOD QUALITY AND SAFETY - Prof. Angel Angelov, Assoc. Prof. Velitchka Gotcheva

angelov@uft-bio.com, gotcheva_v@uft-bio.com

Microbiological methods

Qualitative methods

- Methods of analysis whose response is either **the presence or absence** of the analyte (microorganism or associated byproducts) detected either directly or indirectly in a certain amount of sample

- Based on the isolation, identification, characterization of presumed pathogenic microorganisms

Microbiological methods

Quantitative method

- Method of analysis whose response is **the amount** of the analyte measured either directly (e.g. enumeration in a mass or volume) or indirectly (e.g. color, absorbance, impedance, etc.) in a certain amount of sample

- Based on the enumeration of microorganisms on agar or in liquid medium (most probable number)

Microbiological methods

- A lot of parameters, including sampling, transport and preparation of the sample, pre-enrichment, enrichment and measurement uncertainty
- Analyses depend mostly on their purpose: quality control, production lines, end products, environment, cleaning (HACCP or quality assurance)
- Different methods may be used, the choice varying with the objective

Microbiological methods

- Total viable count
- Presence or absence of certain organism
- Levels (quantity) of indicators
- Presence or absence of specific pathogens
- Levels of specific pathogens

Microbiological methods

- Reference methods
- Routine methods
- Alternative methods
- Internal methods
- Sectorial methods

Microbiological methods

Mission of public and private laboratories

- Official controls - reference methods
- Autocontrols - reference or alternative methods
- Expertises - reference methods
- Analyses performed in case of outbreaks - internal methods and, simultaneously, reference or validated methods for confirmation

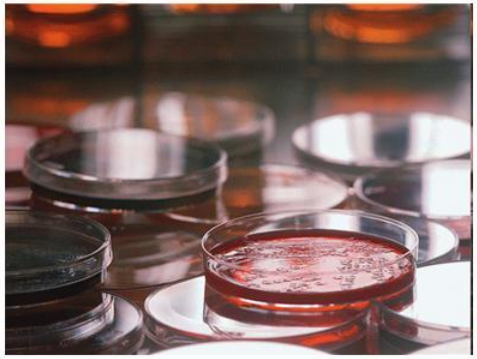
Microbiological methods

- During a long period, methods based exclusively on the use of culture media
- Influence of various factors on the precision of the analysis (sample transport, sample preparation, enrichment, laboratory equipment, supplies, subjective factors)
- Time-, labour- consuming, do not satisfy the requirements for safety in the food chain

Evolution of methods – development of alternative methods

Traditional methods

- Preparation of media, dilution buffers, sterilization of glassware
- Sample homogenisation
- Serial dilutions or enrichment in selective medium
- Plating
- Incubation
- 48 h ÷ week
- Result interpretation?



Conventional counting methods

- Direct count by microscope
- Stain and count under microscope
- Counts **BOTH** live and dead cells

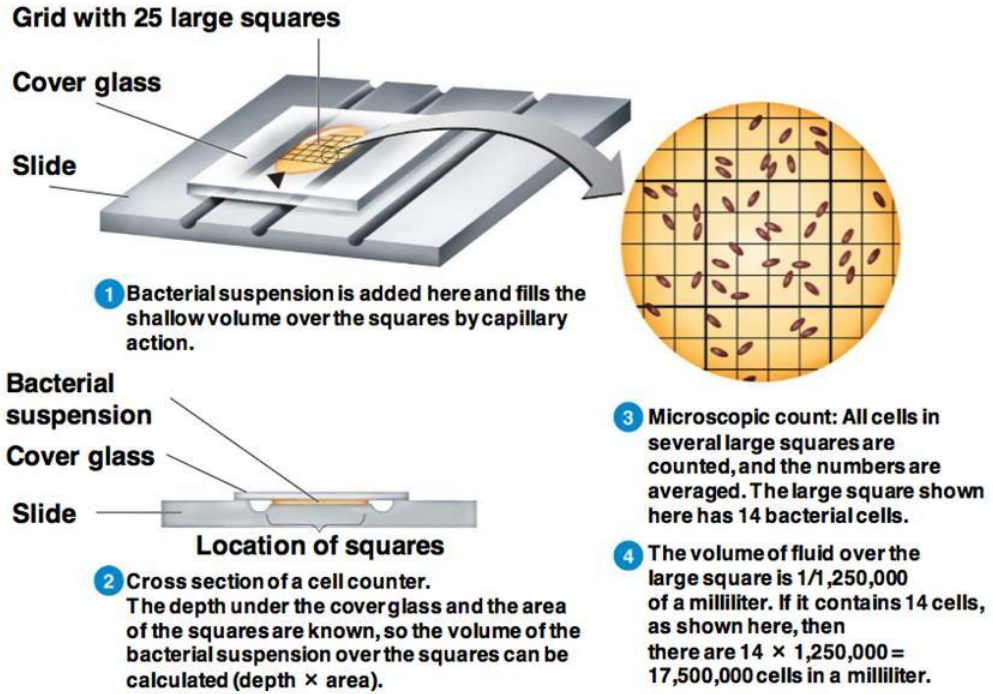
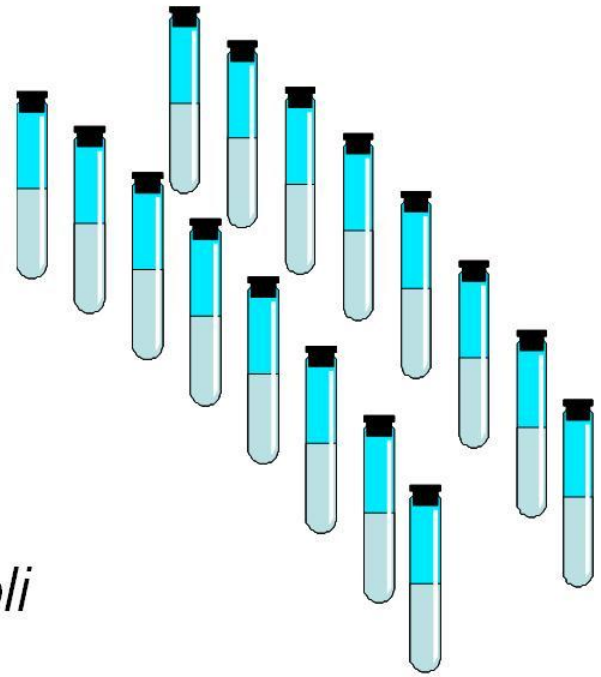


Plate count methods

- Collect sample/ Preparation
- Serial dilutions
- Inoculation in/on specific or nonspecific medium
- Incubation (temperature/time/atmosphere)
- Count colonies
- Calculate results as cfu/g

Most probable number method

- Liquid enumeration
- Serial dilution tests. Replicates (usually 3)
- Bacterial growth in tubes
- Reading of positive/negative tubes
- Final results interpretation according to a statistical table
- Mostly applied for estimation of total coliforms, fecal coliforms, aerogenic *E. coli* in water



Most probable number method

1 ml

1 ml

1 ml

10^{-1}

10^{-2}

10^{-3}

10^{-4}

Incubation

3 +

2 +

2 +

Counting

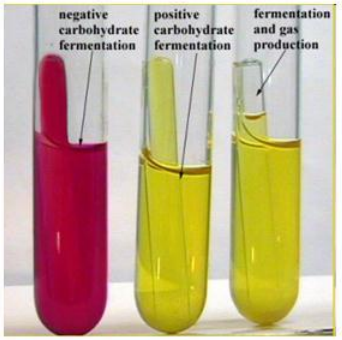
3/2/2

MNP statistical table

$2.1*10^4$ cfu/g

Traditional MO identification

- Morphology
- Gram reaction
- Biochemical tests
- Pigment production
- Nutritional requirements
- Temperature and pH
- Fermentation products
- Antibiotic sensitivity
- Gas requirements
- Pathogenicity
- Serology
- Bergey's Manual: takes time and experience



Rapid methods

Generally, all methods faster than traditional methods

- Reduced time for preparation steps (ready-to-use media, consumables, reduced sample preparation time)
- Sample concentration instead of enrichment
- Shorter time to final results

Criteria for method choice

- Quantitative or qualitative method
- Live / dead cells
- Time to obtain a result
- Type of result - "OK" or value
- Accuracy - false positive or negative results
- Variation in results (confidence!)
- Repeatability of results
- Applicability to reduce risk and protect consumers health

Criteria for method choice

- Need for training
- Safety of performance
- Data availability
- Capacity
- Maintenance of analytical system
- Price
- Validation with standard methods

Improvements - alternative methods

- A large number of analytes - physical structure of MO, metabolites, proteins, DNA, RNA
- A greater number of results for a given time
- Higher specificity, accuracy, reliability, repeatability
- Saving time, labor, human and financial resources
- Rapid response for manufacturing control and verification
- Molecular methods – application in traceability
- Interpretation of results incl. uncertainty of measurement

Official acceptance of alternative methods

- Necessity for validation of alternative methods (around 1990)
- EC regulation 2073/2005 concerning microbiological criteria:

"Tests results are dependent on the analytical method used and, therefore, a given reference method should be associated with a microbiological criterion. However, food business operators should have the possibility to use analytical methods other than the reference methods, in particular more rapid methods as long as the use of these alternative methods provide equivalent results."

Official acceptance of alternative methods

- Alternative methods can be used for official controls if they are validated against the standard methods
- ISO 16140: 2003 Microbiology of food and feed: Protocol Validation of Alternative Methods

Method validation

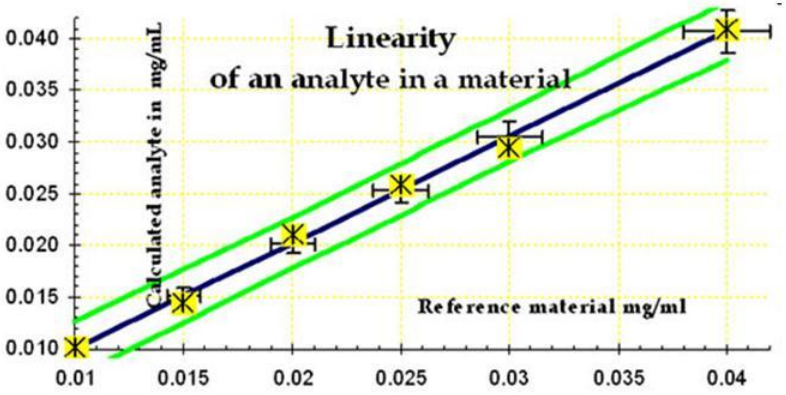
- Confirmation that the analytical procedure employed for a specific test is suitable for its intended use
- Results are used to judge the quality, reliability and consistency of analytical results

Validation of method performance

- The laboratory must be able to match the performance data as described in the standard

Validation of alternative methods

- Demonstration that adequate confidence is provided when the results obtained by the alternative method are comparable to those obtained using the reference method using the statistical criteria contained in the approved validation protocol (Feldsine et al., 2002)



Microbiological analysis - problems

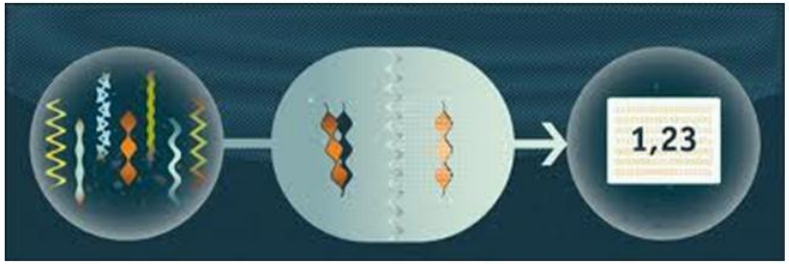
- Difficult, if not impossible, to analyse a number of samples representative of the populations
- The choice of a method, even for a given objective, will always remain a difficult task
- Consideration of possibility to find higher number of positive results by an alternative method compared to the use of a standard method


Patient eats contaminated food
3 - 4 days
Patient becomes ill
Stool sample collected
Time to treatment = 1 - 5 days
Time to diagnosis = 1 - 3 days
E. coli O157 identified
Public health lab receives sample
Shipping time = 0 - 7 days
"DNA fingerprinting" = 1 - 4 days
Case confirmed

Rapid methods

Any method providing faster result

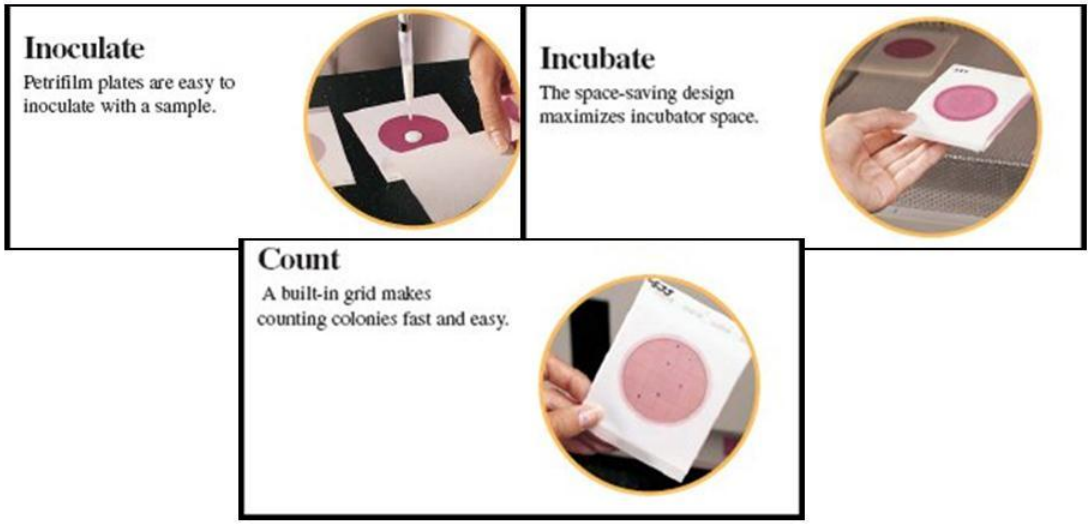
- Ready prepared culture media
- New chromogenic media
- Techniques for membrane filtration
- Biochemical methods
- Immunological methods
- Methods based on impedance
- Methods based on DNA or RNA
- Biosensors
- Nanotechnology
- Automation of laboratory techniques



Rapid methods – ready prepared media

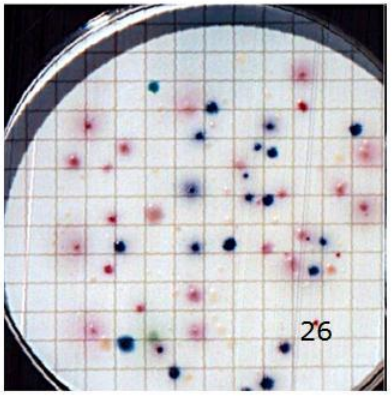
Petrifilm 3M

- Very easy, convenient
- No agar preparation necessary



Redigel

- Agar is liquid until it contacts plate.
- Inoculate liquid agar with sample
- Pour liquid agar onto plate
- The pectin in the liquid agar will solidify due to calcium crosslinkages.
- Can not interchange regular plates!



Rapid methods – ready prepared media

Summary of Dry-Bags Procedure

1. 

Remove Dry-Bag from the outer packaging and place on flat surface.

2. 

Insert sterile Dry-Bag filter (if applicable).

3. 

Connect pump tubing to the filter on Dry-Bag and secure using Tube-Ties.

4. 

To begin filling the Dry-Bag, open the clip and use peristaltic pump to pump water.

5. 

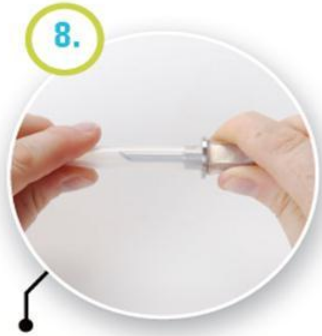
While filling, agitate the Dry-Bag until the powder is dissolved.

6. 

Once the required volume of water is added, turn off water supply and close the clip.

7. 

To dispense medium from Dry-Bag, use a peristaltic pump or diluter.

8. 

Connect peristaltic pump or diluter using Bag Connector, then open clip to allow media to flow.

9. 

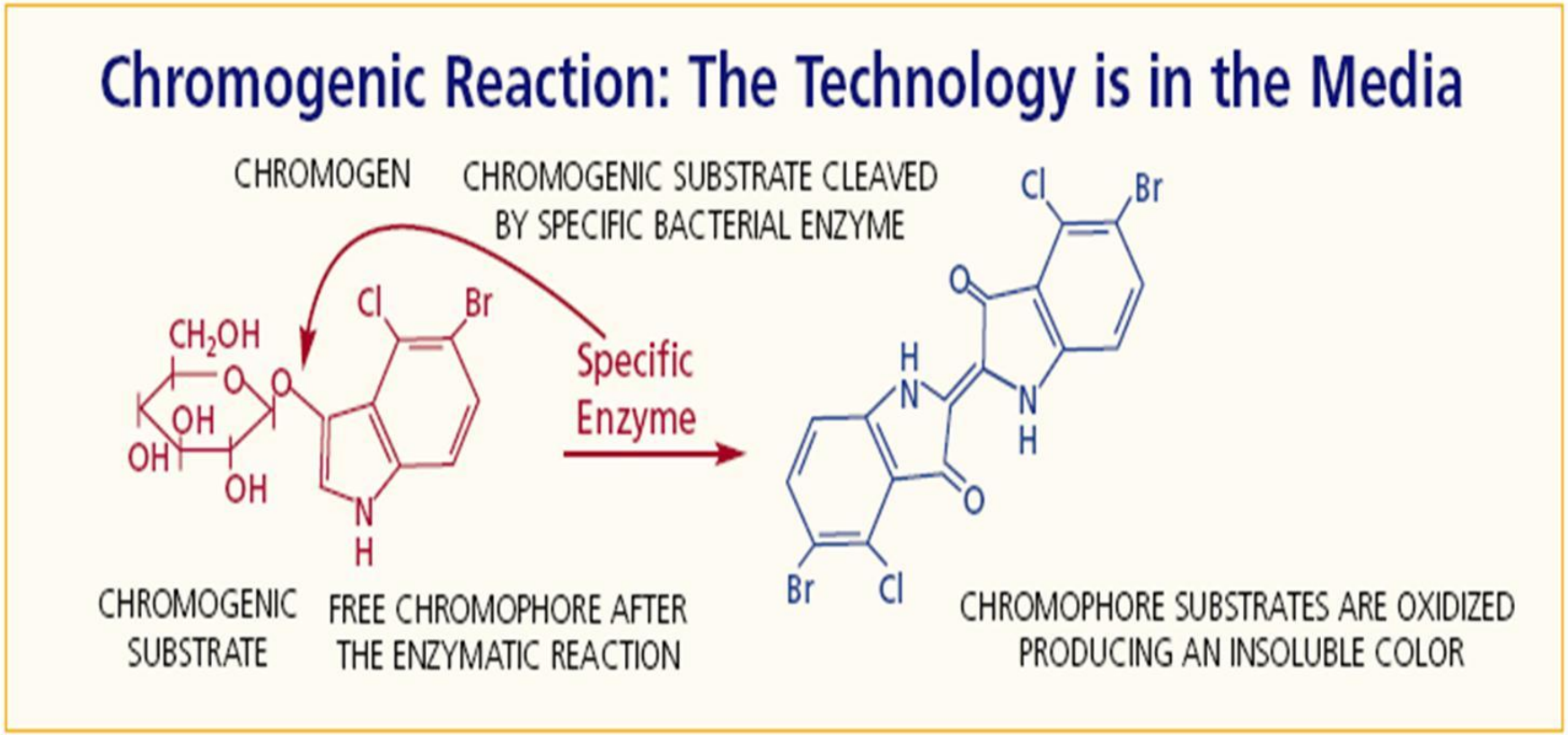
Dispose of empty Dry-Bag with normal laboratory waste.

Rapid methods – new chromogenic media

- Chromogenic material is modified by the microorganisms or their metabolites - change in color or fluorescence

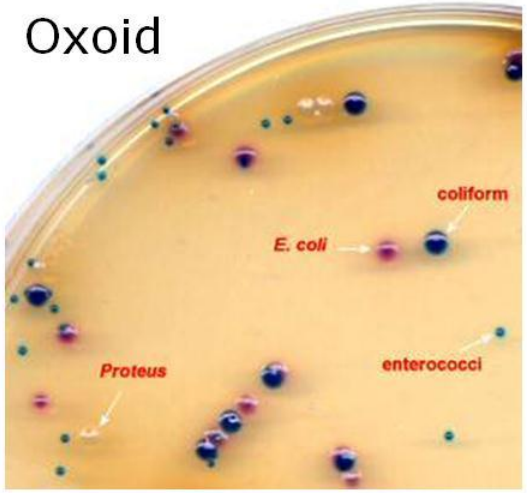
- Easy establishment of colonies target metabolic activity
- Avoiding the need for isolation of pure cultures and confirmatory tests
- A number of ISO methods using chromogenic media

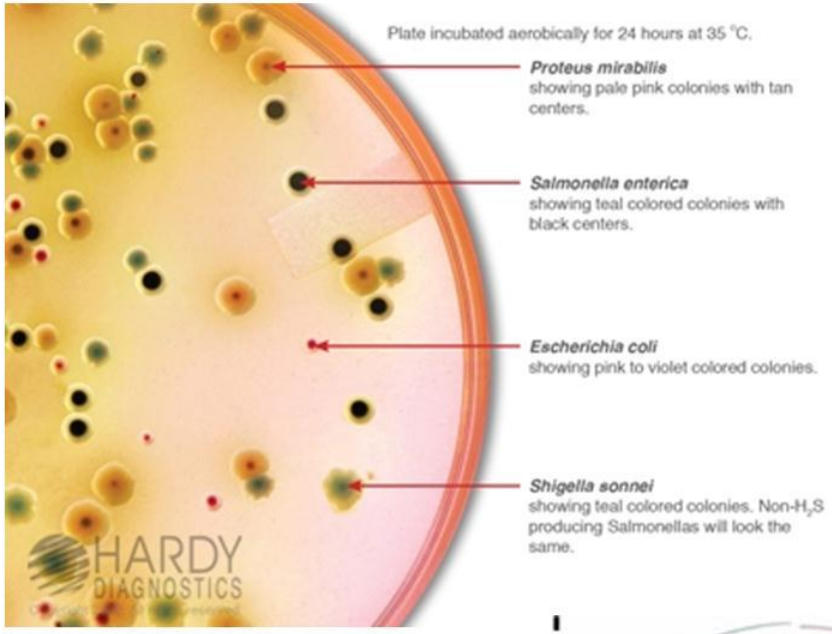
Rapid methods – new chromogenic media

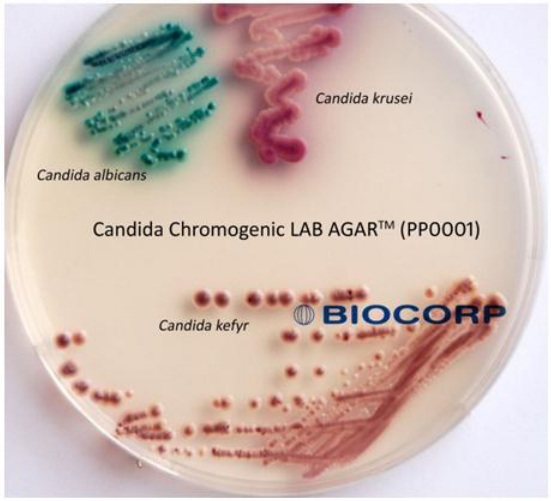


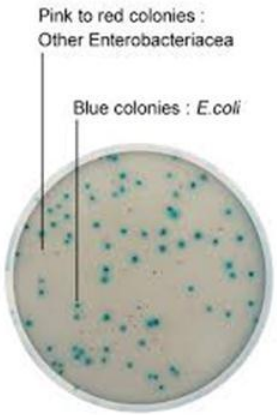
Rapid methods – new chromogenic media

Oxoid









Biochemical methods

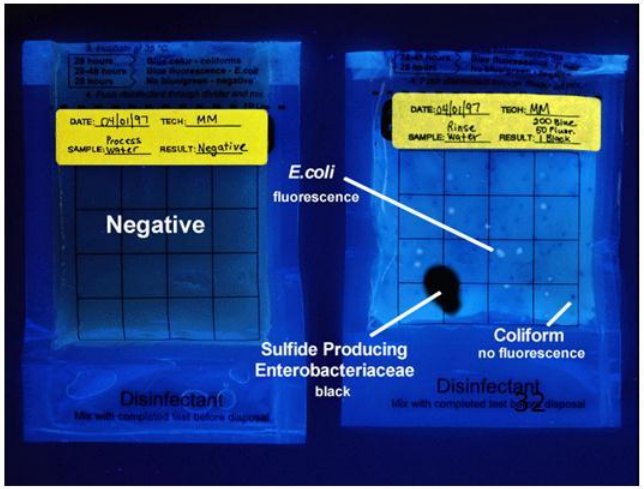
PathoGel (Tylermark)

- Selectively grows, detects and quantitates coliform, *E.coli* and H_2S-producing *Enterobacteriaceae* in food and water
- Single dose microbiological detection system
- No preparation or sample manipulation, incubation at 35°C or 44°C for 28-48 h
- Gram-selective medium with gelling agent solidifies and immobilizes motile bacteria
- Individual CFU are counted and results are comparable to a membrane filter result

Biochemical methods

PathoGel (Tylermark)

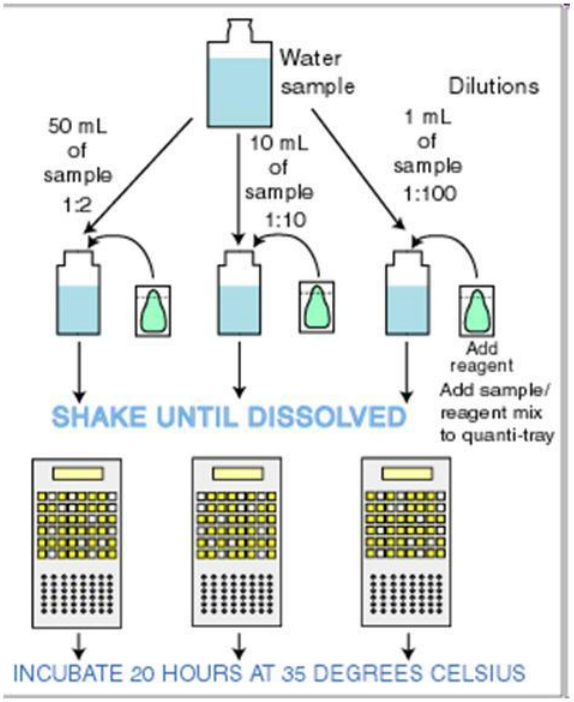
- Coliforms produce galactosidase, metabolize a color indicator forming a blue CFU
- *E.coli* produces galactosidase and glucuronidase, metabolize indicators to form blue/fluorescent CFU
- *Enterobacteriaceae* (sulfide producing), *Salmonella* (sub-group) form black CFU precipitate
- Sensitivity
  - <1 per 100ml for waters
  - <1 per 5ml for milks
  - <1 per 3ml for juices
  - <1 per gram for solid foods

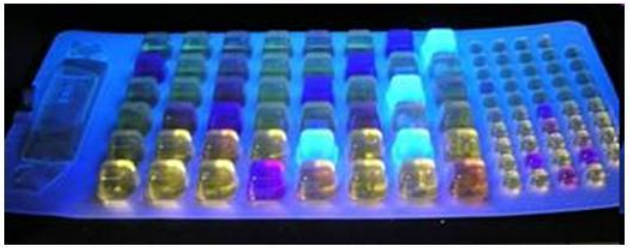


Rapid detection methods

Colilert (IDEXX)

- Detects coliforms and *E. coli* in water in 24 h
- Coliforms cleave ONPG (O-Nitrophenyl-B-d-Galactopyranoside), freeing nutrient from indicator which is then yellow
- *E. coli* metabolizes MUG (4-Methyl umbelliferyl-B-d-Glucuronide) using glucuronidase freeing the indicator portion which fluoresces





Rapid detection methods

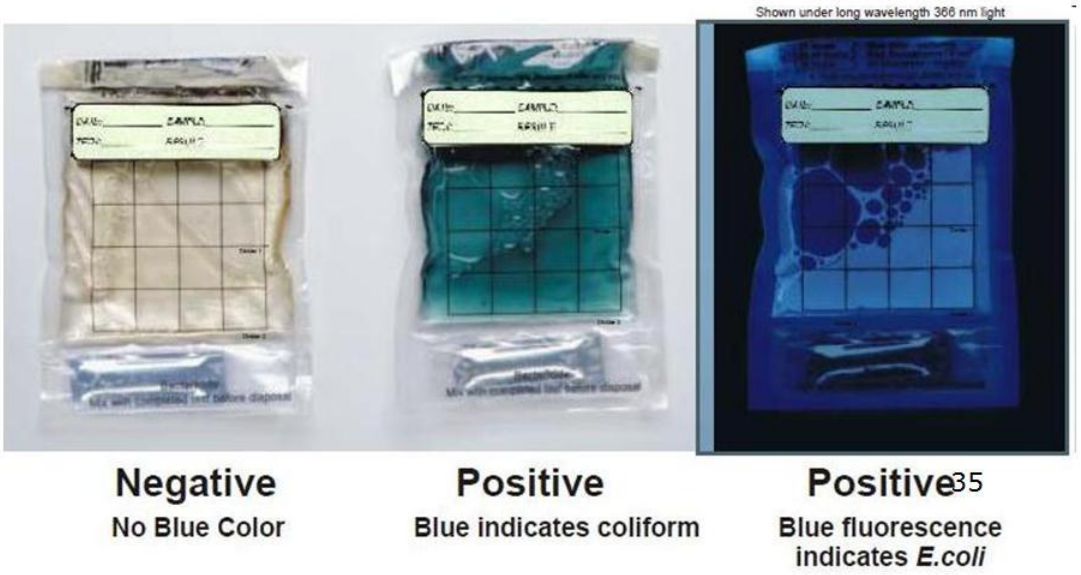
Colilert (IDEXX)

- Advantages of Colilert
  - Time, labor, interpretation, interference
  - Simultaneous detection, identification, and confirmation of *E. coli* and coliforms
  - Clear color change
- U.S. EPA-approved

Biochemical methods

E*Colite (Tylemark)

- Presence/ absence of coliforms and *E.coli* in potable water and other liquids
- Detects a single chlorine-stressed MO in 100ml of water
- US EPA approved



Biochemical kits for MO identification

- Specific biochemical profile for each MO species
- **API (BioMerieux)** – yeasts, molds, bacteria
- **MicroID® (Remel)** – *Enterobacteriaceae, Listeria monocytogenes*
- **RapID™ systems (Remel)** – over 400 clinically relevant MO
- **BBL Crystal™** Rapid Gram-Positive Identification System

API 20E


MicroID®

RapID™

Automated systems for MO identification

- **Vitek (bioMerieux, Inc.)** – cards with 30 wells, 4-12 h incubation
- **MicroFoss и BioSys (BioSys, Inc.)** – separate tubes, 1-512 samples/load
- **Biolog (bioMerieux)** – 1400 species of yeasts, molds, bacteria
- **MicroID® (Remel)** – *Enterobacteriaceae, L. monocytogenes*
- **RapID™ systems (Remel)** – over 400 clinically relevant MO
- **BBL Crystal™** Rapid Gram-Positive Identification System

Vitek

MicroFoss

Biolog

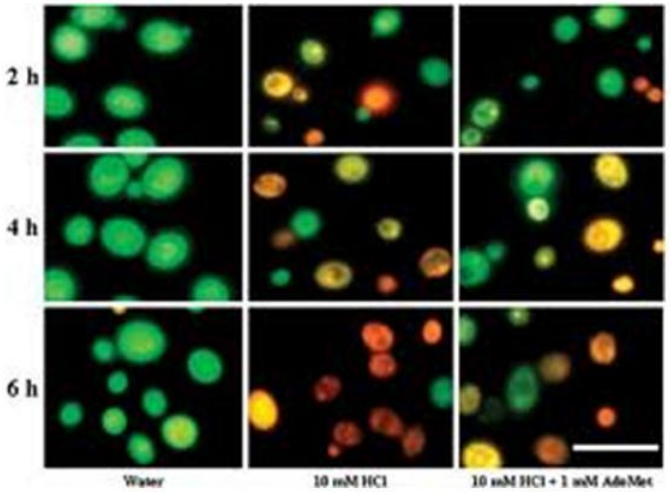
Rapid quantification methods

Direct epifluorescent filtration, filtration through hydrophobic membranes

- Concentration of target MO from a large volume
- Removal of growth inhibitors
- Transfer cells to culture media without physical damaging
- Membranes - nitrocellulose, cellulose acetate esters, nylon, polyvinyl chloride and polyester (thickness of about 10 µm)
- Possibility of direct microscopy

Rapid quantification methods

- **DEFT method** - the concentrated cells on the membrane are stained with acridine orange - red fluorescence upon binding to RNA, and green when bound to a DNA, i.e., living cells fluoresce in green and dead – orange
- Widespread use as a rapid and sensitive method for the determination of live cells in milk and milk products



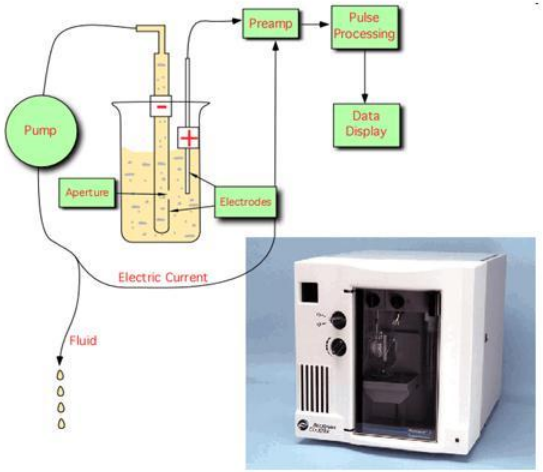
Rapid quantification methods

Hydrophobic membrane filtration (Isogrid)

- Use vacuum source to pull liquid through isogrid filter
- Transfer filter onto solid medium
- Every colony is in a square cell
- Multiply by factor to get MPN
- More than 1 organisms could fall into 1 square!

Automated systems for quantification

- Flow cytometry - electronic particle counter
- Passing the liquid sample through a narrow nozzle
- The particles in the sample is measured and the number
- The signal is dependent on particle size
- Counting live and dead cells



Automated MO detection and quantification

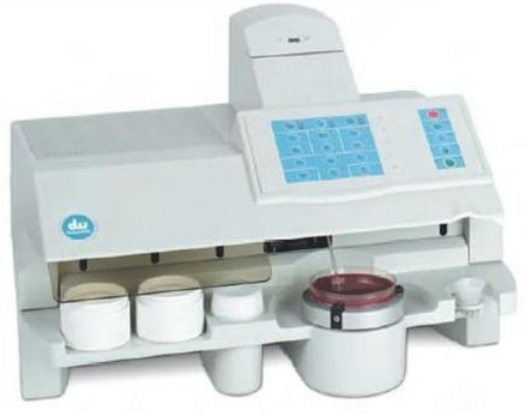
BactoScan FC

- Fully automated counting - flow cytometry
- Individual bacteria count
- 50, 100, 150 samples per hour
- Results in less than 10 minutes - immediate feedback
- Cold/warm samples
- Low reagent cost/samples

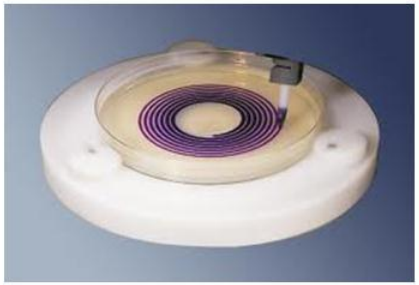
Automated systems for quantification

Spiral Platers

- Cover 3 dilutions
- Use pre-poured agar
- Dispenses known amount of liquid in decreasing volume from center of plate
- Colonies can be hand counted or laser counted
- Laser counter can count, calculate area and convert to cfu/ml
- Autoplater 4000 (Spiral Biotech)
- WASP (MicroBiology Intl.)



WASP (MicroBiology Intl.)



Automated MO detection and quantification

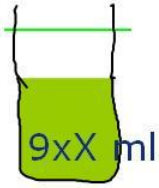
BacT/Alert - Organon Teknika

- Colorimetric detection of carbon dioxide production in liquid (120 or 240 bottles)
- Computer algorithms
- Food is in nutrient bottles with sensor at bottom
- When levels of gas are produced, color changes from dark green to yellow
- Change detected by reflectance colorimetry
- *E. coli* detected in 6 - 8 h
- Considered a 'biosensor'

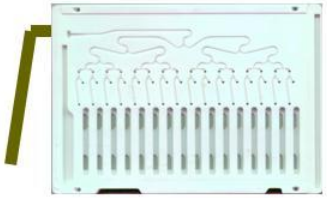
Automated MO detection and quantification

TEMPO® system - based on MPN method

X g product



10^{-1}

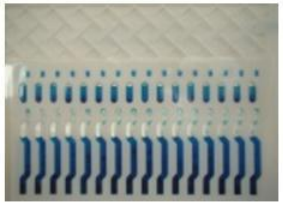


2.5 µl

22.5 µl

225 µl

1/400 TVC, LAB,
1/40 EB, TC, CC, EC, STA,YM



Incubation

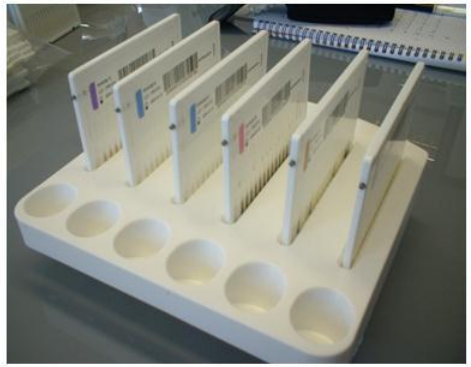
Interpretation 2.1 10^4 CFU/ g

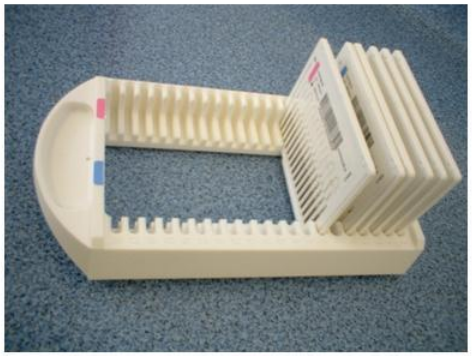
TEMPO® system

- Connect each vial to a card

- Insert in Tempo Filler
- Remove vial

- Incubate





TEMPO® system

Preparation station

Reading station

WiFi connection

TEMPO® system

- TVC Total Viable Count
- EB *Enterobacteriaceae*
- TC Total Coliform ISO 30°C
- CC Coliform Count BAM 35°C
- EC *Escherichia coli*
- LAB Lactic acid bacteria
- STA *Staphylococcus* coagulase +
- YM Yeast and molds

Covering 90% of all QI performed worldwide

Microbiological monitoring of air

- Passive monitoring – petri plates
- Active monitoring – microbiological air sampler
  - Impingers (liquid media)
  - Impactors (solid media)

BioScience International

- < 100 / m^3 = low
- 100 - 300 / m^3 = intermediate
- > 300 / m^3 =high

AES Chemunex

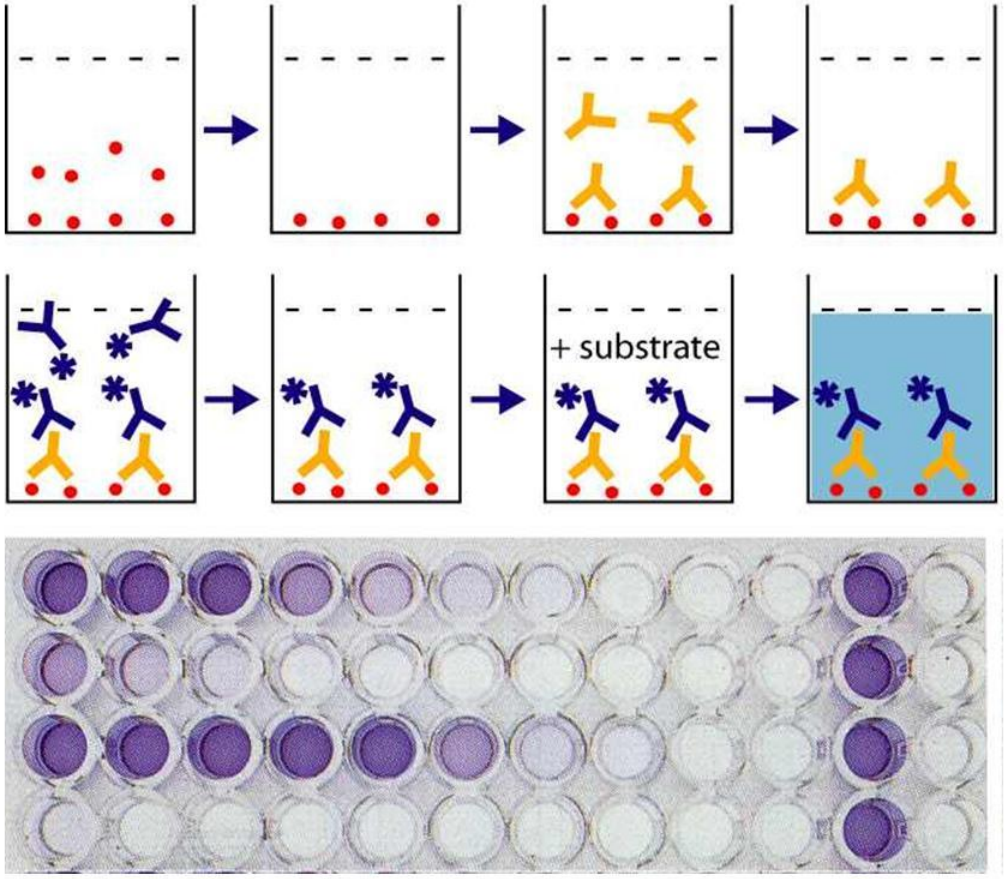
Immunological methods

ELISA (enzyme-linked immunosorbent analysis)

- Microtiter wells coated with specific monoclonal antibodies in which the target MO (antigen) is captured
- Identification by using a second antibody coupled to an enzyme
- Add substrate - visualization of target antigen
- High specificity and possibility for automation
- Lowest concentrations detected - 10^6 CFU / ml ⇨pre-enrichment

Immunological methods

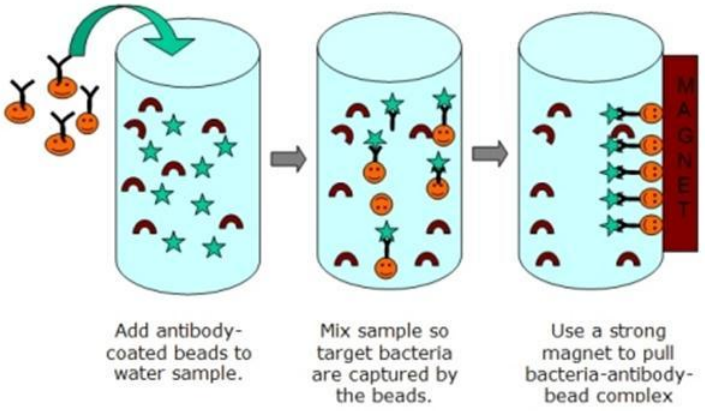
ELISA (enzyme-linked immunosorbent analysis)



Immunological methods

Immunomagnetic separation (IMS)

- Selective capture of target MO in mixed microbial population by the specific antigen-antibody contact ⇨ no need of enrichment
- Capture of injured cells which can not be detected by standard methods
- IMS kits for key pathogens *Salmonella spp.*, *E. coli* O157: H7, *Lysteria monocytogenes*, etc.

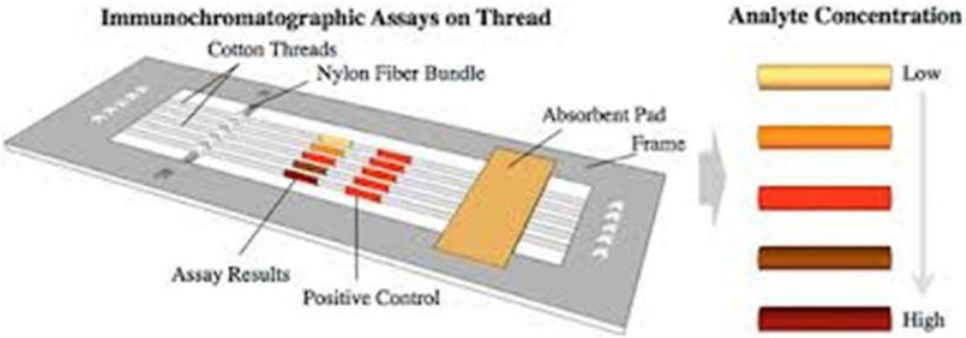


Immunological commercial kits

Lateral flow immunoassays

- Antibody- antigen tests adapted to operate along a single axis to suit the test strip format
- Sandwich tests with two antibody lines for microorganisms (2 lines - a positive result, one line - negative)
- Quick confirmation of bacterial isolates
- Tests for food pathogens (bacteria), bacterial and fungal toxins
- Competitive tests - small molecules (bacterial toxins, mycotoxins)

Immunological commercial kits



Automated ELISA systems

VIDAS (bioMerieux, Inc)

- Fluorescent immunoassay (ELFA)
- ELISA method with a final reading of fluorescent signal
- Result - positive / negative
- *Listeria*, *Salmonella*, *E. coli* O157: H7, staphylococcal enterotoxin, *Campylobacter*

Automated ELISA systems

DIA/PRO™ (Umedik, Inc.)

- Biochip containing ICEfloTM system
  - Enriched sample reacts with fluorescent antibody in DIA/PREPTM applicator
  - Drop of sample applied directly to Biochip
  - Only bacteria can flow through ICEfloTM barrier
  - Biochip into reader to detect pathogen fluorescent antibody complexes
  - After 6h of enrichment - 1 cfu/25g detection in 15 min
- Disposable, rapid, safe, inexpensive

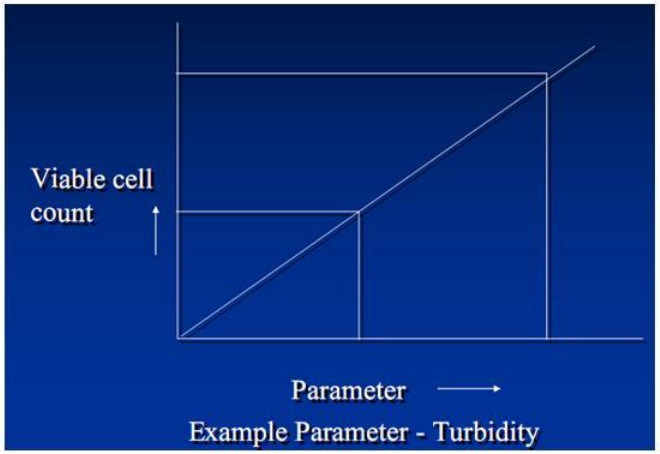
ELISA technology

EiaFoss

- Automated rapid pathogen detection system
- ELISA technology and Immunomagnetic separation
- 24 hours for *Salmonella* and *E. coli* O157
- 48 hours for *Listeria* and *Campylobacter* (incl. enrich.)
- 27 samples at a time; up to 81 samples a day
- 2-step procedure (enrich. phase + automated ELISA)
- Windows-based WinEia
- AOAC-RI Performance TestedSM, AFNOR approval, NorVal approval and EMMAS assessment

Indirect quantification

- Counting - time consuming ➢ methods to estimate the number of microorganisms indirectly
- Estimates must follow the growth curve exactly in order to use this method.
- Indirect estimation assumes that estimate follows the growth curve



Impedance-based methods

- Impedance (conductive) microbiology - impedance is the reciprocal of conductivity and capacity
- Synthesis of ionized metabolites (organic acids, ammonium ions, etc.) → change in electirical conductivity of the environment
- Population of ~5 log/ml causes change
- High populations cause shorter time in changes
- Time of change is inversely proportional to the initial microbial population
- Series of curves to generate 'scattergram' which can be used to estimate population

Impedance-based methods

Bactometer (bioMerieux, Inc.)

- Sample placed into 16-well module, incubated and monitored
- As cells reach 5-6 log, monitor shows impedance changes as growth curve
- Uses heated air as temperature control (35°C)
- Time to reach change determines acceptability
- Can set cut-off point: red, green, yellow
- AOAC approved for total count

Impedance-based methods

RABIT (Microbiology International)

- Rapid Automated Bacterial Impedance Technique
- Uses heated air as temperature control (35°C)
- Uses individual tubes with electrodes instead of 16 well module of Bactometer

Malthus System (Malthus)

- Uses conductance changes of fluid
- Generates conductance curves similar to impedance curves
- Based on mathematical formula
- Uses heated water as temperature control (35°C)
- Has AOAC approved *Salmonella* detection protocol

ATP bioluminescence

- Adenosine Triphosphate (ATP)

$$\text{ATP} + \text{luciferin} + Mg^{+2} \xrightarrow[\text{luciferase}]{} \text{oxiluciferin} + \text{ADP} + \text{light}$$

- Light units used to estimate biomass in solid and liquid foods; reported as 'Relative Light Units'.
- Some fluorimeters can measure as little as 100-1000 femtogram (fg) (10^{-15} g)
- One CFU is reported to have 0.22 - 0.47 - 1.03 fg
- Analysis time – seconds/minutes

ATP bioluminescence

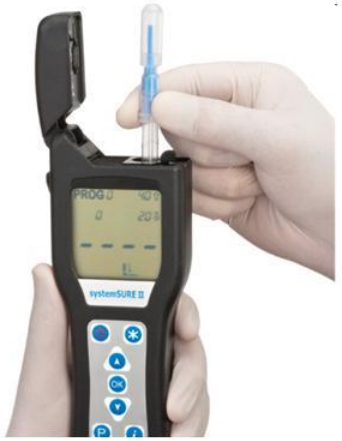
- Non-microbial ATP in environment interferes with ATP measurement
- Used to estimate population, cannot be used to actually measure population
- Swab surface: no ATP = clean
- Portable ATP meters

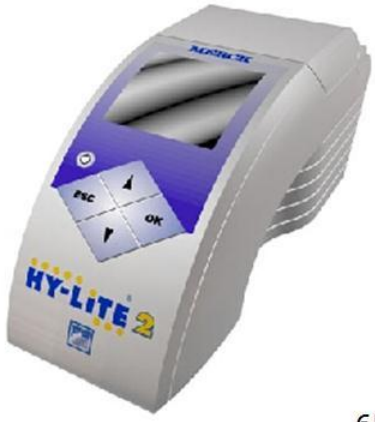
ATP bioluminescence

- Total count can not be estimated
  - Organisms contain different amounts of ATP (Yeast 100 x more ATP than bacteria)
  - Growth stages of an organism differ in ATP
  - Blood and biological fluids interfere
  - ATP is released from injured and dead cells as well
- Systems designed for hygiene monitoring
  - Acceptable, marginal, and unacceptable RLU
  - Simple, compact, computer adaptability, cost, support, documentation of usefulness

ATP instruments (luminometers)

- HY-LiTE® (Merck)
- BioTrace (Plainsboro, NJ)
- Celsis System SURE (Celsis, Cambridge, UK)
- Charm Lum -T (Charm Sciences; Malden, M)
- Lightning (BioControl; Bellevue, WA)
- Lumac (Landgraaf, the Netherlands)

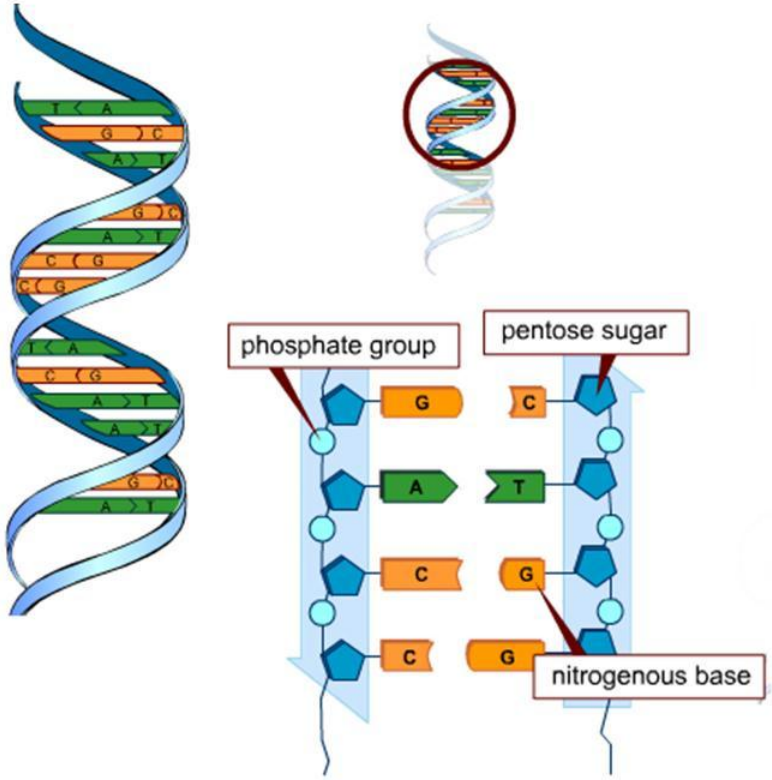




V

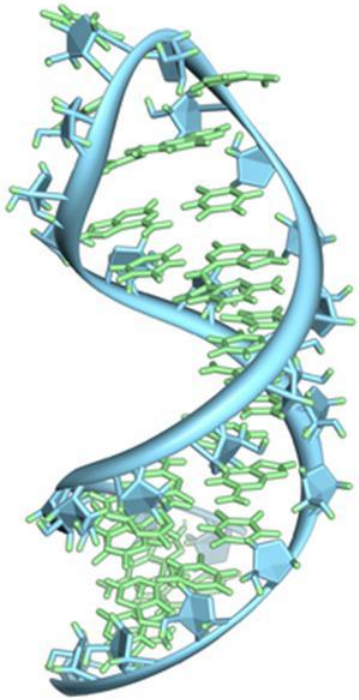
Molecular methods

- DNA carries the genetic information
- Built of nucleotides (nitrogenous base - adenine (A), thymine (T), guanine (G), cytosine (C); deoxiribose; a phosphate group)
- Nucleic DNA is double-stranded (complementary binding A-T, G-C), spiral shape



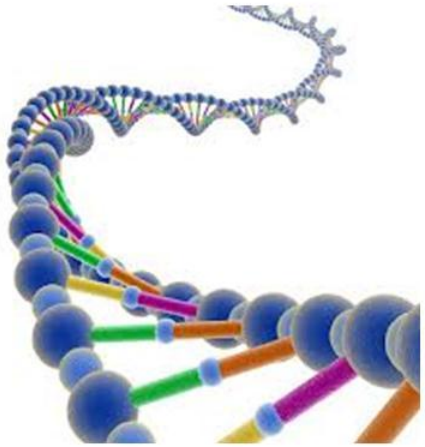
Molecular methods

- RNA - various biological roles in coding, decoding, regulation and expression of genes
- Mostly found in nature as single-stranded, folded onto itself, shorter nucleotide chain
- Contains ribose → less stable molecule
- Universal function – protein synthesis

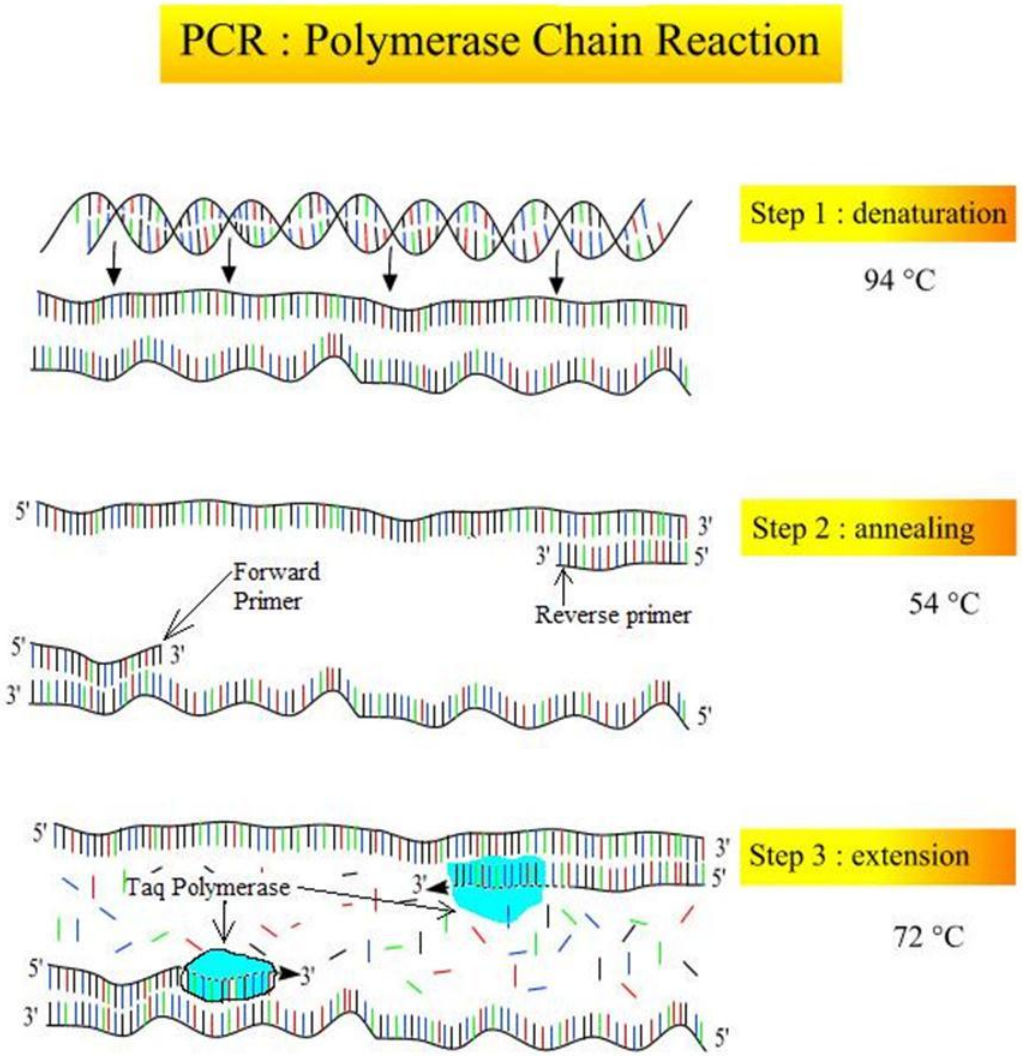


Polimerase chain reaction (PCR)

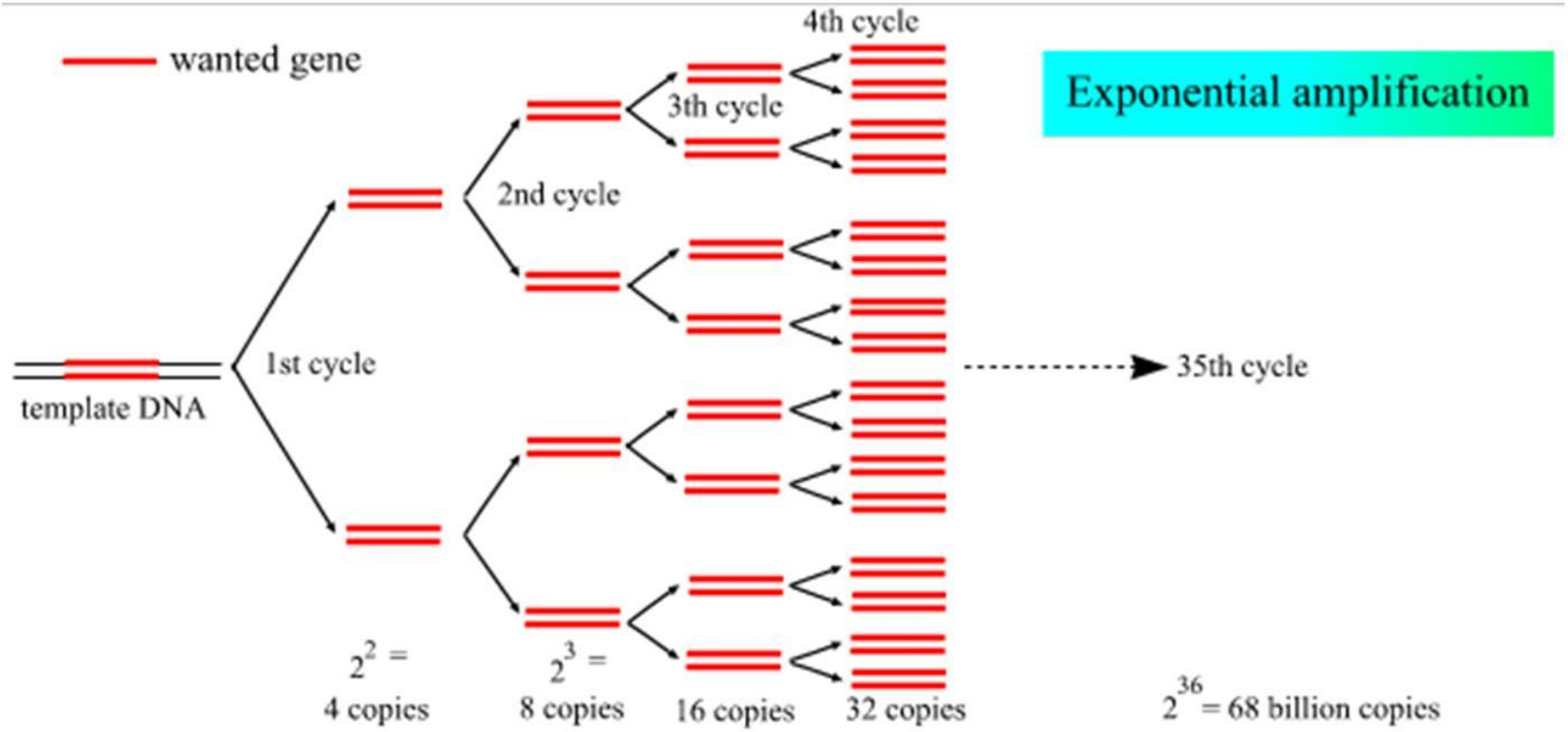
- Specific amplification of a specific DNA sequence to obtain detectable DNA concentration
- The specificity of the reaction is determined by the specificity of the primers
- RT-PCR - PCR with reverse transcription - specific amplification of a specific RNA sequence



Polimerase chain reaction (PCR)



Polimerase chain reaction (PCR)



DNA extraction

- Various in-house methods, commercial kits
- Spectrophotometric determination of DNA concentration, purity and quantity

$A_{260} / A_{280} = 1.8 – 2.0$

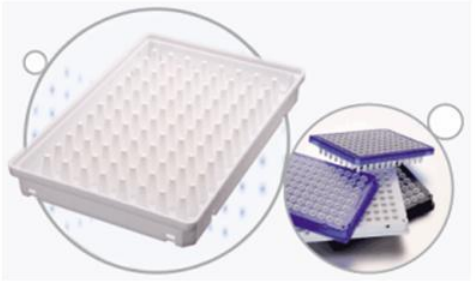
PCR reagents

- Matrix - DNA
- Straight and reverse primer - single stranded DNA fragments of 15-30 bp)
- Taq polymerase - a thermostable enzyme catalyzes the synthesis of new DNA, the activity depends on the presence of Mg^{2+}
- Buffer - optimal conditions for enzyme activity
- $MgCl_2$
- Nucleotide bases - to build a new DNA strands
- Water

PCR process

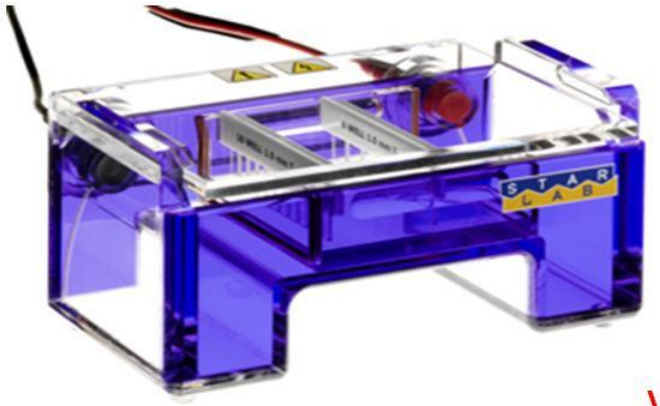
PCR machine

- 96-well plate
- 0.2ml PCR tubes



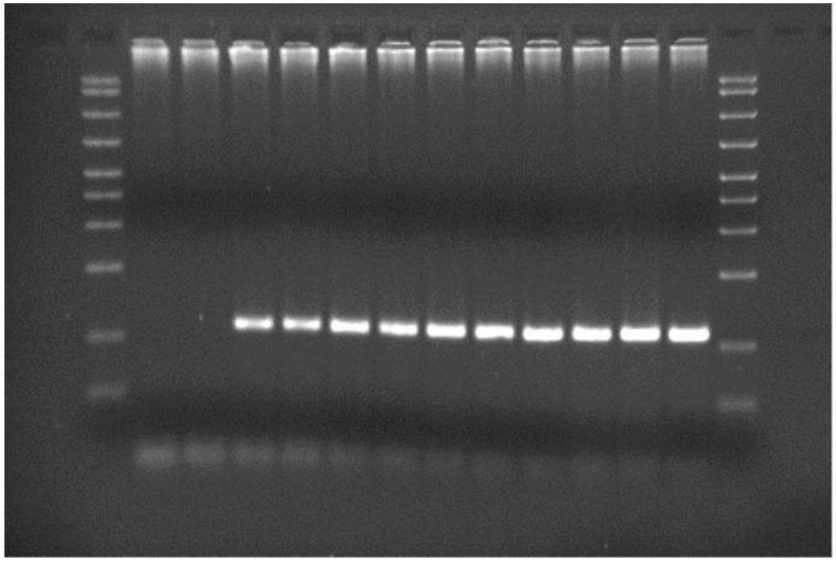
Electrophoresis

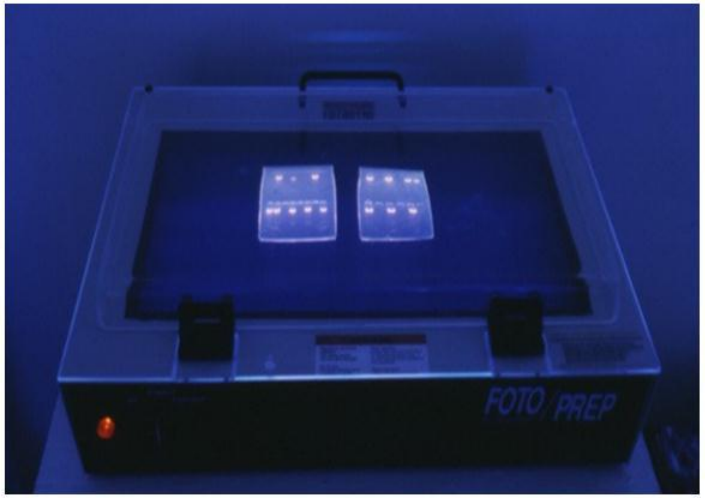
- Separation of molecules of different electric charge in an environment to which is applied an electric voltage;
- DNA has a negative charge → directed to the positive electrode
- The speed of movement depends on the size of the molecule and its conformation → large molecules are moving slower



Visualization

- By ethidium bromide (dangerous!!!)
- By fluorescent dyes





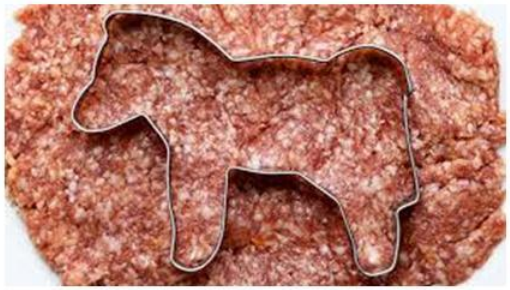
Positive result = PCR product

Negative result = no PCR product

Molecular methods - applications

- Detection of biological objects (starter cultures, pathogens, indicators, GMOs, biological origin of raw materials)

- Diagnosis of animal and plant diseases
- Identification of organisms

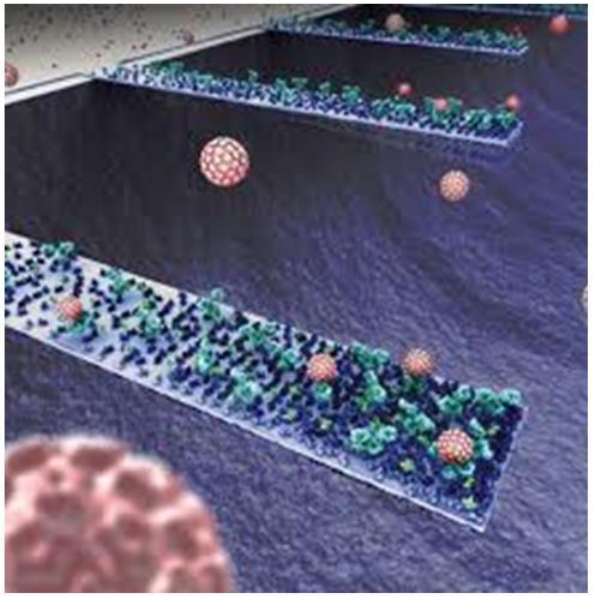


- Establishing of mutations
- Genotyping and mapping of genome
- Sequencing of nucleic acids

- Semi-quantitative analysis, etc.

Biosensors

- Biological derived sensory component
- Biological portion senses analyte (desired target)
- Possible sensors:
  - Antibodies, enzymes, sDNA (stretched DNA), receptor ligand, cells
  - Coupled to a physiochemical transducer that measures and converts the signals (light, heat, voltage, mass) to a digital electronic reading



Biosensors

Analyte	Enzymes, antibodies, microbial cells, metabolites, proteins, oligonucleotides
Detecting element	Cells, receptors, antigens, antibodies, enzymes, tissues, oligonucleotides
Transformer	Electrochemical (amperometric, potentiometric, conductometric, thermistor, optical, piezoelectric)
Signal	

Biosensors

- Transducer depends on analyte, sensor, accuracy needed, and reaction speed
- Pocket size testers for glucose levels (1980)
- Immunosensor - antibody/piezoelectric crystals
  - Crystals vibrate in patterns depending on reaction
  - Detect 10^6 E. coli cells/ml

Automated MO detection and quantification

Electronic nose

- Detects/differentiates based on volatile organic compound emission; artificial membranes change color

IMS – Electrochemiluminescence

- Improved sensitivity with IMS capture
- 10^2-10^3 cfu E. coli /ml

SensorFreshQ

Detects presence of bacteria producing gases

Green light – fresh meat

Yellow - "ok"

Red – do not eat!

Automated MO detection and quantification

BiaCore® Q

- Uses surface plasmon resonance (SPR) to detect and measure biological interactions
- Surface plasmon waves are excited at the metal/liquid interface of the sensor chip
- Incident light excites plasmons (electron charge density waves) in a gold film
- Characteristic absorption of energy occurs and SPR is seen as a drop in intensity of the reflected light
- Changes in solute concentration at the surface cause changes in the refractive index of the solution which can be measured as a change in the SPR

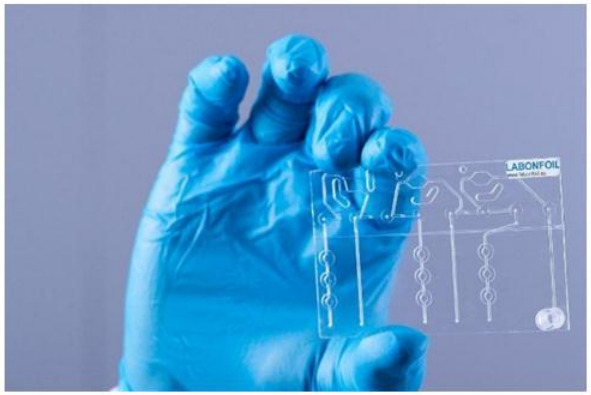
Biacore® Q

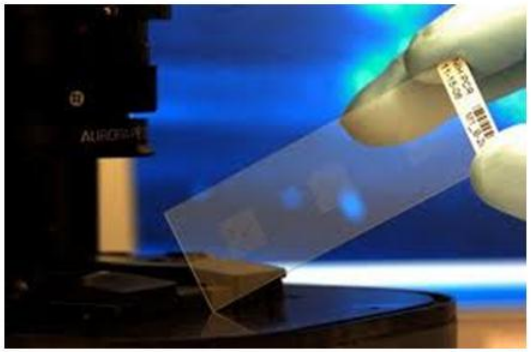
Microarrays

- 'Biochips' or 'microchips'
- Miniaturization technology
- 50,000 spots, each spot having millions of copies of a DNA probe on a special microscope slide
- Fluorescent targets can be hybridized to the spots and be detected
- Can imprint antibodies against specific pathogens for simultaneous detection of *Salmonella*, *Listeria*, *E. coli*, *Staphylococcus aureus* on same chip
- Market value estimated to be ~$5 billion by middle of decade (Elaine Heron, Applied Biosystems)

V

Microarrays





Labonfoil
FP7, EC
cpmpleted 2012

Nanotechnology +
Multiplex PCR

Nanotechnology

- Zhu et al. (2012) - a platform for detection of *E. coli* in liquid samples that attaches to a mobile phone
- Compact and inexpensive design
- Based on the anti-E. coli O157: H7 antibodies immobilized in glass capillaries
- Applicability with other pathogens using appropriate antibodies

Camera phones could be used for pathogen detection within a year - creator

By Joe Whitworth, 02-Mar-2012

A microscope to detect food pathogens that can be attached to a mobile phone could be commercially available within a year says its creator.

The lightweight technology is a miniaturised microscope without a lens that attaches to a phone and uses the camera to calculate the concentration of E.coli in a liquid sample within 30 minutes.

LUCAS (Lensless Ultra-wide-field Cell monitoring Array platform based on Shadow imaging) is formed on the idea that light

The device attached to a camera phone

Nanotechnology

- NanoRETE, Inc. (USA) – real-time detection of pathogens with nano-biosensors
- Result after 1 hour
- Easy and convenient to use platform
- Tests for anthrax, *E. coli*, *Salmonella*, tuberculosis, etc.
- Possibility for multiplex determination

nanoRETE has developed a field-operable, nanoparticle-based biosensors for the rapid detection of multiple pathogens and toxins. Our custom biosensor platform, X-MARK™, provides unique advantages over competitive technology.

Perspectives

- Nano-devices for detection of pathogens in the food chain (farm - industry - logistics - users)
- Household kits alert / rapid testing for pathogens
- Need for training of users!
- Fully automated microbiological control labs

Lavetan

Perspectives

Thank you!!!

See you in Plovdiv! ☺