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Functionalized surfaces for the detection of circulating tumor cells (CTC) and bacteria

1- Background and issues

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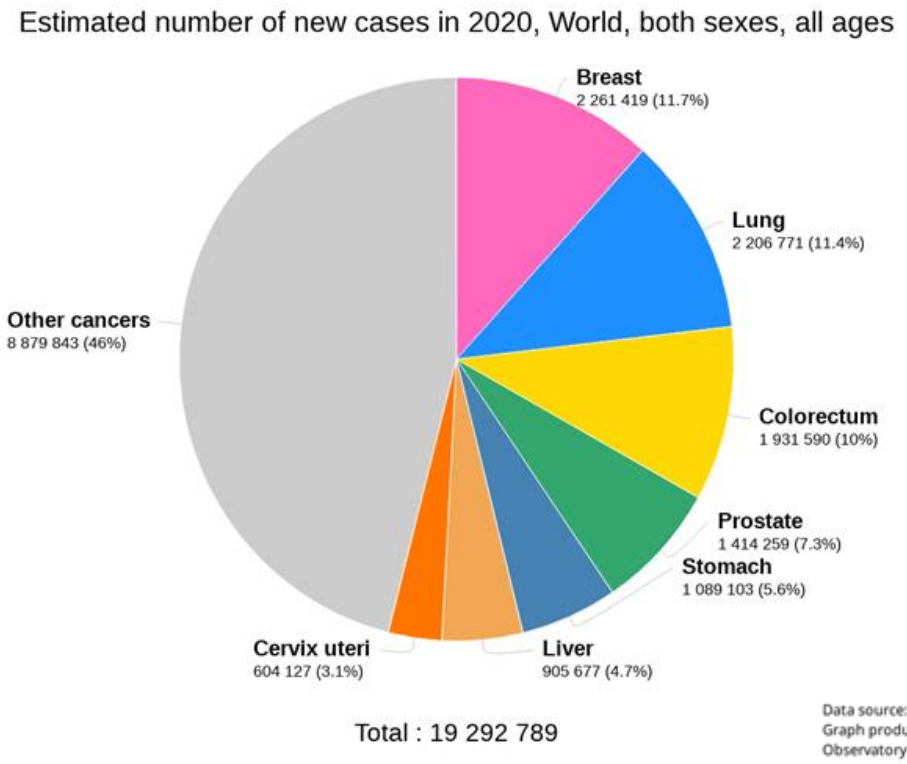
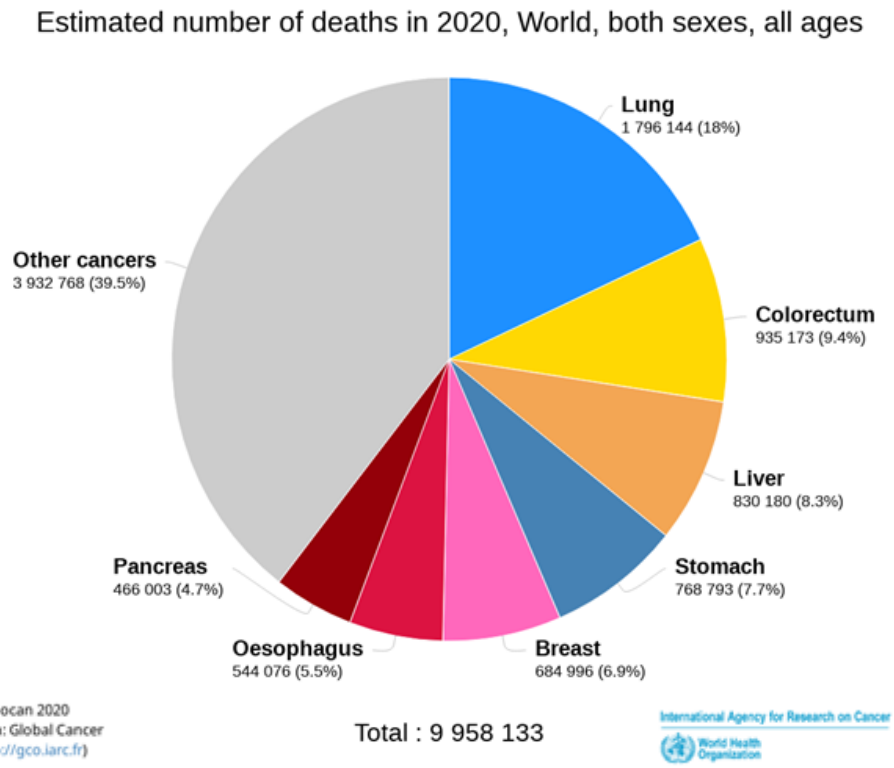
Some data about cancer



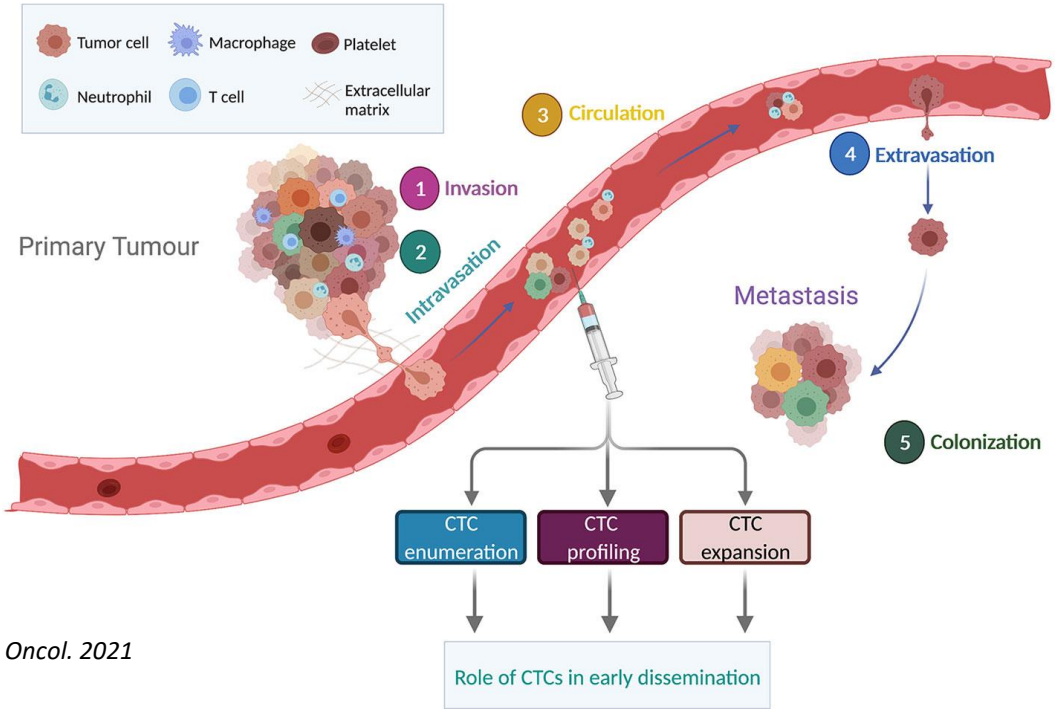


- 3rd cause of death in world (1st: cardio-vascular diseases; 2d: bacteria infection)
- 1st cause of death in France

☞ Challenges in cancer management:

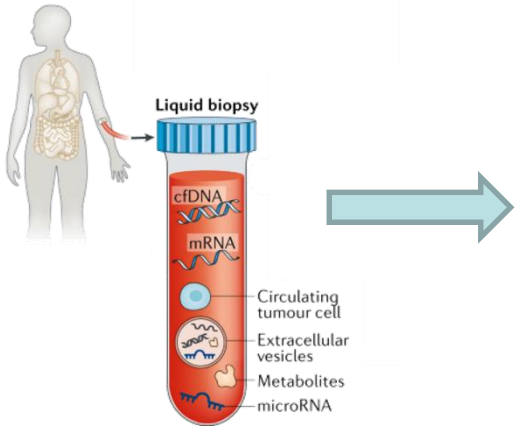
☒ screening and early diagnosis
☒ metastasis (responsible for 90% of cancer death)
☒ novel therapies and personalized treatment

☞ Interest in circulating tumor cells (CTC)



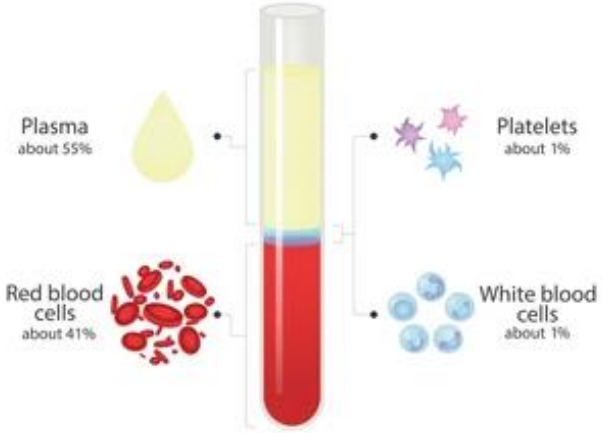
Chemi et al., Front. Oncol. 2021

☞ The liquid biopsy: non invasive collection of tumor cells and tumor markers



☒ Early diagnosis
☒ Prognosis
☒ Treatment monitoring

☒ 1 µL of blood contains:
- 4 to 6.10^6 red blood cells
- 5 to 7.10^3 white blood cells
- 2 to 3.10^5 platelets



☒ 1 to 10 CTC / mL of blood

☞ **A major issue: "find a needle in a haystack" and identify it**

Characteristics of CTC

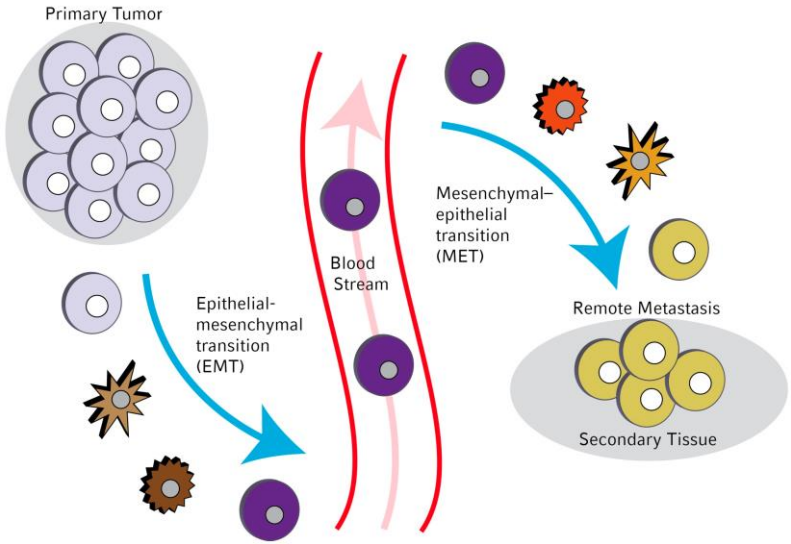
- Size
  - ☒ 17µm < CTC< 50µm
  - ☒ 6µm < RBC < 8µM
  - ☒ 7µm < WBC < 15µm-20µm

- Heterogenous cell population

- Lack CD45 and CD15 cell surface markers compare to WBC

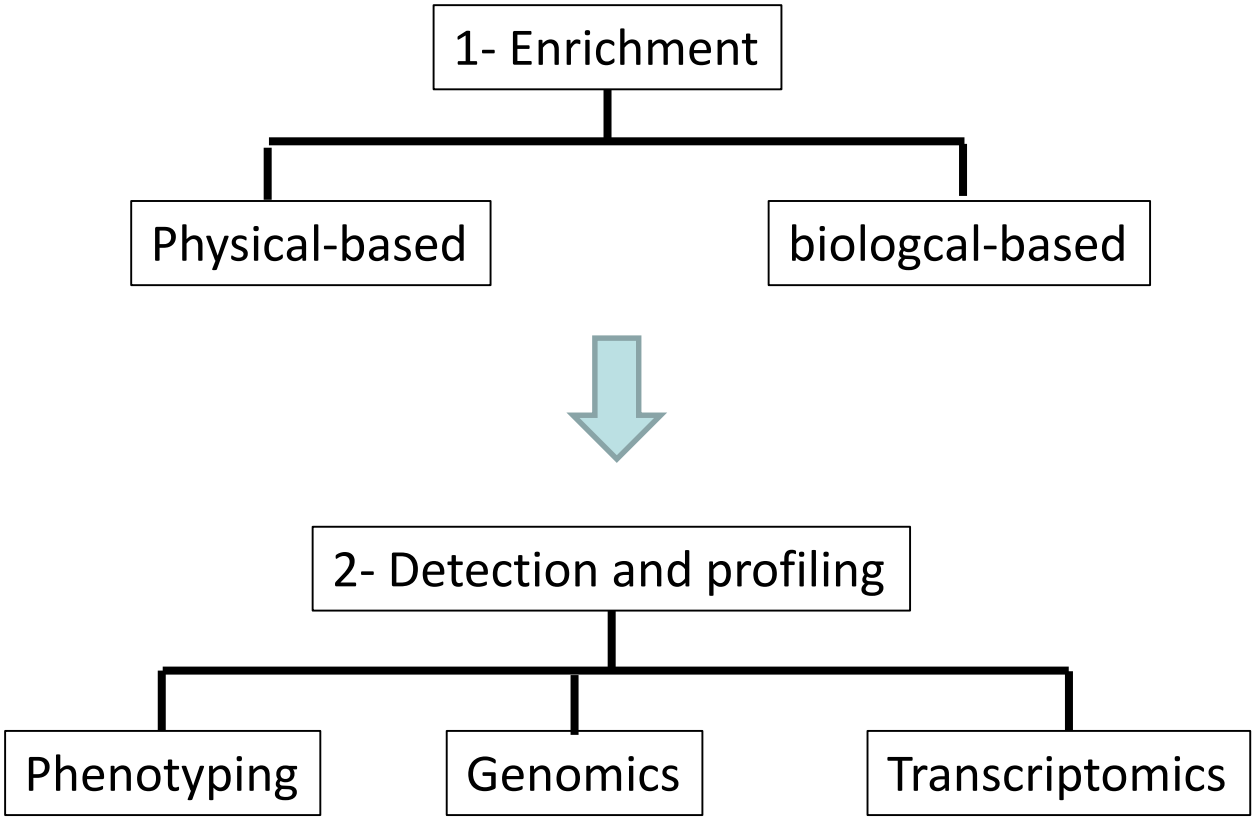
- Specific epithelial type markers
  - ☒ EpCAM, Cadherin
  - ☒ Cytokeratin (CK8, CK18, CK19)

- Specific mesenchymal type markers
  - ☒ TGF-β, Fibronectin
  - ☒ c-Met, Integrin, N-Cadherin
  - ☒ Vimentin, TWIST, SNAIL



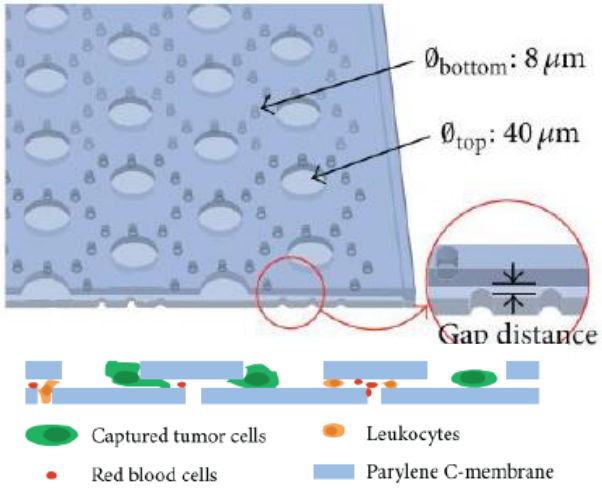
Kholbl et al., Int. J. Mol. Sci. 2016

Classical CTC detection methods



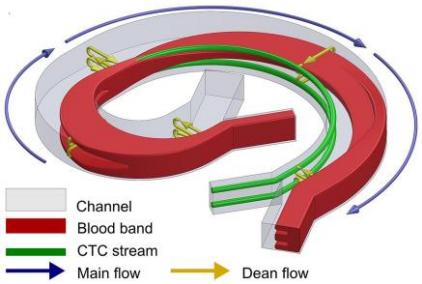
CTC enrichment based on physical properties

☞ size-based: ISET®, ScreenCell®, ClearCell®



Zhou et al., Sci. Rep. 2014

- 75 % - 85% recovery
- 70% viability

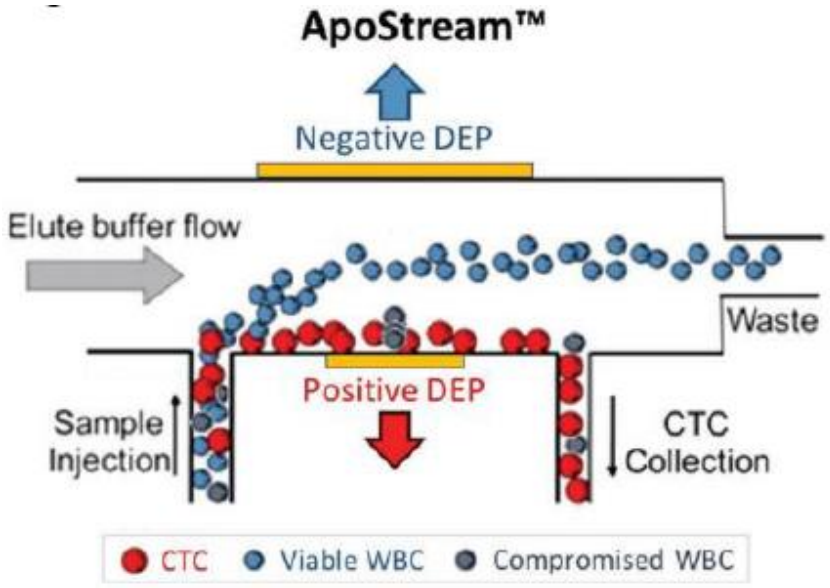


Hou et al., Sci. Rep. 2013

- 85% recovery
- 90% viability

☞ density (Ficoll): AccuCyte

☞ electric field (DEP): ApoStream™



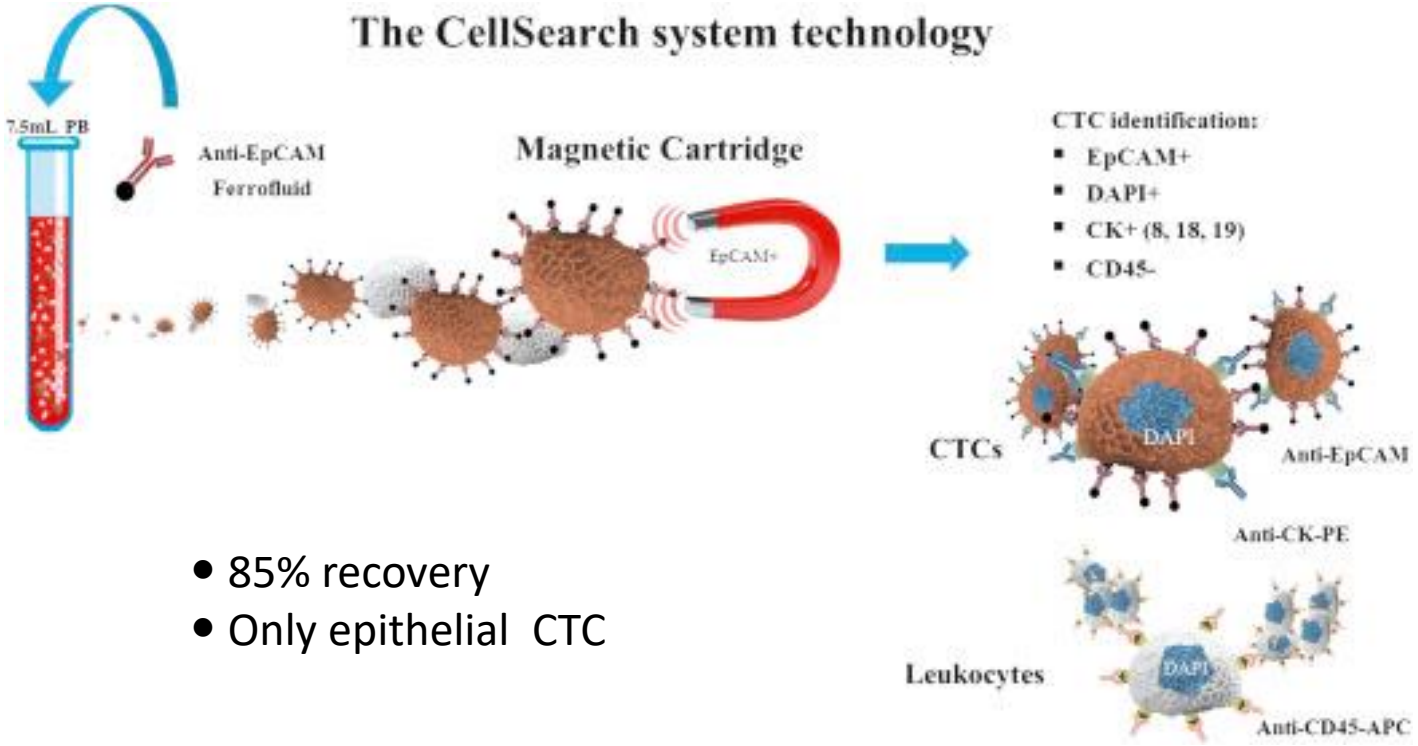
Gupta et al., Biomicrofluidics 2012

- 70% recovery
- 97% viability

CTC enrichment based on biological properties

☞ Positive immuno-selection based on EpCAM targeting

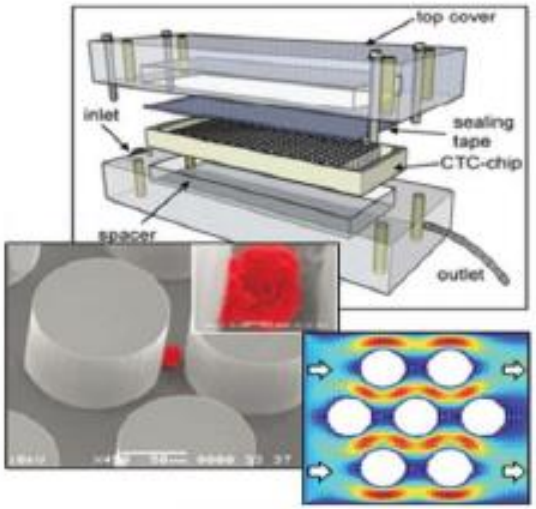
☒ CellSearch® (FDA approuved) : « Gold standard »



- 85% recovery
- Only epithelial CTC

Cristofanilli et al., J. Clin. Oncol. 2005

☒ CTC-Chip

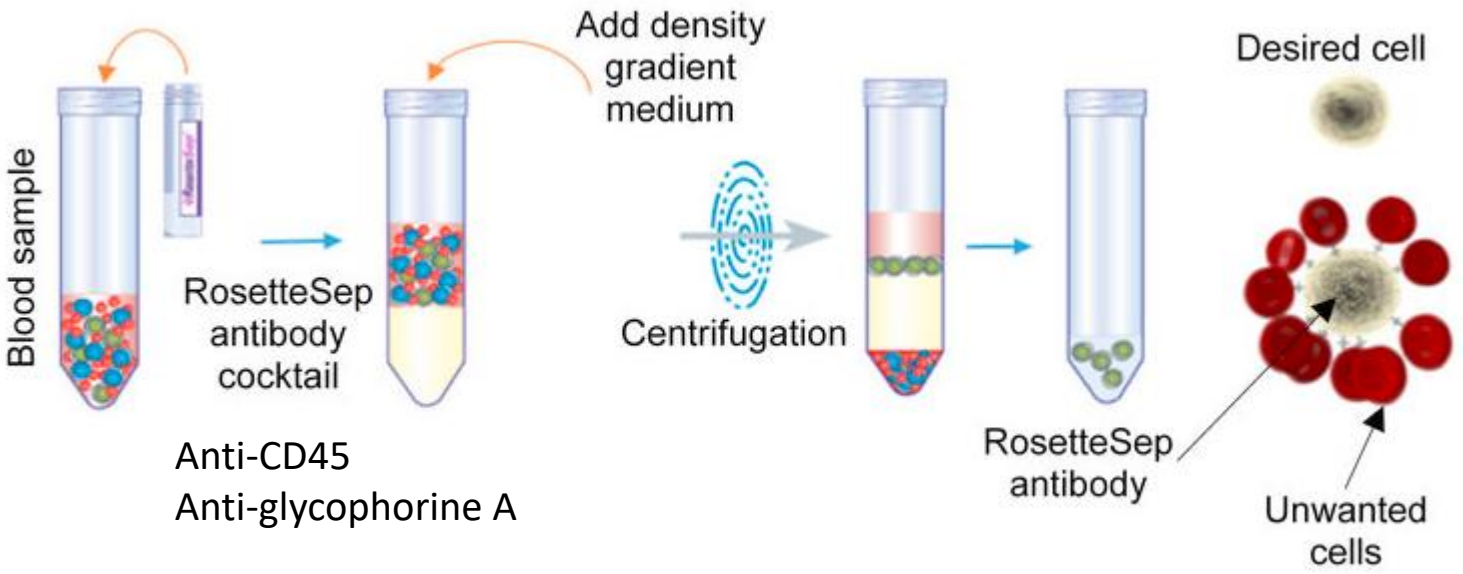


Nagrath et al., Nature 2007

- 78000 coated anti-EpCAM micropillar (100µm dia, 50µm spacing)
- 80% recovery
- No release of CTC for subsequent analysis

☞ Negative immuno-selection

☒ RosetteSep™



Anti-CD45
Anti-glycophorine A

- 36% recovery
- Loss and morphological damages to CTC

Naume et al., Cytotherapy 2004

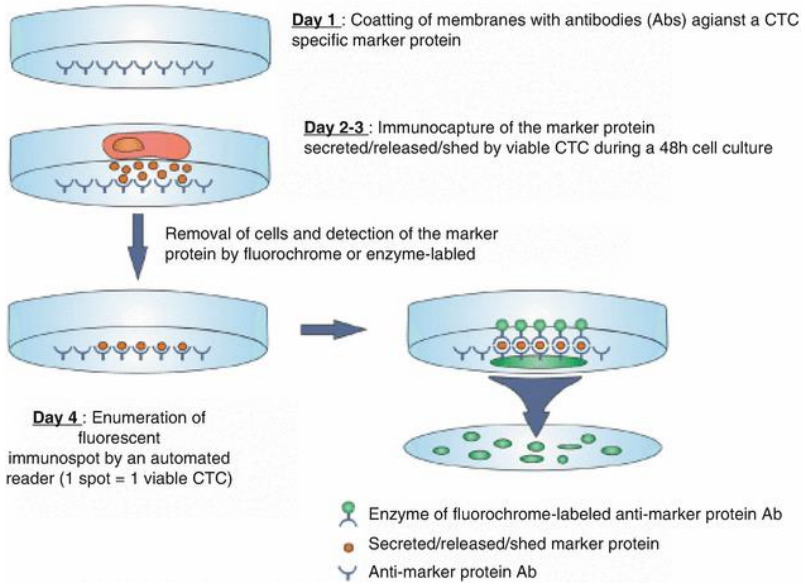
☒ Dynabeads®, EasySep® : immuno-magnetic depletion

Chen et al., Biosens. Bioelectron. 2016

- 24-44% recovery

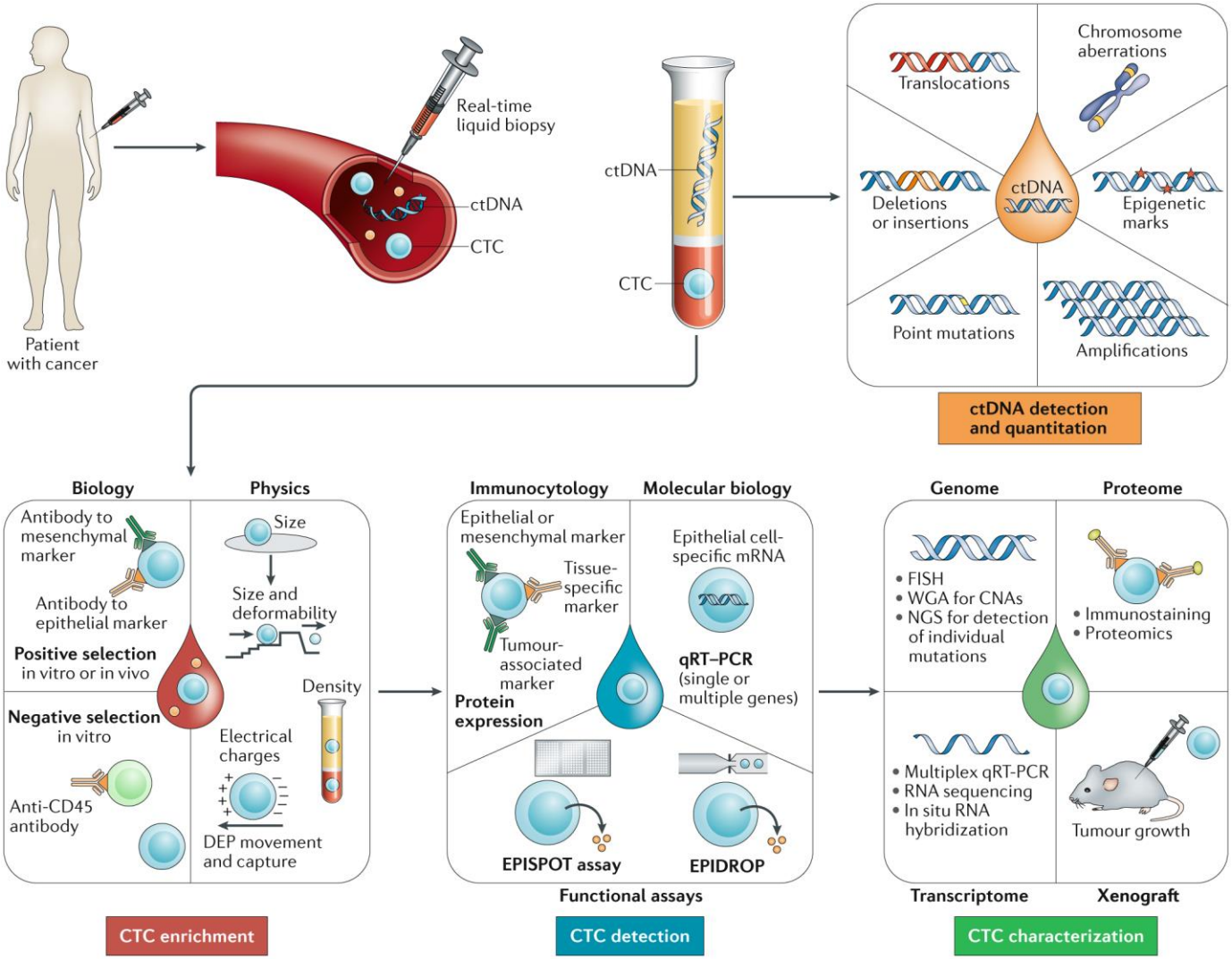
CTC detection and profiling

- Molecular analysis by sequencing technologies and FISH
  - ☒ genomic and transciptomic informations
- Phenotyping analysis and counting by immunohistochemical methods
  - ☒ fluorescent microscopy, flow cytometry
- Fonctional assay: EPISPOT
  - ☒ CK19 and MUC1 secretion
  - ☒ only alive CTC



Alix-Panabières., Recent Results Cancer Res. 2012

Summary for CTC analysis workflow



Pentel et al., Nature Rev. Clin. Oncol. 2019

Clinical applications of CTC detection and challenges

- CTC as prognostic markers in cancer
  - ☒ low cut-off value of CTC enumeration
  - ☒ improvement of recovery rate after enrichment/purification
  - ☒ development of standardized detection techniques
- CTC as chemotherapy indicators in cancer
  - ☒ markers of targeted chemotherapy selection
  - ☒ markers of treatment resistance
  - ☒ markers of treatment sensitivity
- Discovery of novel biomarkers and drug development
  - ☒ rarity, heterogeneity, fragility, cluster formation
  - ☒ single-cell analysis
  - ☒ recovery rate of alive CTC

Some data about bacteria infection

☞ 2d cause of death in the world (7,7 millions)

☞ Growing of antimicrobial resistance

☞ Major issue in health, environment, agri-food and security

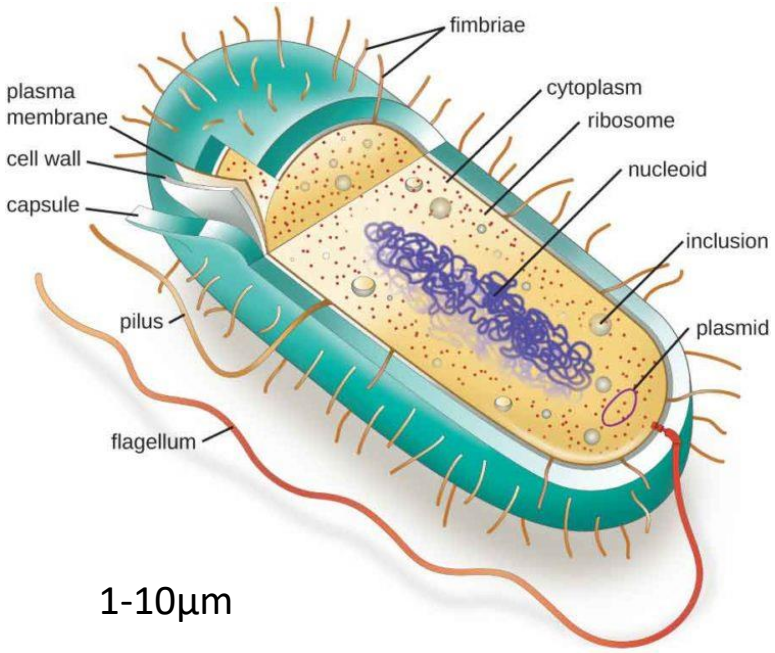
- Public health: precise diagnosis and rapid screening, treatment efficacy
- Environment / security: protection and monitoring
- Agri-food: food quality and monitoring of production

☞ Nosocomial infection (5% of patients): *E. coli, Staph aureus, Pseudomonas aeruginosa, Streptococcus, Enterococcus, Clostridium difficile*

☞ Food and waterborne infections (10% of people): *E. coli, Campylobacter, Legionella, Salmonella, Shigella*

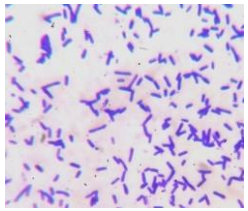
Bacteria characteristics

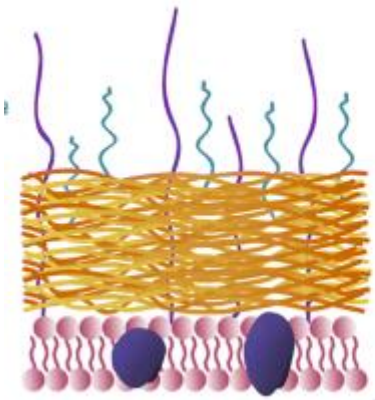
☞ Bacteria are procaryotic cells: 2 major groups



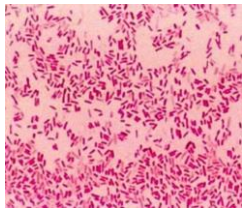
1-10µm

Gram positive (G+)
coque, bacille
Listeria, Staph aureus, pneumococcus, streptococcus





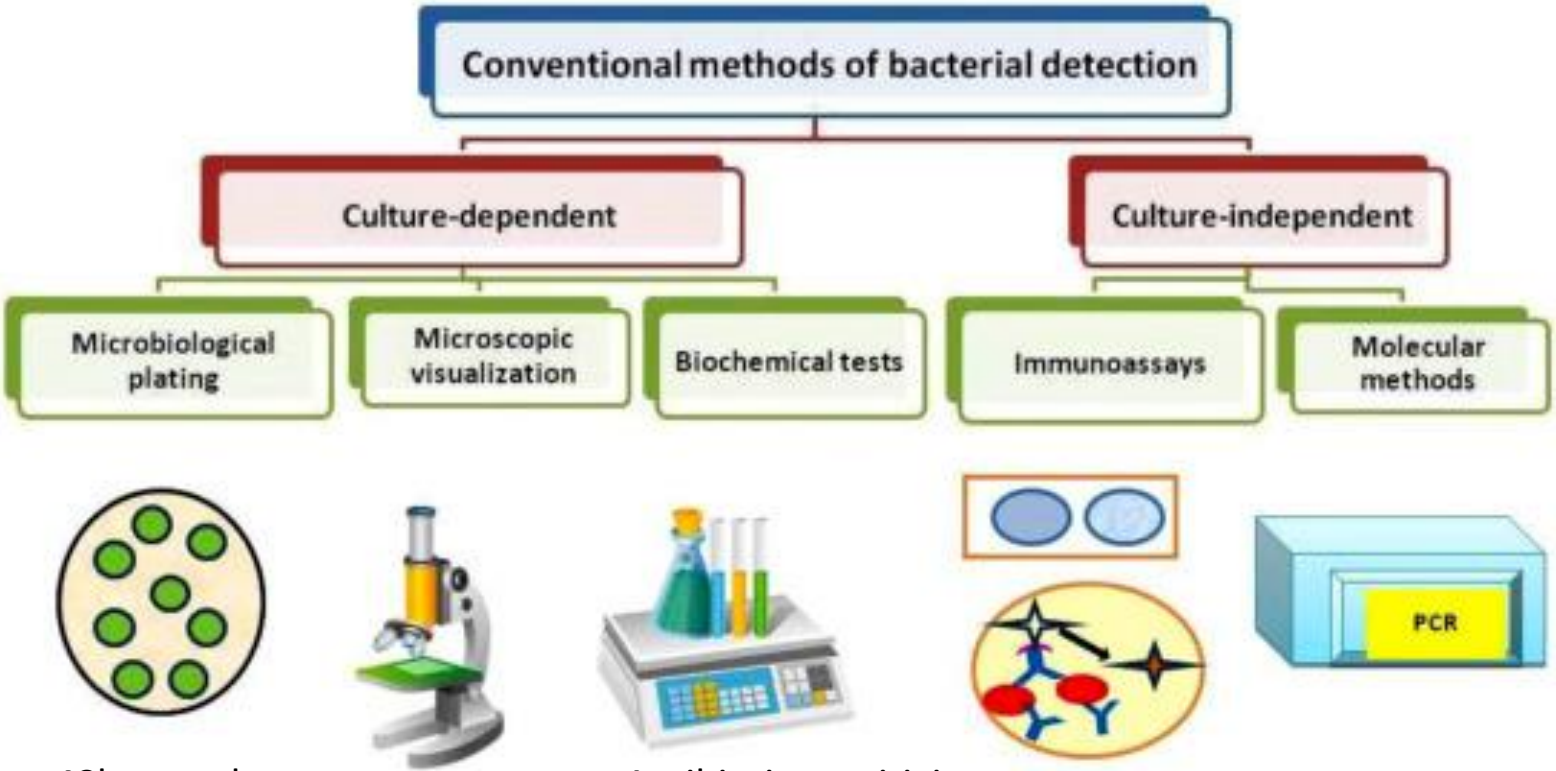
Gram negative (G-)
coque, bacille, spirochete
Legionella, Pseudomonas, Salmonella, E. coli



☞ Specific metabolism of bacteria and enzymatic activities

- ☒ nitroreductase (NTR) widely expressed in G+ and G-
- ☒ alcalin phosphatase (ALP) highly expressed in E. coli
- ☒ β-lactamase highly expressed in antibiotic resistant bacteria
- ☒ capsase-I activated in infected human cells
- ☒ catalase-positive Staphylococcus/catalase-negative Streptococcus
- ☒ Coagulase test for pathogenic bacteria

How to detect bacterial infection?



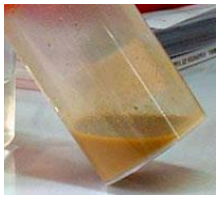
>48h growth

Gram staining
Ziehl-Neelsen staining

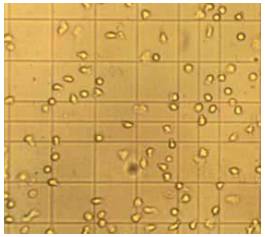
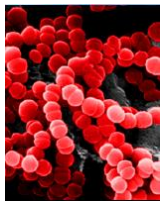
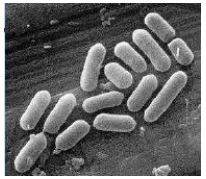
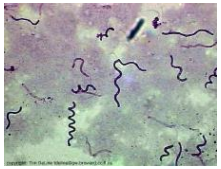
Antibiotic sensitivity
C-reactive protein test
Enzymatic test
Metabolic assay

Culture-dependant detection methods: « the gold standard »

- Macroscopic observation
  - ☒ presence of RBC, WBC
  - ☒ smell
  - ☒ consistance, cloudy solution




- Microscopic observation
  - ☒ shape, mobility, association
  - ☒ Staining
  - ☒ enumeration

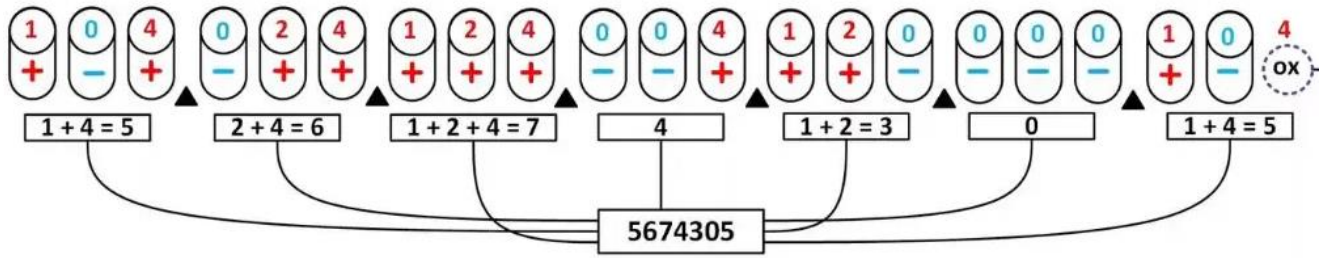
- Microbiological culture on specific conditions
  - ☒ liquid or solid medium
  - ☒ selectif medium
  - ☒ t°, aerobic or anaerobic

☞ API 20E (Analytical Profil Index) biochemical tests: bacteria identification

☒ 20 biochemical assays for culturing bacteria

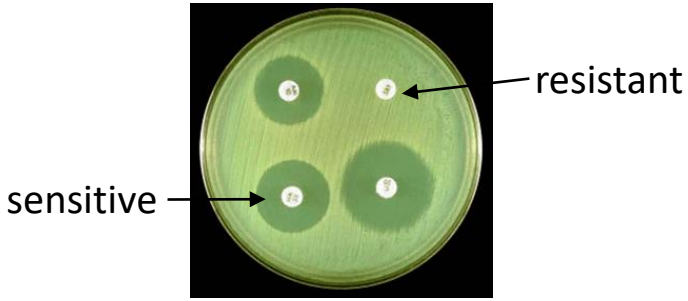
Negative test

Positive test



Enter the numerical profile in the apiweb to obtain the identity.

☞ Antibiotic sensitivity

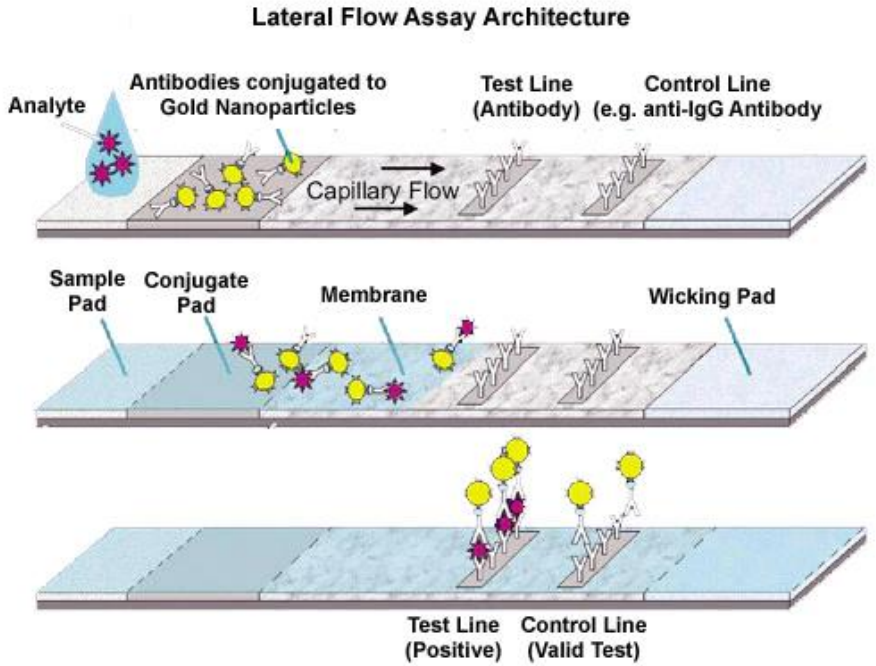


☞ Culture-dependant methods are:

☒ Cost-effective methods with good specificity (>80%)
☒ Time consuming (>48h) and low sensitivity (10^3 CFU/mL)
☒ Not possible for non-cultivable pathogens

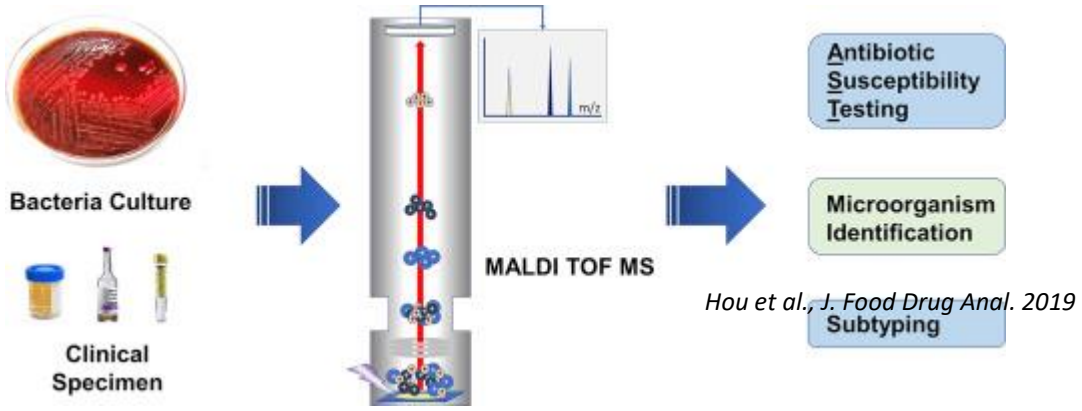
Culture-independant detection methods

- ☞ Immunoassay-based methods (Numerous commercially available kits)
  - ☒ ELISA: Enzyme-Linked Immuno Sorbent Assay
  - ☒ FLISA: Fluorescent-Linked Immuno Sorbent Assay
  - ☒ LFIA: Lateral Flow ImmunoAssay



- Specific and sensitive (10 CFU/mL)
- Easy to operate and cost-effective
- Allow detection of bacterial toxins
- Rapid method compare to cell culture

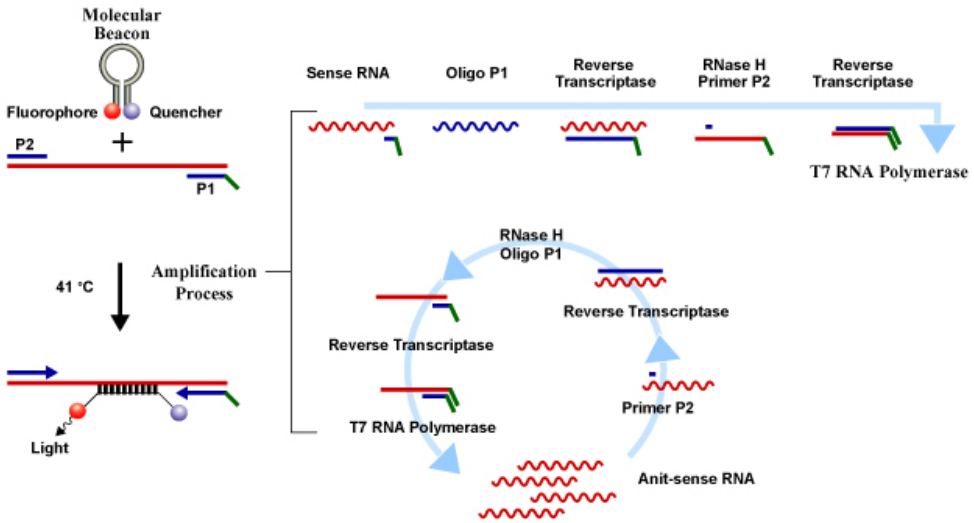
- Mass spectrometry-based methods
  - ☒ LC-MS
  - ☒ MALDI-TOF



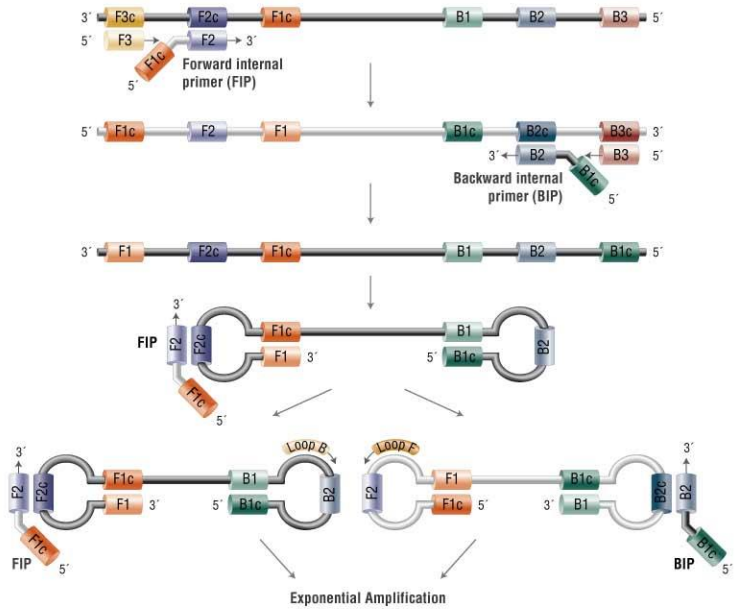
Hou et al., J. Food Drug Anal. 2019

- Rapid (few hours) and accurate analysis (70% sensitivity, 90% specificity)
- First need bacteria culture and sample preparation

- Nucleic acid-based methods
  - ☒ PCR, multiplex PCR, quantitative PCR: 10^2-10^3 CFU/mL
  - ☒ NASBA (Nucleic Acid Sequence-Based Amplification): <1 CFU/mL
  - ☒ LAMP (Loop-Mediated isothermal Amplification): <10 CFU/mL



NASBA principle



LAMP principle

- Only NASBA allows detection of alive bacteria
- Highly sensitive and specific methods
- NASBA and LAMP are low-cost and fast (<2h)

Challenges of bacteria detection

☞ Bacteria infection is a major public health issue due to growing antibiotic resistance

☞ Several bacteria detection and identification methods are available

- ☒ specificity and sensitivity
- ☒ cost
- ☒ material requirement
- ☒ time to result

☞ Challenges still to be resolved

- ☒ pre-treatment of samples
- ☒ integrated biosensing approaches for POC detection
- ☒ microfluidic sensors for rapid detection
- ☒ label-free detection