Navigating Challenges Associated with PCR Technology in a Cannabis Laboratory

Presented by:
PJLA President, Tracy Szerszen
Pat Bird of PMB BioTek Consulting

Thursday, June 10, 2021
1:00-2:00 PM EDT

PJLA

Presentation Overview

Tracy Szerszen
President
Perry Johnson Laboratory Accreditation (PJLA)

17025 Requirements regarding selection of appropriate methods and method validation criteria

Challenges Associated with PCR Technology for laboratories

Questions & Answers

PJLA

Webinar Housekeeping

- This webinar will be recorded
- All PJLA webinars are made available on our website & YouTube channel
  - https://www.pjlabs.com/training/pjla-webinars/past-webinars
- All attendees are muted
- Please utilize the question tool bar to submit questions
  - To be answered at the end of presentation

ISO/IEC 17025:2017 - 7.2
Selection Verification and Validation of Methods

- Methods shall be appropriate for the test
- Methods shall be up to date and available to personnel
- Labs shall use the latest version of the method unless its not appropriate to do so
- It is recommended to select methods that are published either in international, regional or national standards, or by reputable technical organizations, or in relevant scientific texts or journals, or as specified by the manufacturer of the equipment
- Method Validation – shall be planned activity, trainer personnel, re-verified periodically
- *a) calibration or evaluation of bias and precision using reference standards or reference materials;*
- *b) systematic assessment of the factors influencing the result;*
- *c) testing method robustness through variation of controlled parameters, such as incubator temperature, volume dispensed;*
- *d) comparison of results achieved with other validated methods;*
- *e) interlaboratory comparisons;*
- *f) evaluation of measurement uncertainty of the results based on an understanding of the theoretical principles of the method and practical experience of the performance of the sampling or test method.*

ISO/IEC 17025:2017 - 7.2
Selection Verification and Validation of Methods

- *7.2.2.4 The laboratory shall retain the following records of validation:*
- *a) the validation procedure used;*
- *b) specification of the requirements;*
- *c) determination of the performance characteristics of the method;*
- *d) results obtained;*
- *e) a statement on the validity of the method, detailing its fitness for the intended use.*

Accreditation Expectations

- Follow 17025 in regard to method validation
- Utilize available resources when developing methods; other industry standards –etc. food, environmental
- Demonstrate during the assessment your competency in regard to the development and performance of the internal method
- Have data available to us to review

Welcome and Introductions

Guest Speaker:
Patrick Bird
PMB BioTek Consulting
consulting@pmbbiotek.com

As the principal consultant at PMB BioTek Consulting, Pat works with method developers, contract laboratories and industry to identify solutions to emerging microbiological issues, optimizing method workflows and designing validation studies. Pat is an active member of the AOAC CASP initiative, previously serving as co-chair of the microbiology contaminants working group and is a co-conveyor for several projects within the Food Microbiology Method Validation Working Group (WG3) within ISO/TC34/SC9. Pat holds a BS in Microbiology from the Ohio State University in Columbus, Ohio and a MS in Food Safety from Michigan State University in East Lansing, MI.

How accurate is my method?

Understanding the Strengths and Limitations of Molecular Technologies

Patrick M. Bird

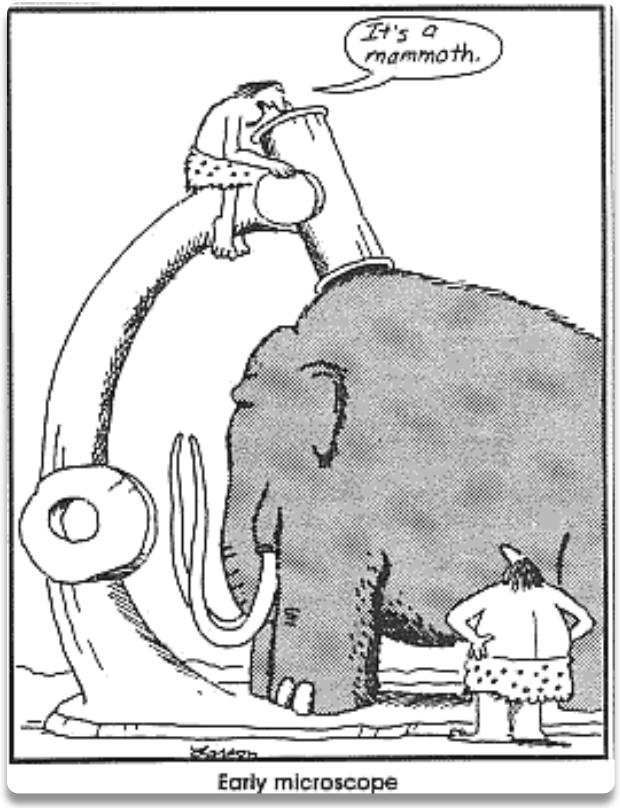
PMB BioTek Consulting

Consulting@pmbbiotek.com

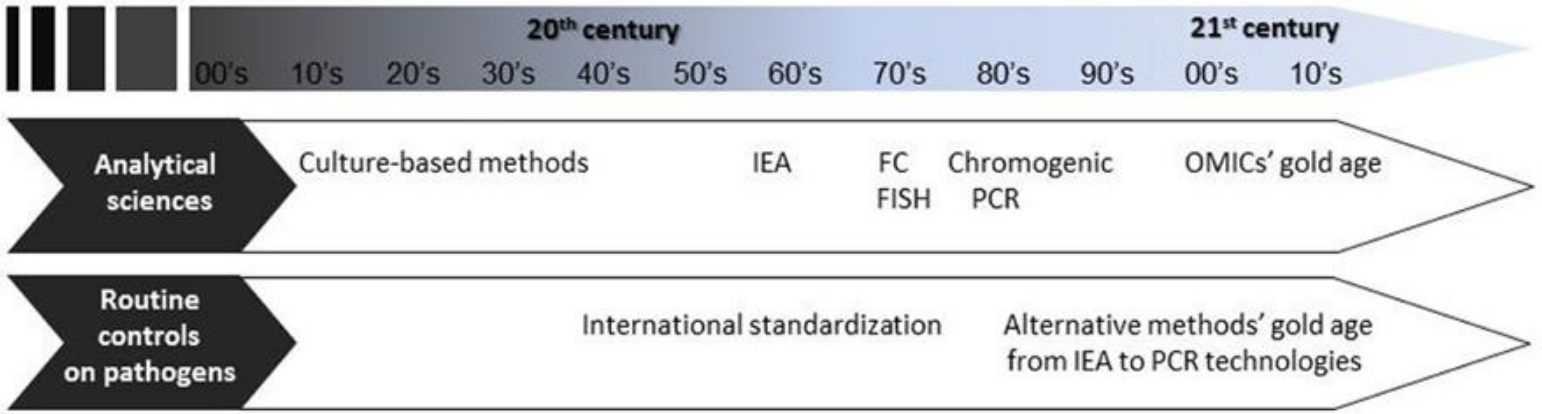
Overview

- **Evolution of microbiology testing**
  - **How has testing evolved?**
  - **How do we improve the accuracy of molecular methods?**
- Testing modifications to eliminate “spurious” signals
- Improvements in testing technology to focus on live-cell detection

History of Microbiology

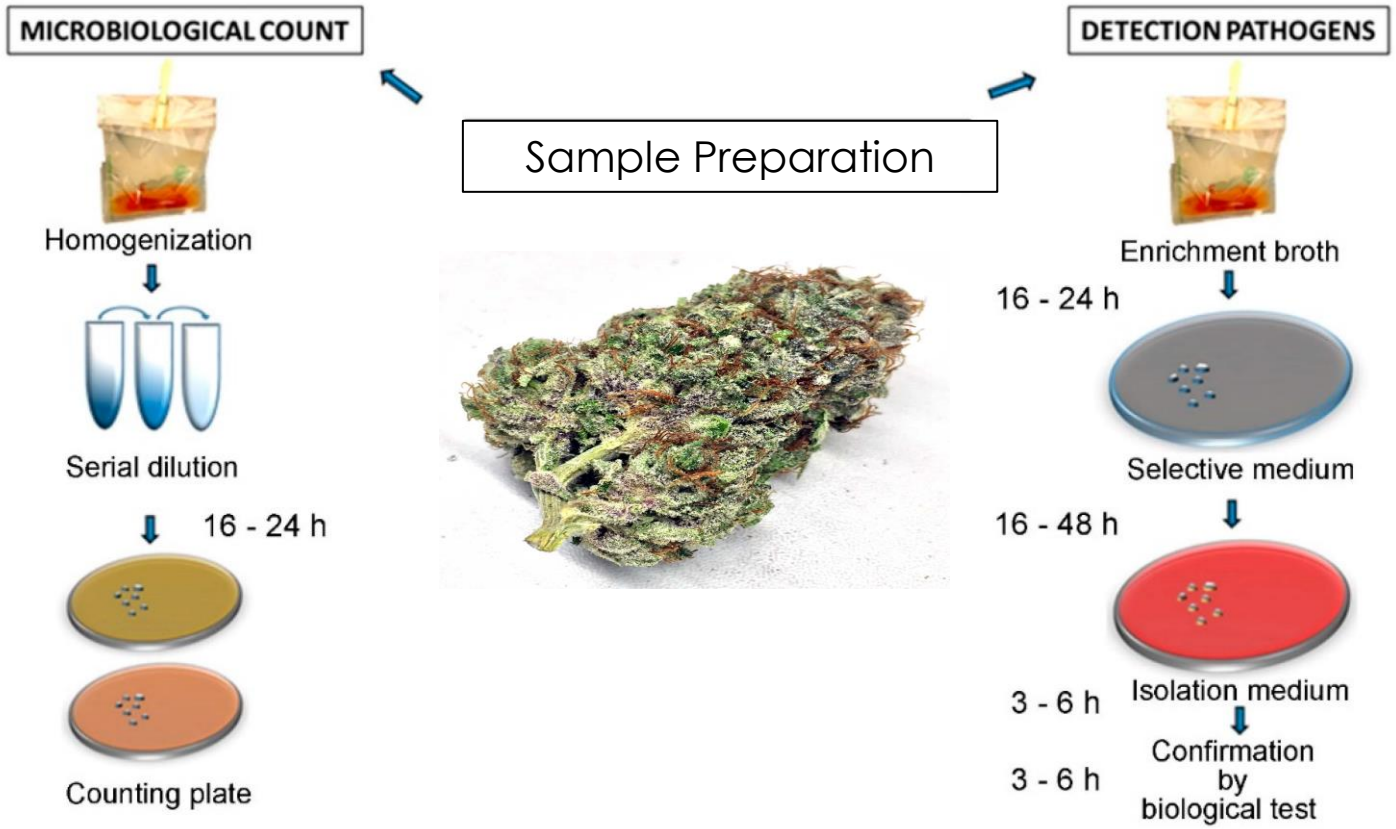


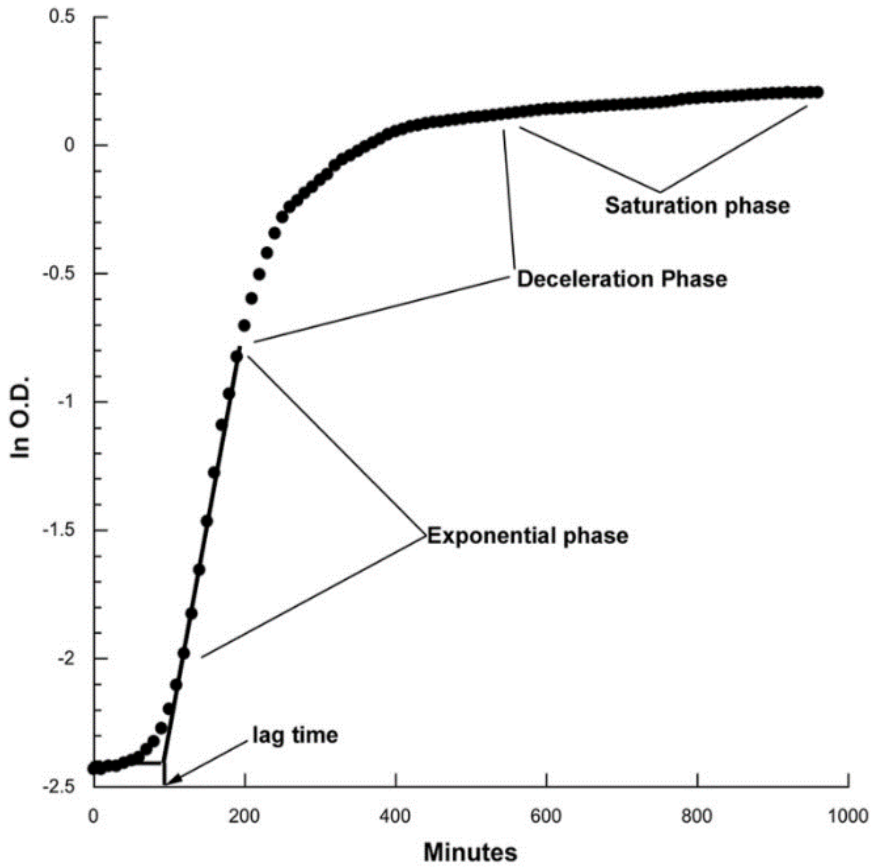
Early microscope



Sohier D, Pavan S, Riou A, Combrisson J, Postollec F. Evolution of microbiological analytical methods for dairy industry needs. *Front Microbiol*. 2014;5:16. Published 2014 Feb 7. doi:10.3389/fmicb.2014.00016

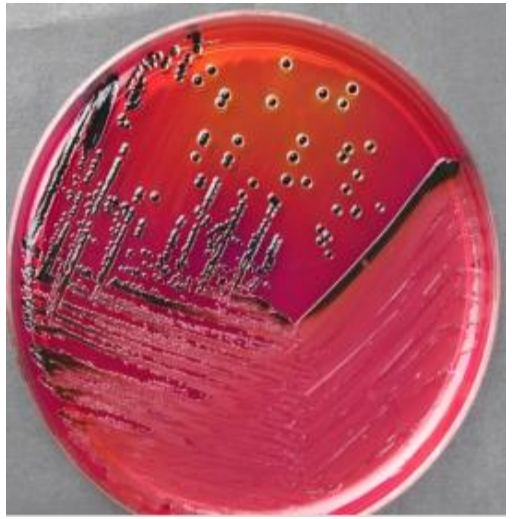
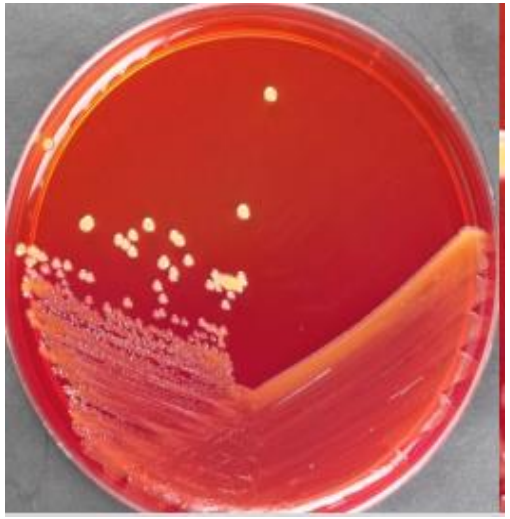
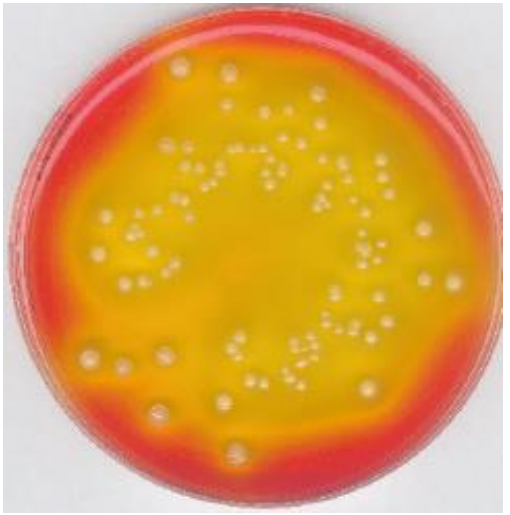
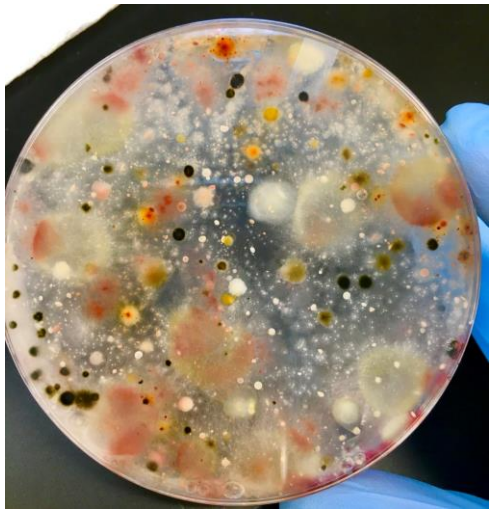
Culture Based Techniques





Hall, B Acar Kirit H, Nandipati A, Barlow M. Growth Rates Made Easy. Mole. Bio. & Evo. 2013; 31 10.1093/molbev/mst187

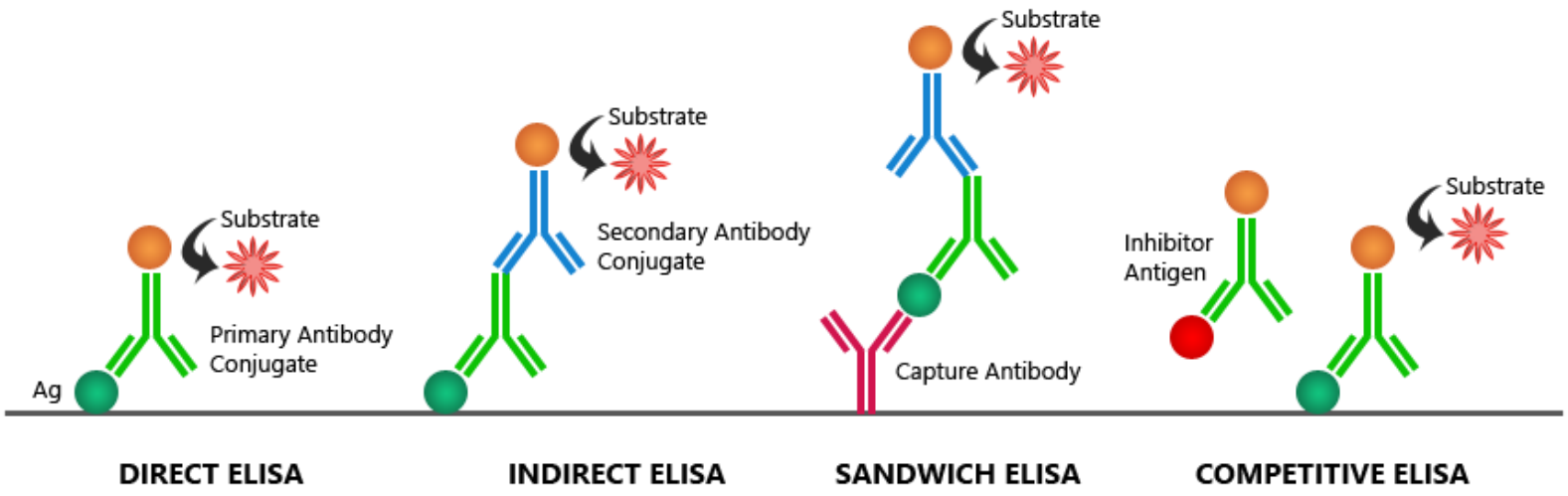
Culture Based Techniques

https://www.fda.gov/files/food/published/%3Ci%3ESalmonella%3C-i%3E-Flipbook.pdf

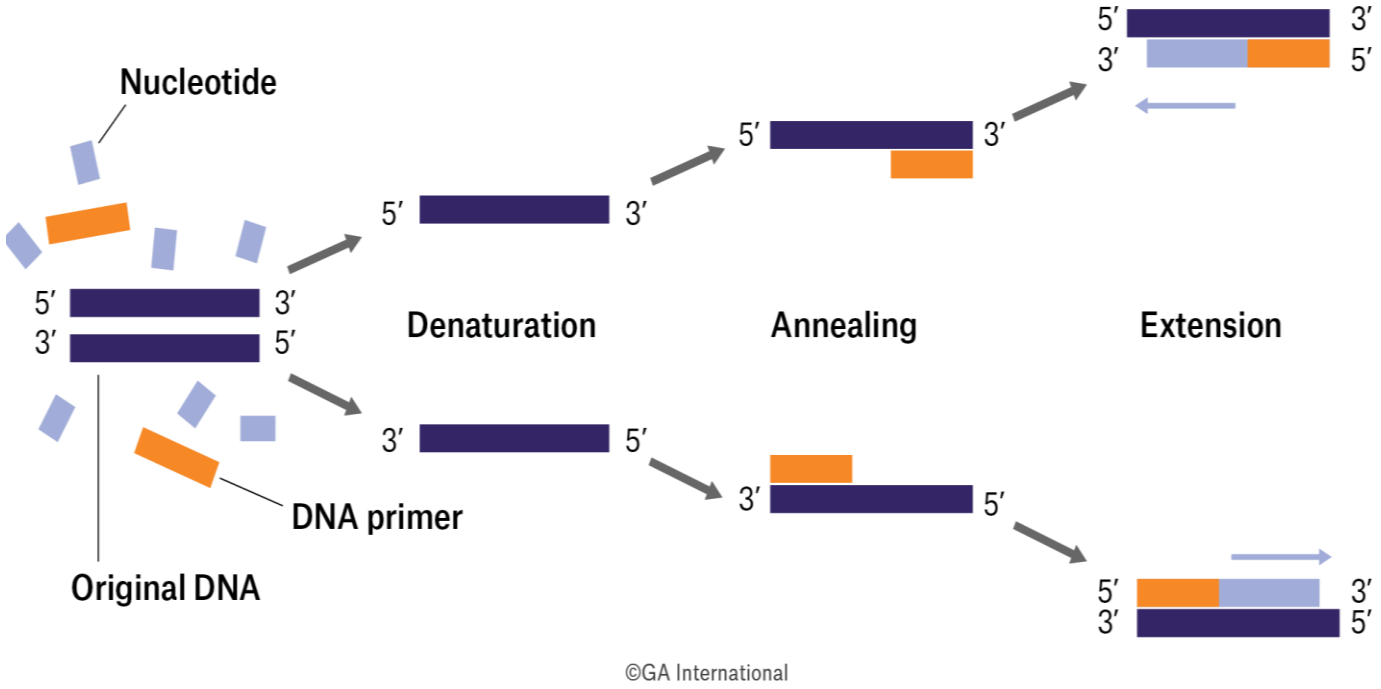
Immunoassays

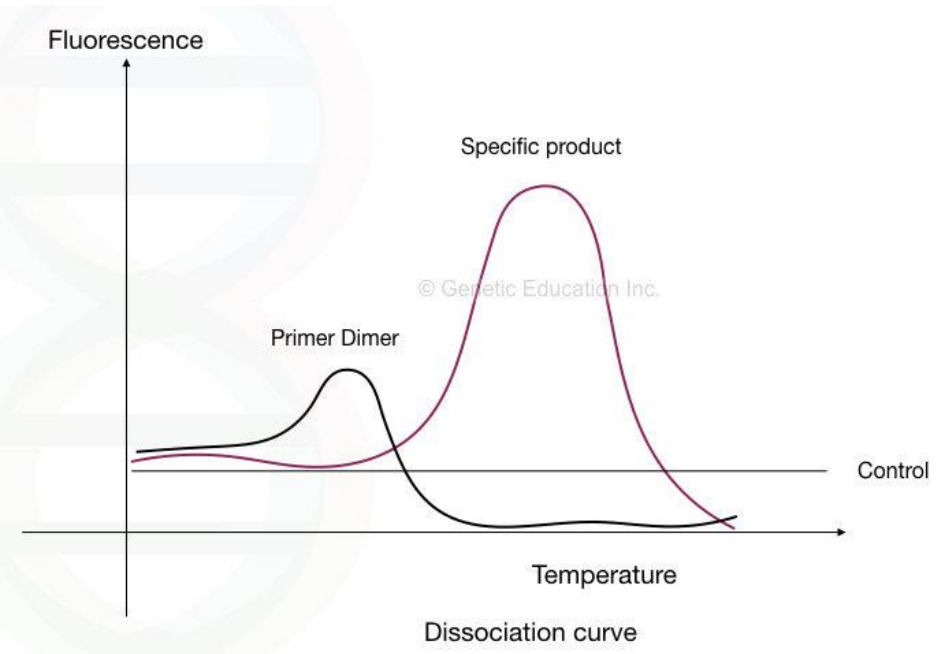
- Quicker TTR
- Moderate sensitivity
- Improved workflows

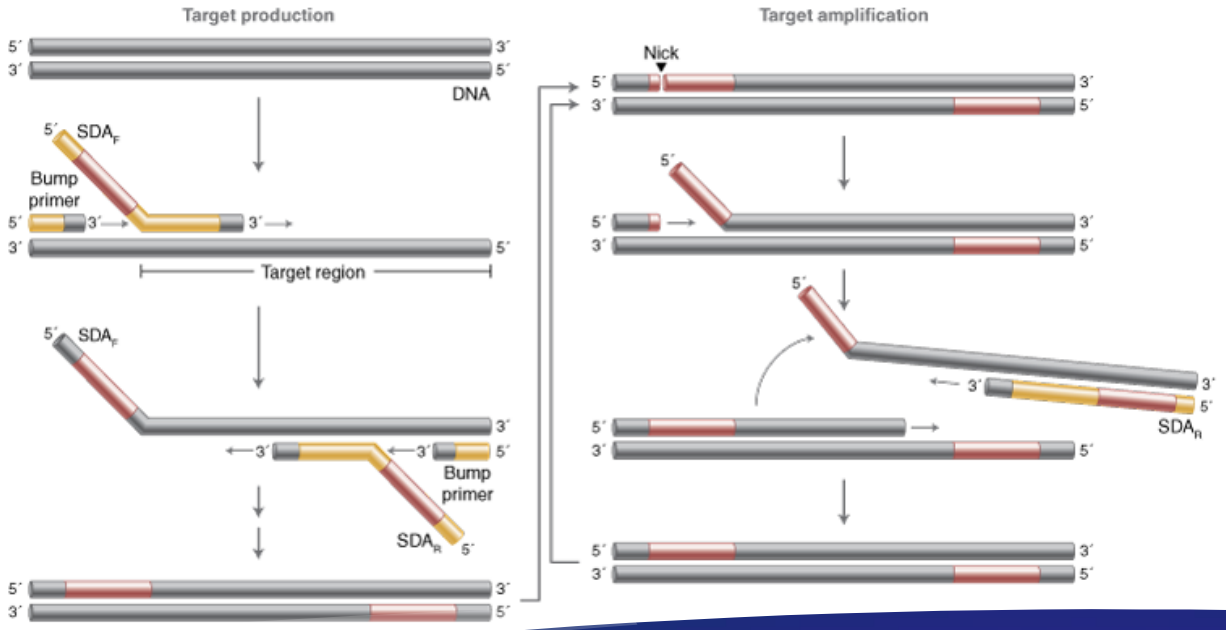


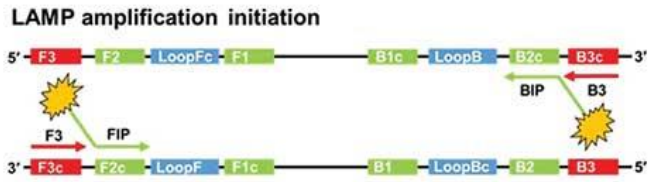
Microbe Notes. *Enzyme-linked immunosorbent assay (ELISA)*, Oct 19, 2017.

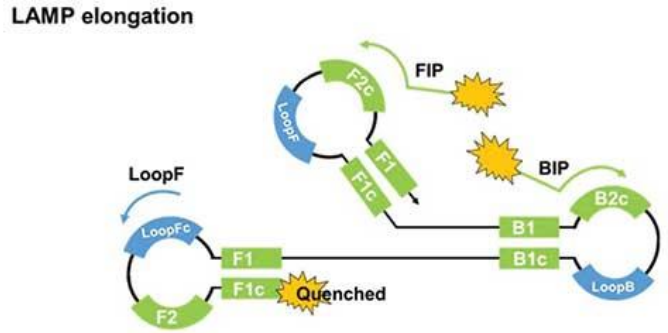
Polymerase Chain Reaction (PCR)

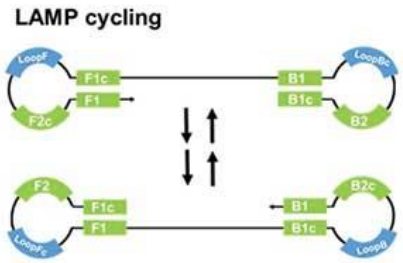










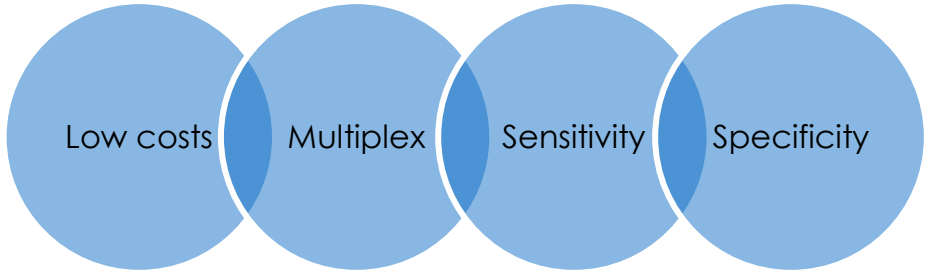


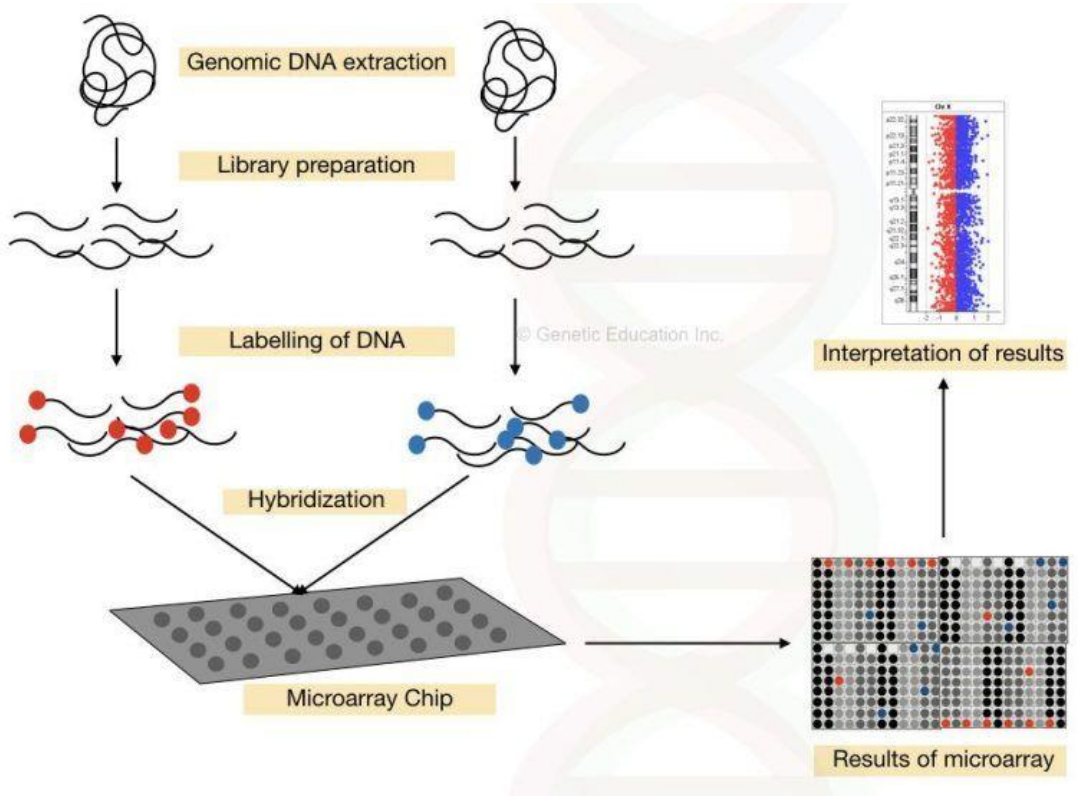
Loop-mediated Isothermal Amplification (LAMP)

ISO Thermal Amplification: LAMP & NEAR

Microarray Technology

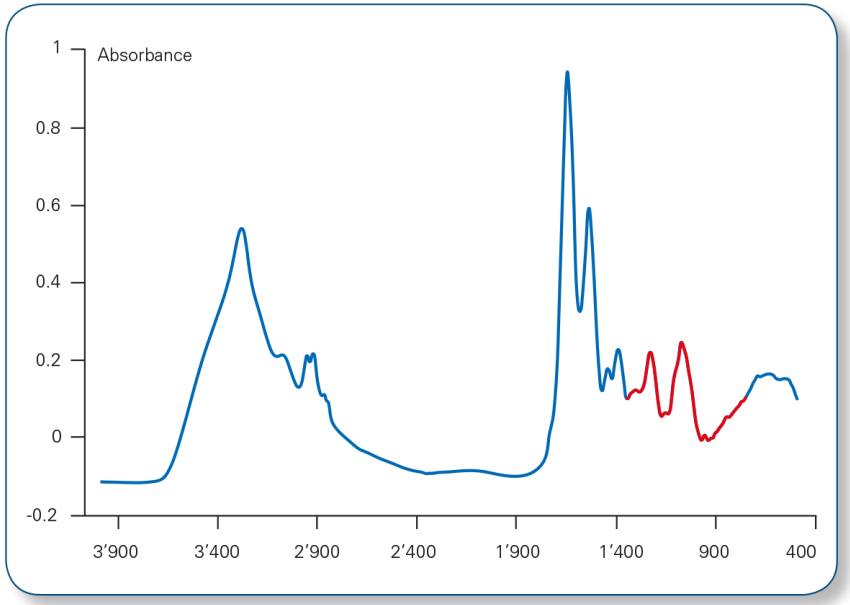
- Two common paths for DNA/RNA Detection:
  - Sample enrichment
  - PCR (single or tandem)
- Labeled with target probes
- Hybridized to complimentary sequences on microchips



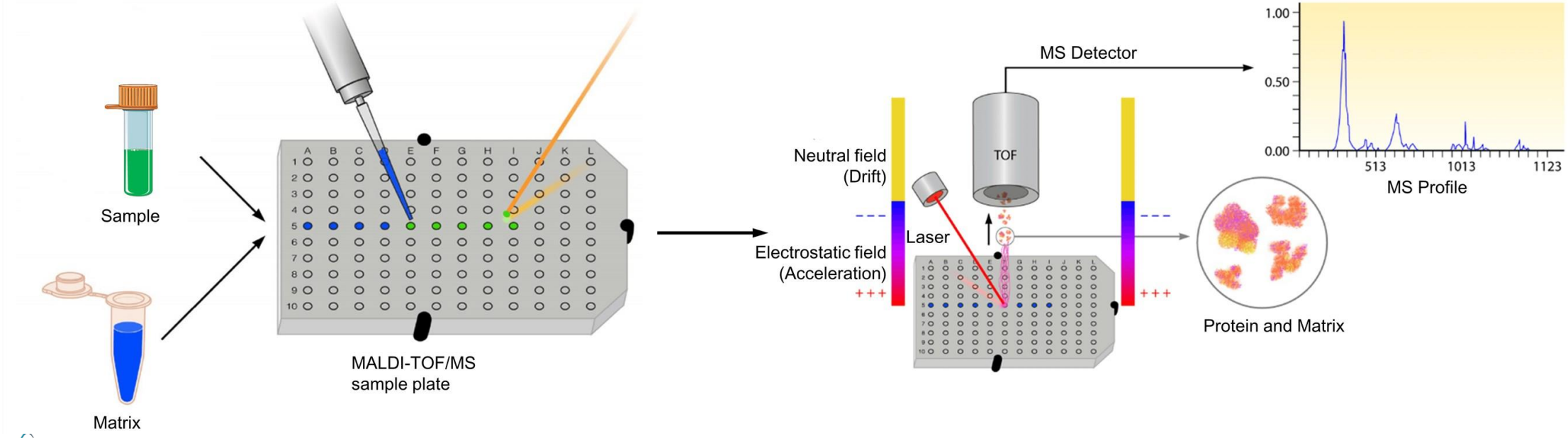


MALDI-TOF

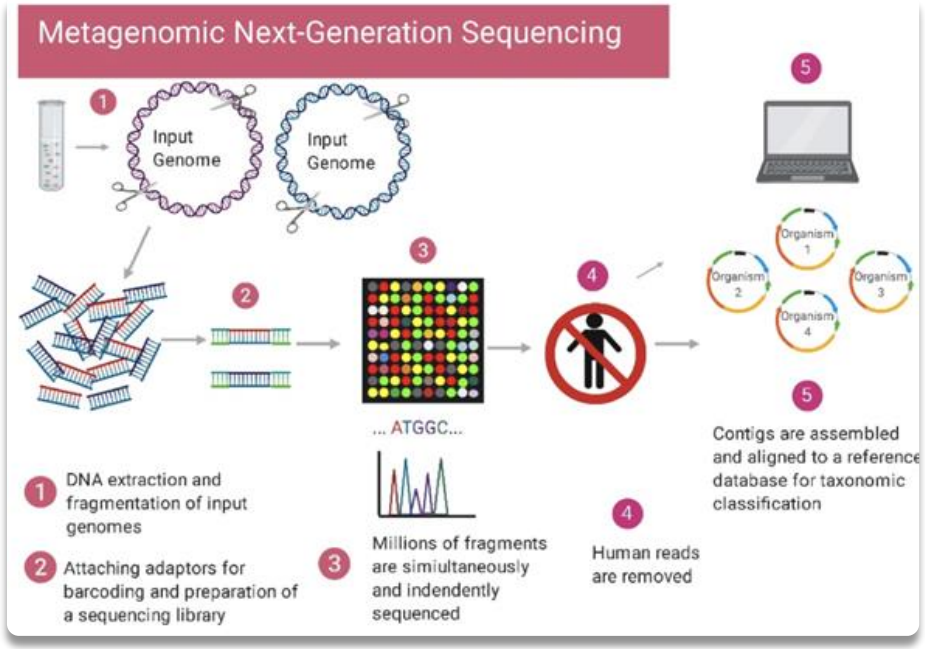
Matrix- assisted laser desorption/ionization



Infrared (IR) Spectroscopy



Next-Generation Sequencing

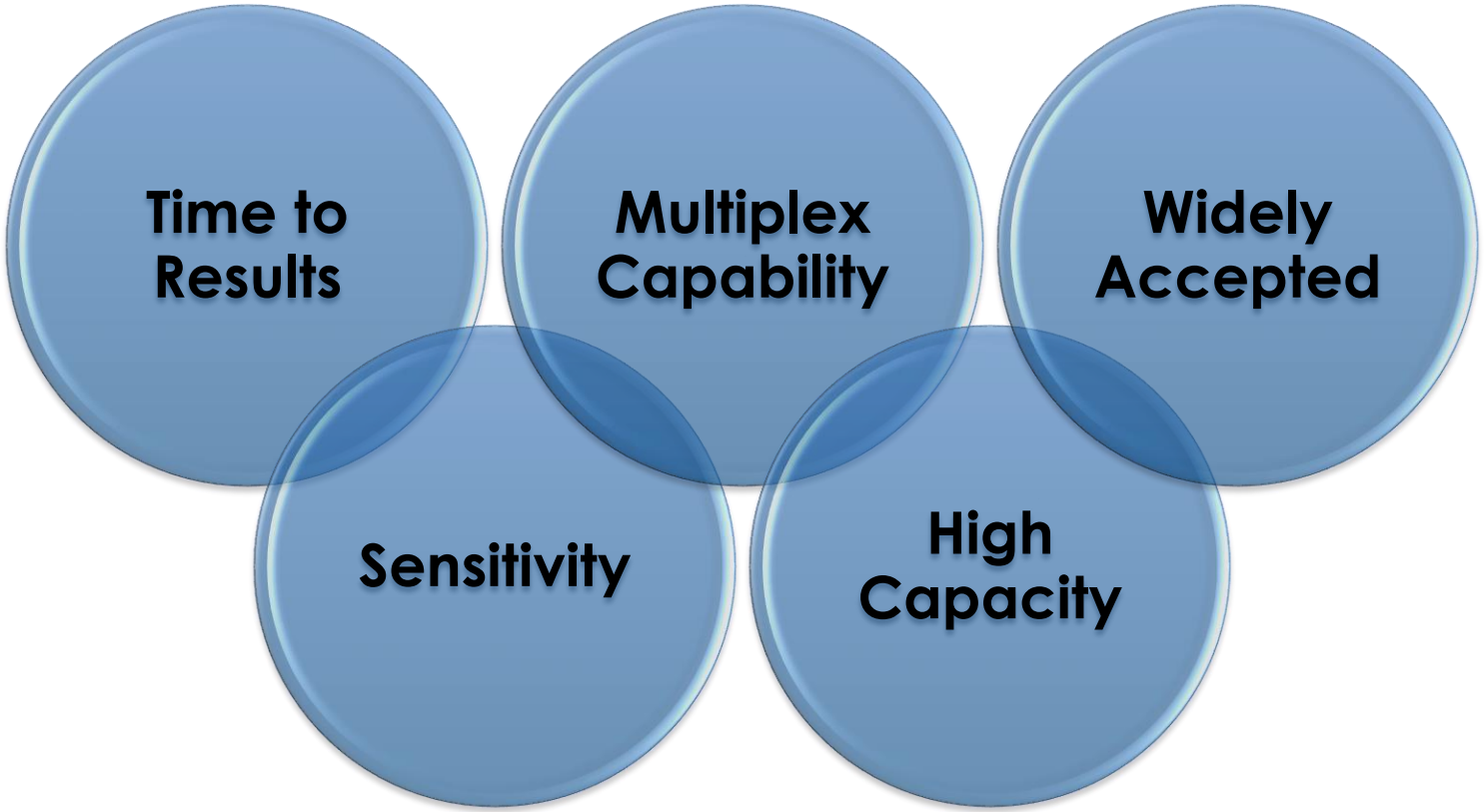


Metagenomic Next Generation Sequencing: How Does It Work and Is It Coming to Your Clinical Microbiology Lab?

American Society of Microbiology

Nov 4, 2019.

Pros with Molecular Methods



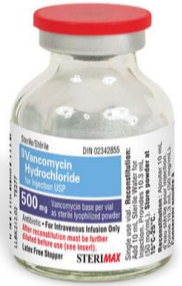
Cons with Molecular Methods

- Cost
- Technical Skill
- Spurious signals
  - Inhibition
  - False positive result

Overview

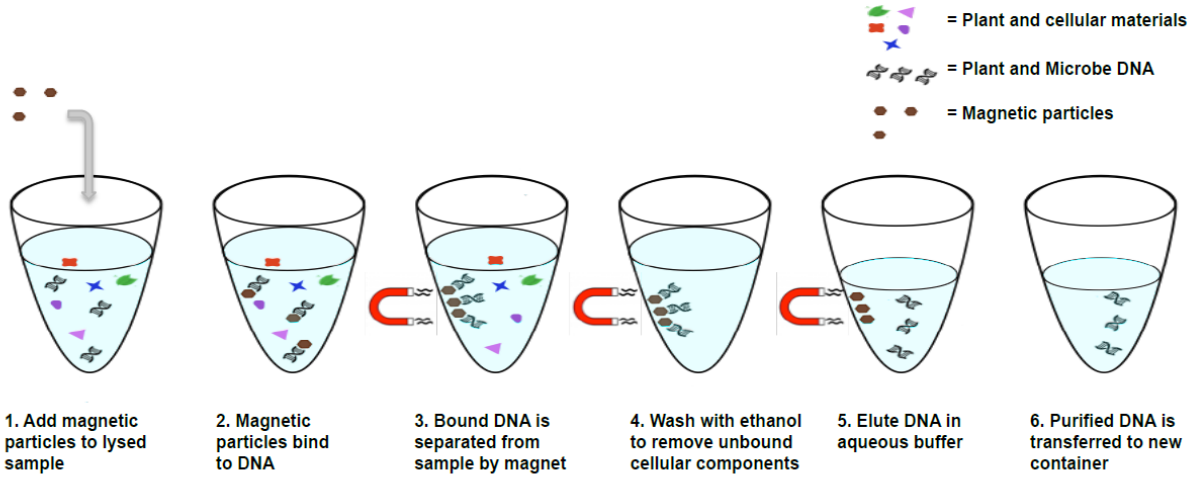
- Evolution of microbiology testing
  - How has testing evolved?
  - How do we improve the accuracy of molecular methods?
- **Testing modifications to eliminate “spurious” signals**
- Improvements in testing technology to focus on live-cell detection

Enrichment Media and Supplements

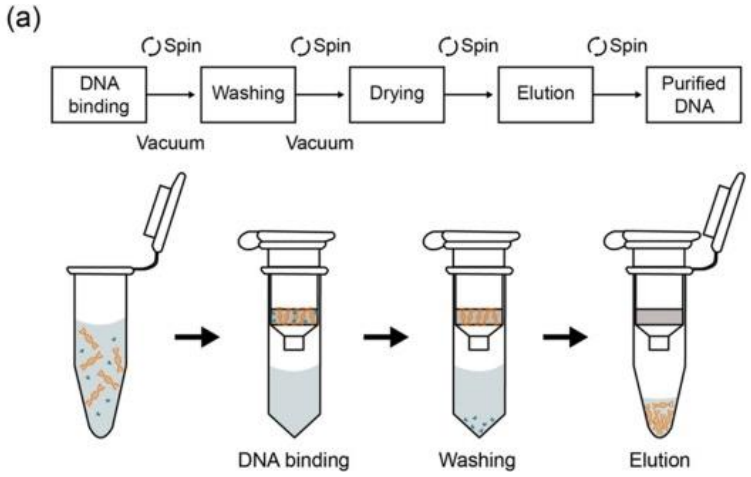


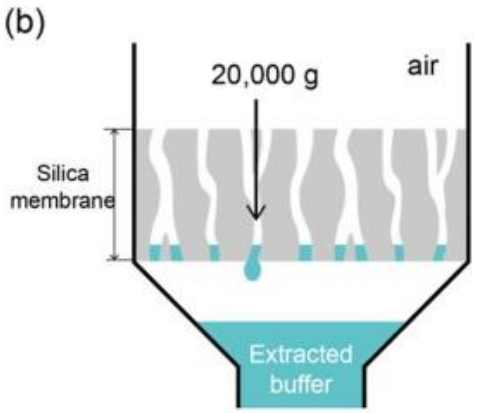
Extraction Processes

Process Overview



Filtration Processes





Improved Lysis/Purification Reagents

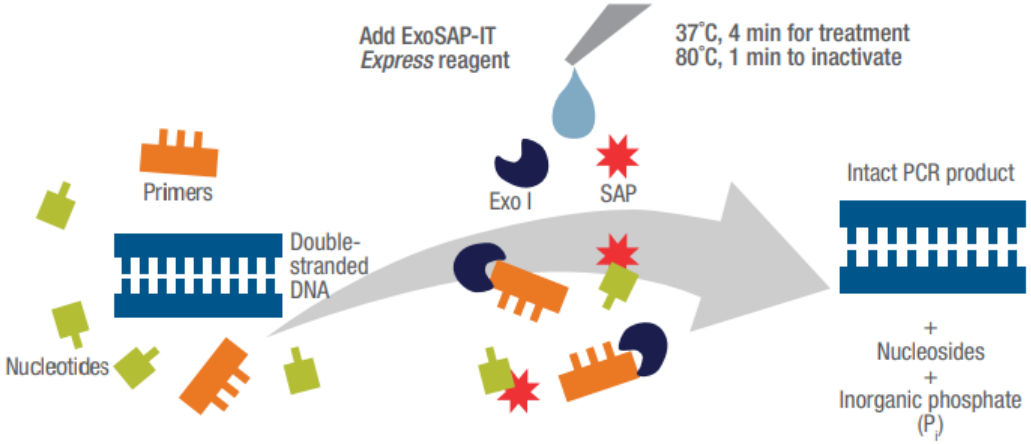
Enrich 1 gram matrix in 15ml mPDB

Transfer 500ul to 1.5ml tube and heat

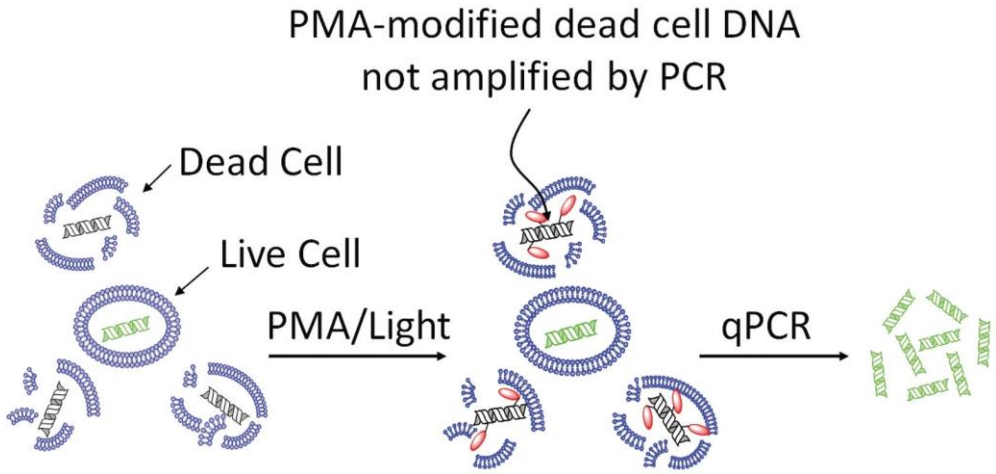
Add 50 µL to DIGEST tube, place tube in cycler and run DIGEST program (40 min)

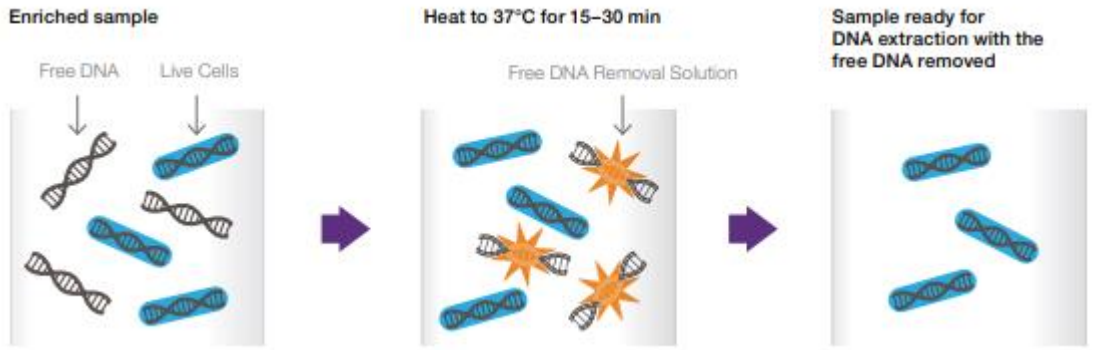
Add 5 µL to PCR tube, place tube in cycler and run VERIPRO *Aspergillus* program (2 hr)

GENE-UP®



Environmental DNA Removal Solutions



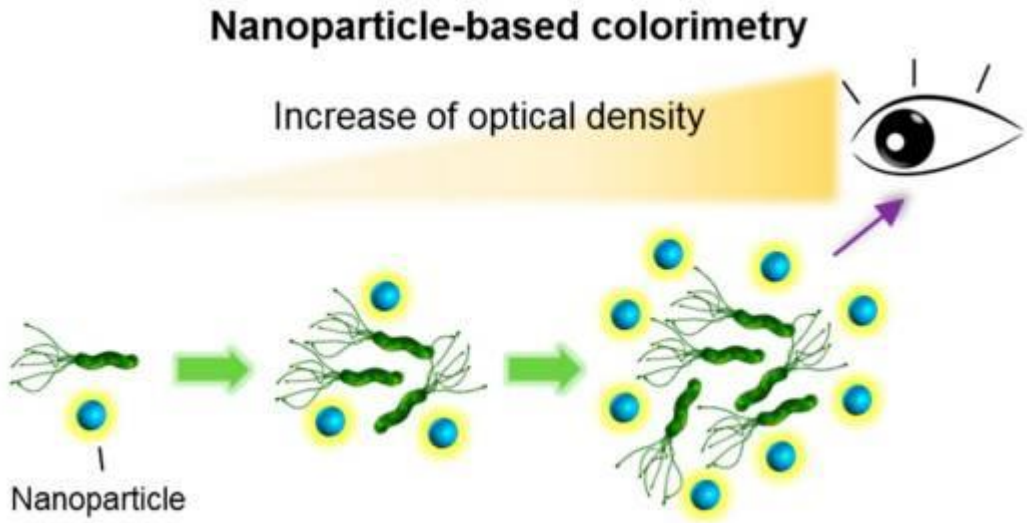


Overview

- Test sensitivity and (how) has it changed over the years
- Testing modifications to eliminate “spurious” signals
- **Improvements in testing technology to focus on live-cell detection**

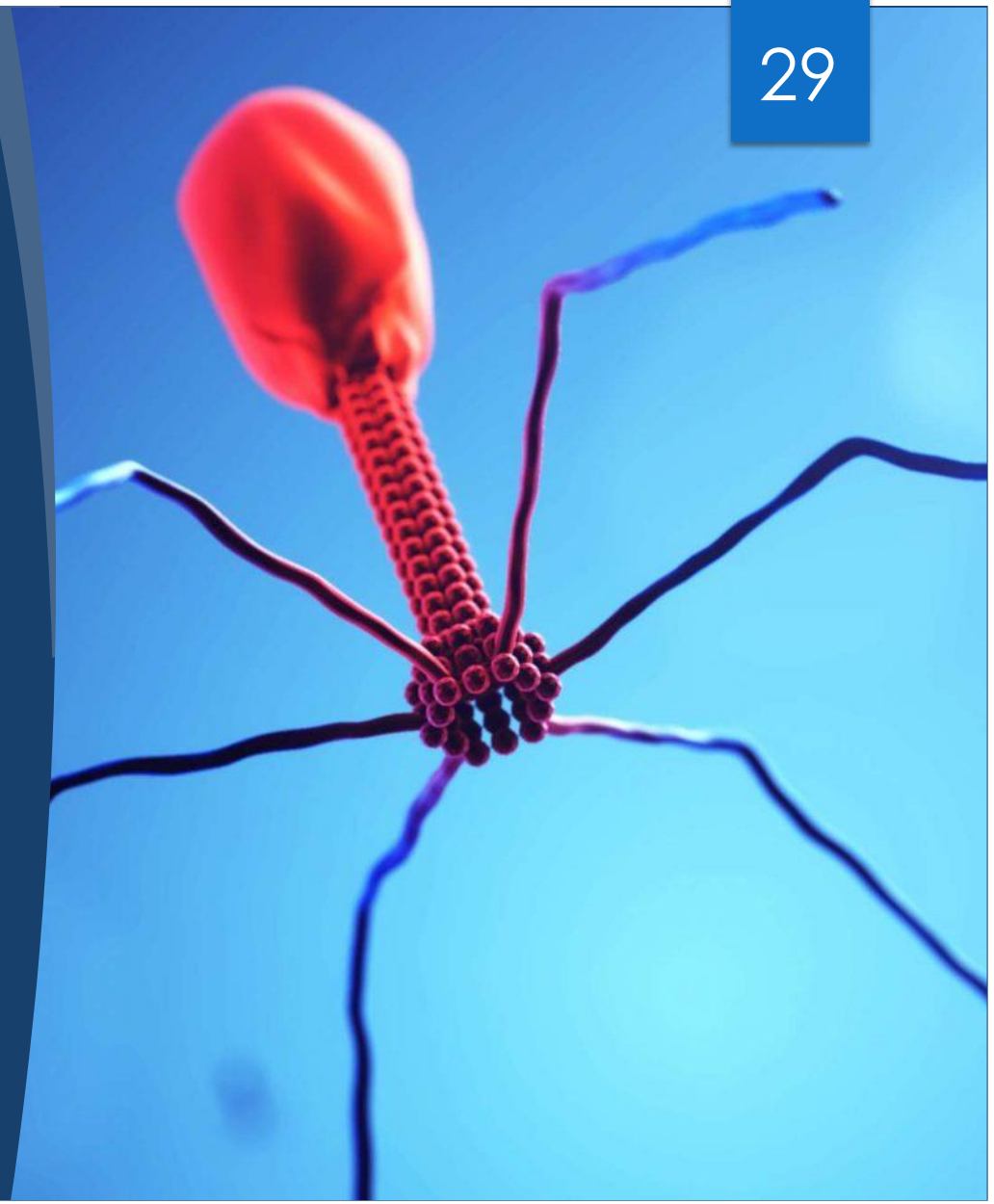
Enhanced Colorimetric Based Methods

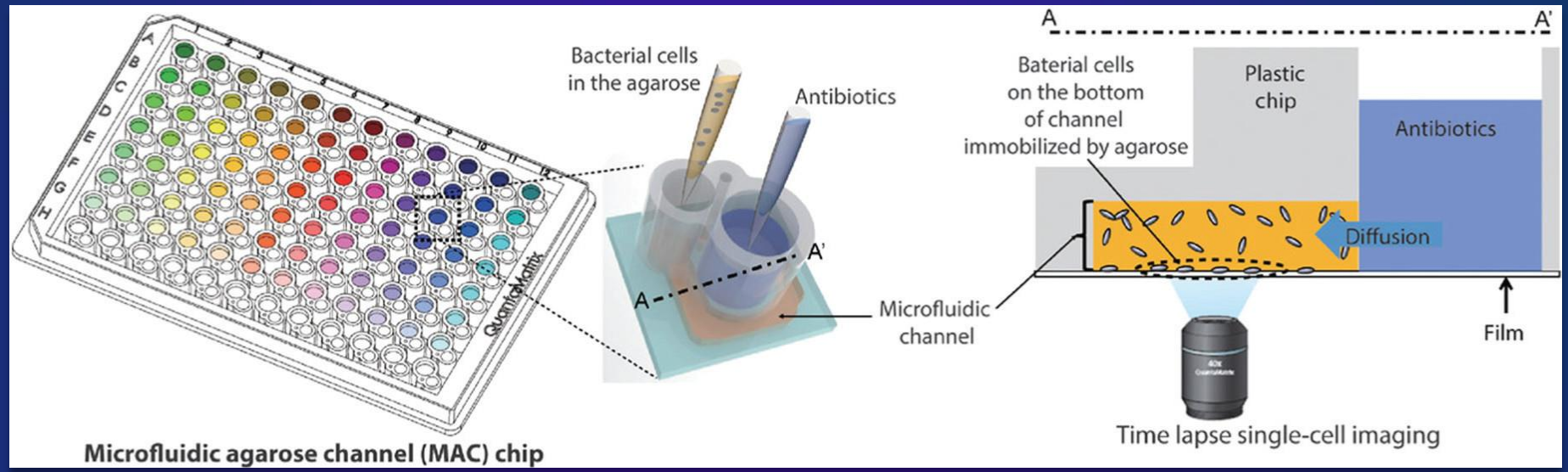
- Combine aspects of traditional colorimetric assays with the sensitivity of nanoparticles
- Target of nanoparticles can be varied with intent to capture viable bacteria or enzymes from viable bacteria
- Complexity of food matrix may lead to interference (non-detection/inhibition)
- Current validated methods for environmental surfaces and select matrices



Bacteriophage

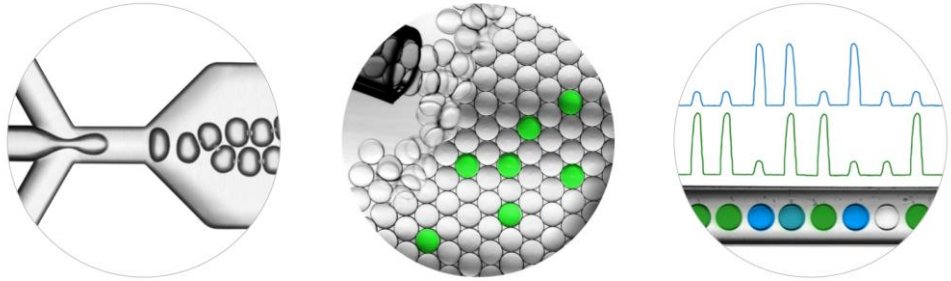
- Bacteriophage or bacteriophage receptor binding protein can be used for highly-specific bacterial separation from food matrices
- Bacteriophages can only replicate inside living cells, meaning that phage-based methods can be tests to demonstrate cell viability

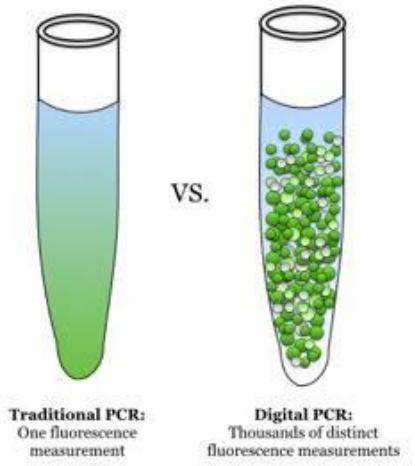




Microfluidics

Droplet Digital PCR



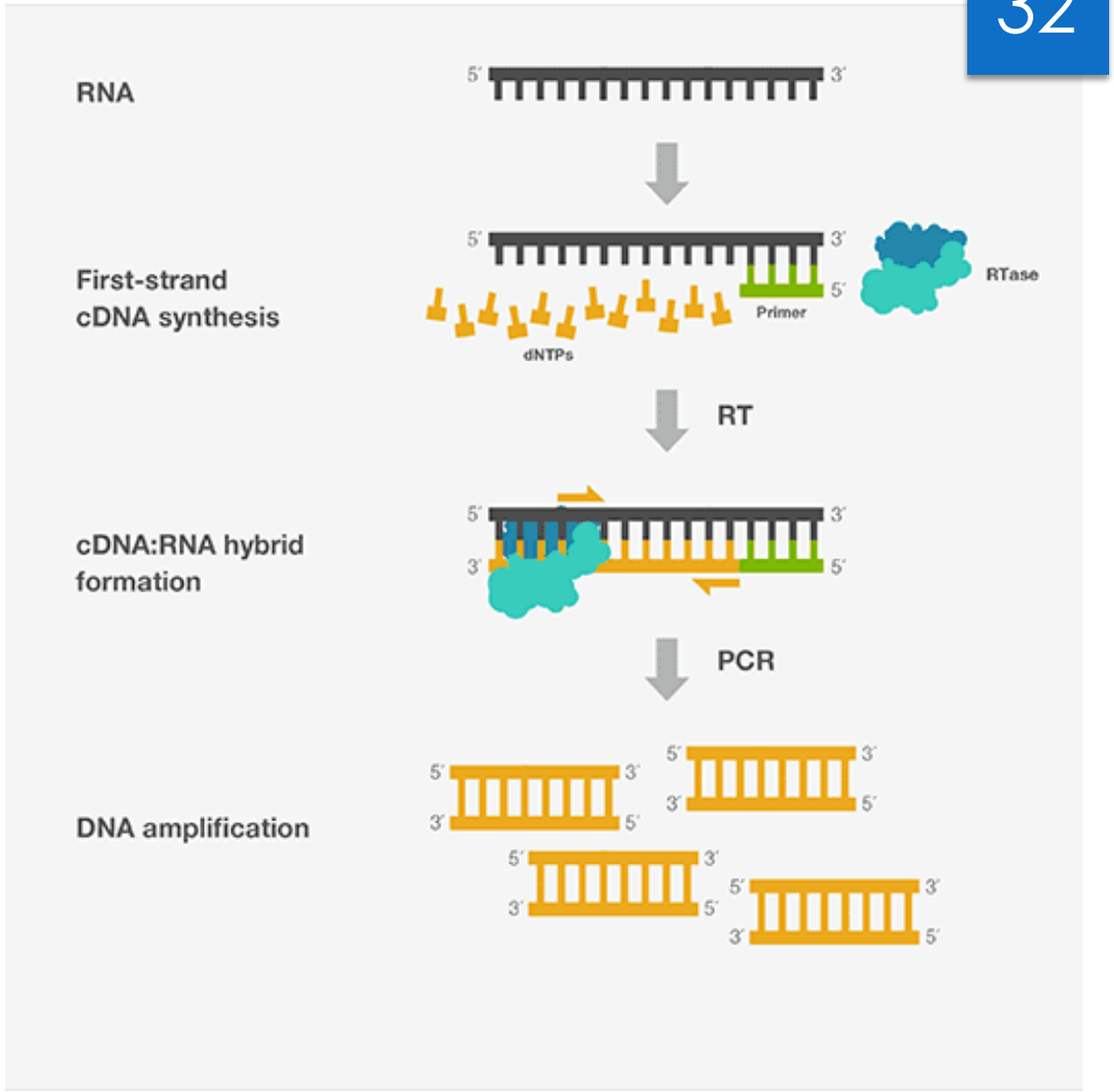


RNA/Reverse Transcription-PCR

The detection of messenger RNA (mRNA) is considered a better indicator of cell viability than DNA, since this molecule is only present in metabolically active cells.

Reverse transcription-PCR (RT-PCR) uses the reverse transcriptase enzyme to convert originally extracted mRNA into complementary DNA (cDNA).

The newly synthesized cDNA is then used as a template for exponential amplification using conventional PCR (RT-PCR) or quantification using quantitative PCR (RT-qPCR).



Questions???

consulting@pmbbiotek.com

Contact Information

Perry Johnson Laboratory Accreditation, Inc.

755 West Big Beaver Road, Suite 1325

Troy, MI 48084

Tel: (248)-519-2603

Website: www.pjlabs.com

Tracy Szerszen

PJLA

tszerszen@pjlabs.com

Patrick Bird

PMB BioTek Consulting

consulting@pmbbiotek.com