Reducing the risk of misdiagnosis with Quality Control

Marc MAURO
Technical Support Specialist Europe, Middle-East, Africa
mmauro@microibologics.com

Microbiologics®

Contents

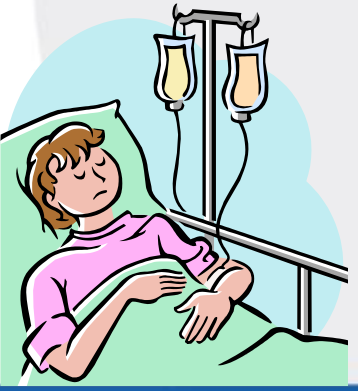
- Introduction
- Best practices for quality controls
- Quality controls and Proficiency testing
- Microorganisms maintenance
- Conclusion

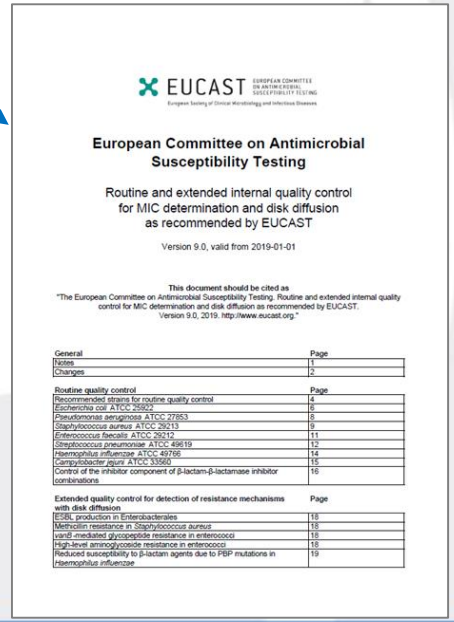
Introduction

Microbiologics®

Clinical Microbiology Laboratory

- Needs to know:
  - What microorganism is making the patient sick?
  - Which drug will make the patient better?
  - That their results are correct!





EUCAST

European Committee on Antimicrobial Susceptibility Testing

Routine and extended internal quality control for MIC determination and disk diffusion as recommended by EUCAST

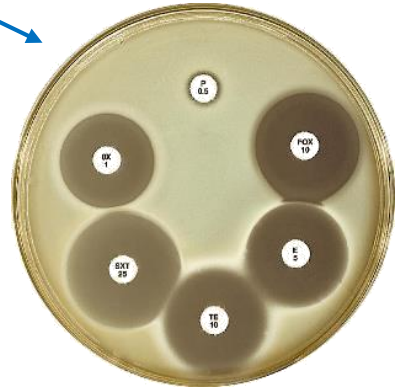
Version 9.0, valid from 2019-01-01

This document should be cited as
"The European Committee on Antimicrobial Susceptibility Testing. Routine and extended internal quality control for MIC determination and disk diffusion as recommended by EUCAST. Version 9.0, 2019. http://www.eucast.org."

General	Page
Notes	1
Changes	2

Routine quality control	Page
Recommended strains for routine quality control	4
Escherichia coli ATCC 25922	6
Pseudomonas aeruginosa ATCC 27853	8
Staphylococcus aureus ATCC 29213	9
Enterococcus faecalis ATCC 29212	11
Streptococcus pneumoniae ATCC 49619	12
Haemophilus influenzae ATCC 49766	14
Campylobacter jejuni ATCC 33560	15
Control of the inhibitor component of β-lactam-β-lactamase inhibitor combinations	16

Extended quality control for detection of resistance mechanisms with disk diffusion	Page
ESBL production in Enterobacterales	18
Methicillin resistance in *Staphylococcus aureus*	18
vanB-mediated glycopeptide resistance in enterococci	18
High-level aminoglycoside resistance in enterococci	18
Reduced susceptibility to β-lactam agents due to PBP mutations in *Haemophilus influenzae*	19



Microbiologics®

Technology evolution, which risks/benefits for the lab?

- Time to results ↘
- Multiplexing ↗
- Syndromic approach ↗
- Number of parameters per test ↗
- Specificity ↗
- Sensitivity ↗
- Effect of transport, storage… ↗
- Cost of failure ↗
- Kit manufacturer dependency ↗

Microbiologics®

Make sure evolution doesn’t become a risk

Feature	Risk
Specificity	Can vary from batch to batch
Sensitivity	Can vary from batch to batch
Affected by transport, storage…	Performance decrease: false negative, if detected by QC, no cost
Cost of failure	False negative/positive results, impact time to result and cost
Kit manufacturer dependency	Failure not detected by the manufacturer, if detected by QC, not cost

HIGH RISK traceability drift

- Cultures may not actually be those you thought they were:
  - Initially identified incorrectly
  - Contaminated on subculture
  - Genetically adapted to environment
  - Mislabeling

- Ramifications
  - Failure of selective media
  - Misinterpretation of sensitivity results
  - Incorrect identification of isolates
  - Failure of efficacy of antimicrobials
  - Failure in audits and loss of certification
  - Loss of clientele

Best practices in QC: ISO 15189 and EUCAST

Microbiologics®

ISO 15189 overview

- ISO standard written by medical lab professionals
- Set specific requirements for quality and competence in clinical labs
- For accreditation, not certification
  - Certification: gives written assurance od confirmation of specified requirements
  - Accreditation: confirms technical competence

What Are these Best Practices Based On?

- ISO 15189:2012 Medical laboratories - Requirements for quality and competence
  - Standard has been adopted by 60 countries including Canada, France, and the United Arab Emirates

- CLSI - Clinical Laboratory Standards Institute
  - Provides global standards and guidelines for the clinical laboratory

- EUCAST - European Committee on Antimicrobial Susceptibility Testing

1. Use QC materials with known value

- ISO 15189 Ch. 5.6.2.1: “The laboratory shall design quality control procedures that verify the attainment of the intended quality of results”

- EUCAST: “Use specified control strains to monitor the performance of the test”
- CLSI: “Each QC strain should be obtained from a recognized source (eg ATCC)”

- Use controls recommended by:
  - Standards: EUCAST, CLSI…
  - Manufacturer: Identification systems, PCR, serology…

2. Perform QC tests as required

- EUCAST: “Control strains should be tested daily until performance is shown to be satisfactory at which stage testing frequency may be reduced to once a week.”

- CLSI: “Routine QC is performed each day the test is performed unless an alternative QC plan has been established”…“Upon successful completion, the laboratory can convert from daily to weekly QC testing”.

- Manufacturers recommend to perform QC with every batch or delivery

3. Perform QC tests as required

- 5.6.2.2 Quality control materials
  - The laboratory shall use **quality control materials that react** to the examining system in a manner **as close as possible to patient samples**.
  - Quality control materials shall be periodically examined with a frequency that is based on the stability of the procedure and the risk of harm to the patient from an erroneous result.
  - Use of **independent third party control materials should be considered**, either instead of, or in addition to, any control materials supplied by the reagent or instrument manufacturer.

Internal control is not third party quality control

- In 2017 Roche had recalled several batches of consumable for diagnostics purpose in UK

- The control provided was designed to always pass the QC
- Need for a third-party control provider

- Internal controls
  - Often included in the kit
  - Target a different target a different sequence
  - Do not provide the guarantee that the detection is possible
  - Just demonstrate the absence of inhibition

4. Monitor media, reagents, stains, antigens

- ISO 15189 Ch 5.3.2.3: "**New lot or shipment, shall be verified for performance before use in examinations**".
  - Ready to use media
  - Bottled media
  - Laboratory prepared media

- Physical and chemical parameter can influence the results
  - Storage temperature (and transport)
  - pH
  - Lot to lot composition difference
  - Prep date and expiration
  - Manufacturer

5. Monitor equipment

- ISO 15189 Ch 5.3.12: “The laboratory shall verify upon installation od before use that the equipment is capable of achieving the necessary performance”

- Follow manufacturer recommendations for:
  - Preventive maintenance
  - Quality control

6. Train and monitor personnel

- ISO 15189 Ch 5.1.5: “The laboratory shall provide training to all personnel”
  - Education program activities for the technicians
  - Use of reference material: book, slides, strains, parasite suspensions

- ISO 15189 Ch 5.1.6: “The laboratory shall assess the competence of each person”
  - Direct observation
  - Monitoring work records and results
  - Testing unknowns

7. Participation in proficiency testing

- ISO 15189 Ch 5.6.3.1: “The laboratory shall participate in an inter-laboratory comparison program such as an external quality assessment program or a proficiency testing program”

- Proficiency tests for bacteriology labs may include
  - Gram stain
  - Antigen testing
  - Identification
  - Susceptibility testing

Quality control and proficiency testing

Microbiologics®

Definitions: Proficiency Testing

- Key element in the laboratory accreditation process, enabling labs to monitor the quality of their analytical results as stipulated in ISO accreditation

- Determines the performance of individual laboratories for specific tests or measurements and is used to monitor laboratories' continuing performance.

- Proficiency testing is also called interlaboratory comparison or External quality assessment (EQA). As this term implies, proficiency testing compares the measuring results obtained by different laboratories.

Definitions: Quality Control

- A process by which the lab review the quality of all factors involved. This approach places an emphasis on three aspects:
  - Elements such as controls, job management, defined and well managed processes, performance and integrity criteria, and identification of records
  - Competence, such as knowledge, skills, experience, and qualifications
  - Soft elements, such as personnel and quality relationships.

- The quality of results is at risk if any of these three aspects is deficient in any way.

- In molecular, internal amplification control is not Quality control
  - Only check that a negative test is not a false negative: amplification inhibition…

Quality Control vs Proficiency Testing

Quality control (QC)

- Verifies a test or equipment is providing correct results
- Should be conducted on a routine basis
- Cultures have been tested and designed for quality control testing
- Quality Control material producers can be accredited by a number of accreditations – Microbiologics is ISO 9001, ISO 17025, and ISO 17034 accredited

Proficiency Testing (PT)

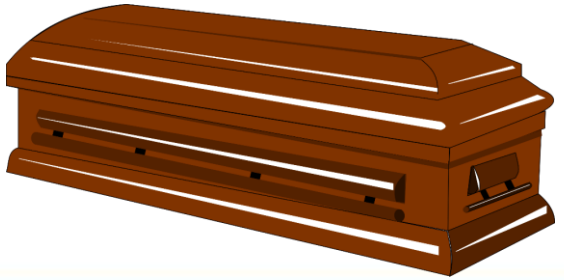
- Verifies an individual is performing a test properly and obtaining the correct results
- Should be conducted periodically
- PT samples are designed as unknown samples to be identified and then discarded
- Proficiency Providers are ISO/IEC 17043 or NELAC accredited

Quality Control vs Proficiency Testing

	Quality control	Proficiency testing
Source of QC material	Recognized collections EUCAST and manufacturer's instructions	Wild types
Frequency	EUCAST: every week Manufacturers: every batch or delivery	Vary: 2, 3, 4 times per year depending on the program
Results comparison	To standard known values	To the group of participants
Outcome	Make sure each step is under control Prepare for PT	Confirm all steps together work as requested

False negative vs false positive

- **False positive**: announcing to the patient he has a multi-resistant *Staphylococcus aureus* when he hasn't
  - Unnecessary treatment: cost for the patient and the healthcare, may favor the emergence of resistant strain
- **False negative**: announcing to the patient he doesn't have a multi-resistant *S.aureus* when he has
  - Absence of necessary treatment: the patient will not be cured and will come back for another test, with consequences on his health
- In both cases:
  - High risk for the patient's health
  - Loss of trust in your laboratory services



Royal treatment

- Make your results and group results inaccurate
- Only fool yourself
- It will not inform you about any deficiency
  - Technique
  - Equipment
  - Personnel competence

- Not giving the royal treatment to PT sample only has benefits
  - Good results: give you the insurance that you are working well
  - Wrong results: will not lead to loss of accreditation and will help you to understand where the improvements needs to be

Microorganisms maintenance: saving with compliance

Microbiologics®

Why is it important to know the number of passages?

- A passage is defined as a subculture involving growth of the viable microorganism with fresh medium.
- Microorganisms for standard protocols should be used within five passages of the ATCC reference culture.
- Many guidelines state a maximum number of passages are allowed
  - Additional passages have been demonstrated to show mutations
  - Freezing and thawing an organism can lead to mutations
  - This has been demonstrated clearly when testing for antibiotic sensitivities

Why Cryobeads Should Be Avoided!

- Risk of contamination
- Risk of mislabelling
- Risk of mutation
- Risk of incorrect temperature storage
- Needs to be validated for
  - Each strain
  - During the shelf life you plan to use them and before using them
- Lack of traceability due to broken chain
- No longer represents a control from outside
- Does not really save on cost

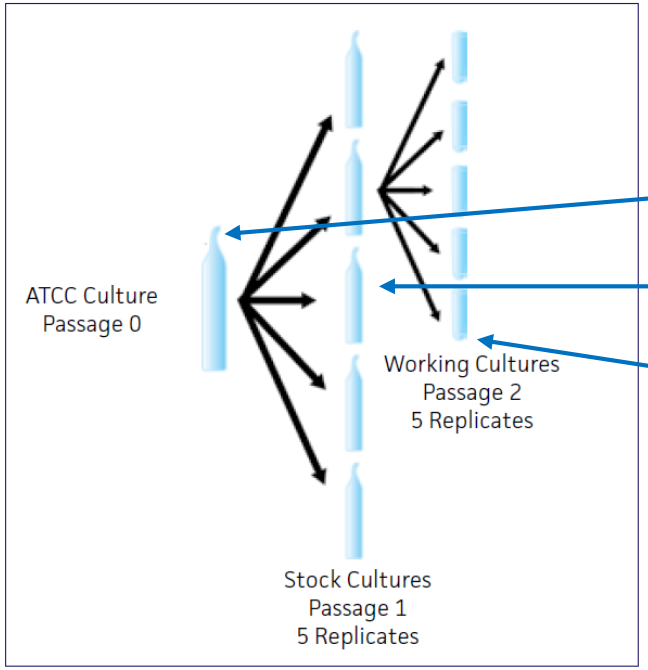
- Plasmid mediated resistance
- Fastidious organisms

Microbiologics®

Why is it important to know the number of passages?

- Stock cultures can be subcultured for working cultures weekly, typically kept as slants. A seed lot system is recommended.

Figure 1. Seed lot system

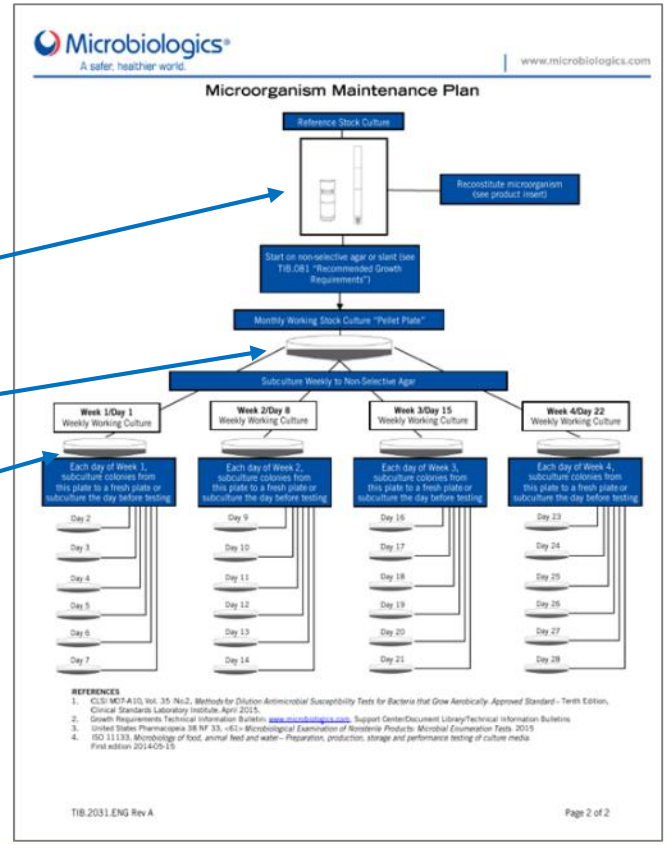


Monthly maintenance protocol

Original culture

Monthly stock culture

Working culture



Strain maintenance: the real cost

Frequency of test	52	weeks per year
Nb of strains used	12	
Culture media cost	0,20 €	/plate
Labour cost	13 €	/h
Identification cost	5 €	/strain

Frequency according to EUCAST

Average number of strains used

Average labor cost in Estonia

Time

Frozen cultures

	Time (min)
Culturing from KWIK STIK on mother plate	1
Sub-culturing	2
Aliquoting on beads (for 1 year stock)	15
Thawing 1 bead and culturing	4
Sub-culturing to get working culture/year	104
Identification control after thawing and subculturing	520
Total maintenance time (h/year)	**129,2**
Cost of maintenance frezing the strains	**1 680 €**

Microbiologics Maintenance protocol

	Time (min)
Culturing from KWIK STIK on mother plate	1
Sub-culturing to get working culture /week	2
Total maintenance time (h/year)	**23,2**
Cost of maintenance with maintenance protocol	**302 €**

Resources

Nb agar plates/year	1248
Cost of culture media for strain maintenance	249,60 €
Cryobeads, price per unit	1,20 €
Freezer, cost per day	2,00 €
Price per year for all the strains	748,80 €
Cost of identification reagent/year	3 120,00 €
Material cost/year	**4 848,40 €**

Nb agar plates/year	768
Cost of culture media for strain maintenance	153,60 €
Material cost/year	**153,60 €**

Microbiologics®

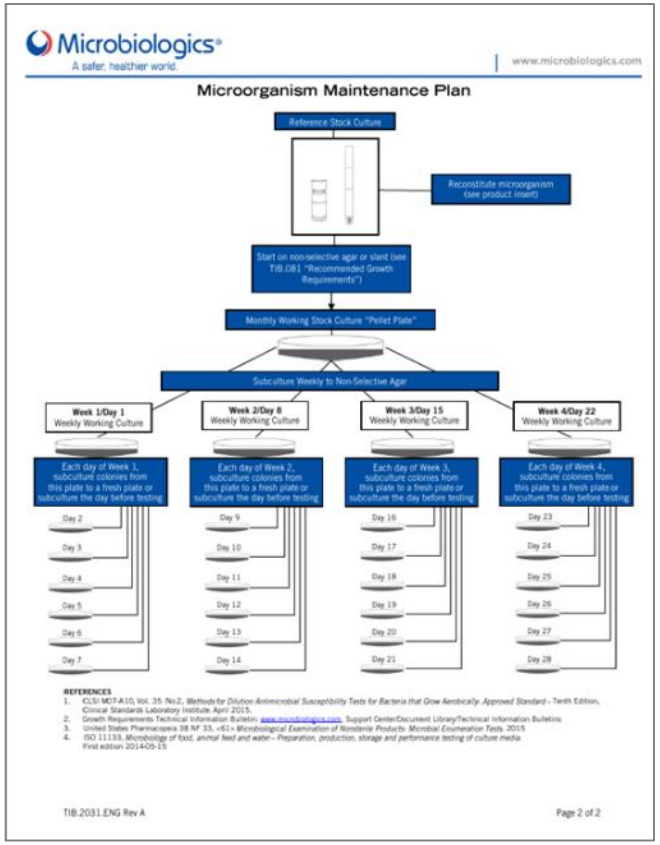
Strain maintenance: the real cost

- Yearly impact of our maintenance protocol, developed according to ATCC and CLSI

- **Time saved: 15,1 working days** per year
- **Money saved: 6073€** per year

- What would you do with **3 weeks of technician time and 6073€ per year**?

- And it doesn't take in account the cost of possible QC failure



Microbiologics®

Conclusion

Microbiologics®

YOU CANNOT AFFORD not to do Quality Control

- **ISO 15189** can help to get the best practices for the lab, optimizing the lab's workflow
- **Proficiency Testing** and Quality Control are not the same but both need to be done. Good QC leads to high scoring PT and no risk of misdiagnosis!
- **Strain maintenance**, when done properly, can have a significant impact on time and budget
- **Molecular controls** should be done as well as the culturable controls, the cost of misdiagnosis will be higher than for traditional methods when you will need to retest
- **The cost of non conformity**: curative is always more expensive than preventive

Cultural methods: KWIK STIK

Packaging/contents

- Each self-contained unit includes a microorganism pellet, hydrating fluid and a swab. Packaged in a foil pouch
- Available in packs of 2 or 6
- Ready to use
- 3 passages
- Store at 2-8°C
- CE IVD

Applications/specifications

- Culture QC
- QC ID Instruments
- QC Antimicrobial Susceptibility Tests

Microbiologics®

Molecular QC Sets: QC for commercial diagnosis kits

Packaging/contents

- Instrument or test specific
- Inactivated organisms
- Multiplex kit
- Developed with the main players

Applications/specifications

- Full process control
- Sensitivity & Specificity
- Validation & Verification
- Training
- Proficiency Testing
- Lot-to-Lot Testing
- QC of Molecular Instruments

Cepheid A better way. BD Luminex® BIOMÉRIEUX INDUSTRY … Microbiologics®

Conclusion

- The common points between an aircraft pilot and an healthcare microbiologist?

- They are responsible for other's life
- They are regularly trained to deliver the best quality of results
- They don't skip quality controls

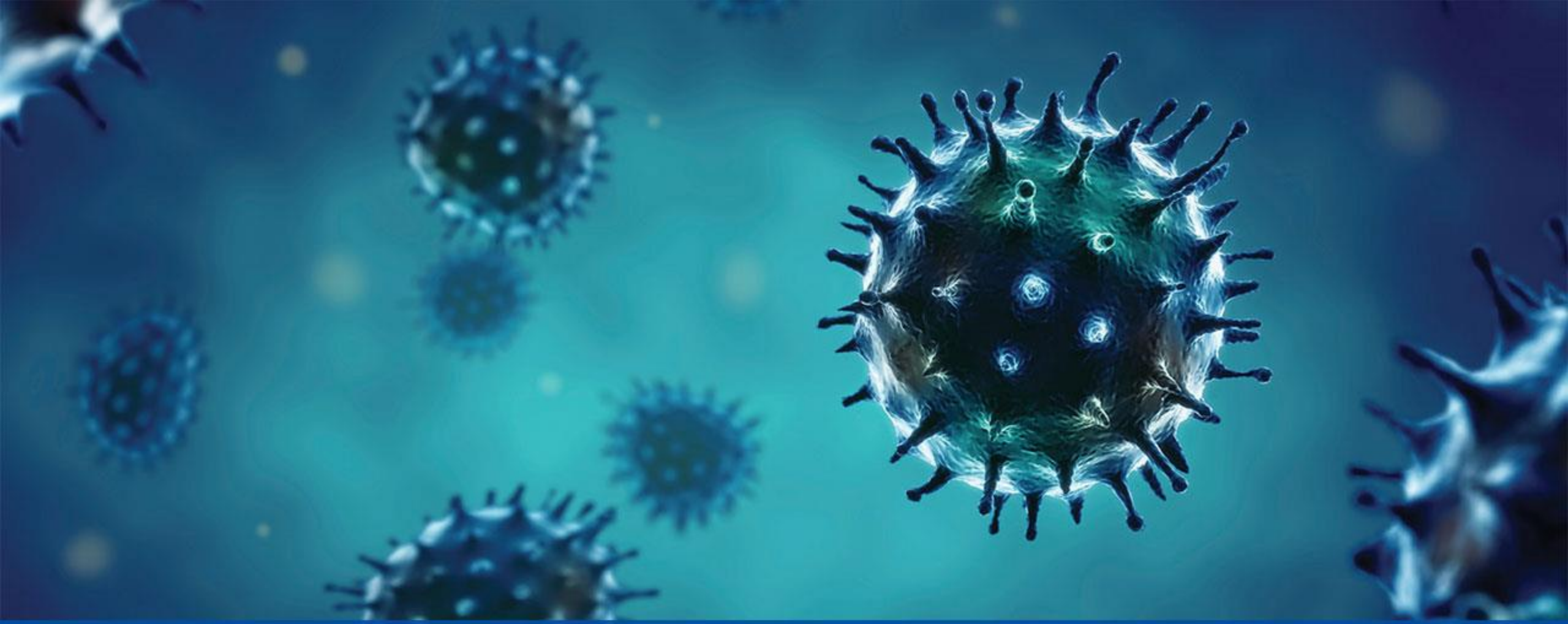
Microbiologics®

And never forget…

- Controls are the foundation of product manufacturing
- They do represent a small budget in any lab

- We are all patients some time, expecting having safe and reliable analysis to be cured the right way

Microbiologics®



Thank you for your attention

Microbiologics®