Past, Present, and Future State of Mycoplasma Testing

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Presentation Overview

1. **Mycoplasma Biology**
2. **Past State**
   A. Broth/Agar
   B. DNAF
3. **Current State**
   A. Real-time PCR
   B. Validation Guidelines
4. **Future State**
   A. In-line/At-line Testing
   B. Next Generation Sequencing
   C. New Technology Implementation Considerations

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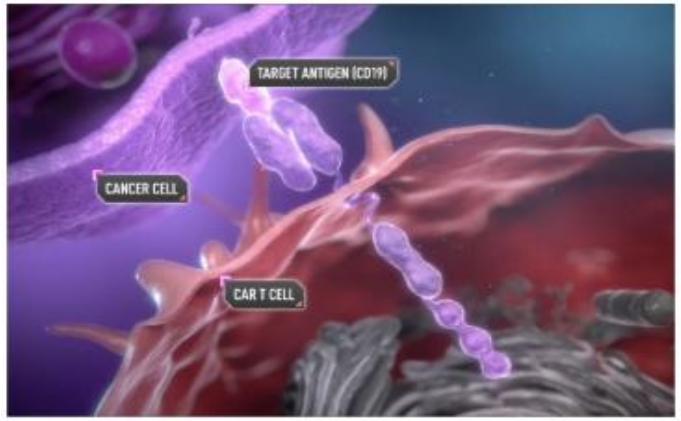
PERSONALIZED, SINGLE-INFUSION, CAR T IMMUNOTHERAPY

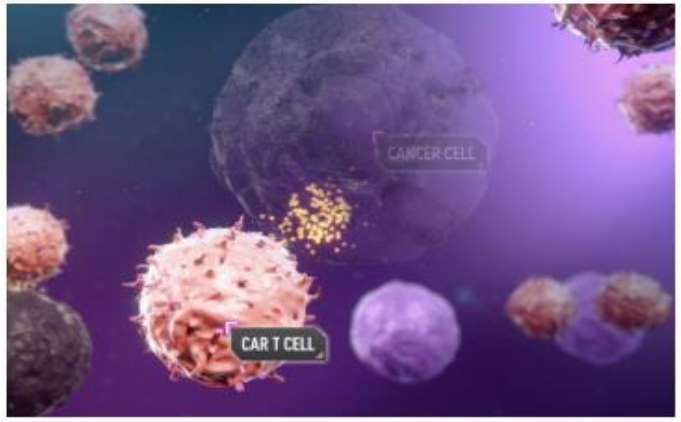


TARGET CD19

Axi-cel uses an anti-CD19 extracullular domain to target and bind to CD19 on the surface of healthy and cancerous B cells

ACTIVATE CELLS

Following antigen binding, the CD28 costimulatory domain works with the CD3ζ activation domain to enhance activation and proliferation of CAR T cells.



ELIMINATE B CELLS

CAR T activation allows the release of inflammatory cytokines and chemokines that leads to the elimination of CD19-expressing cells.

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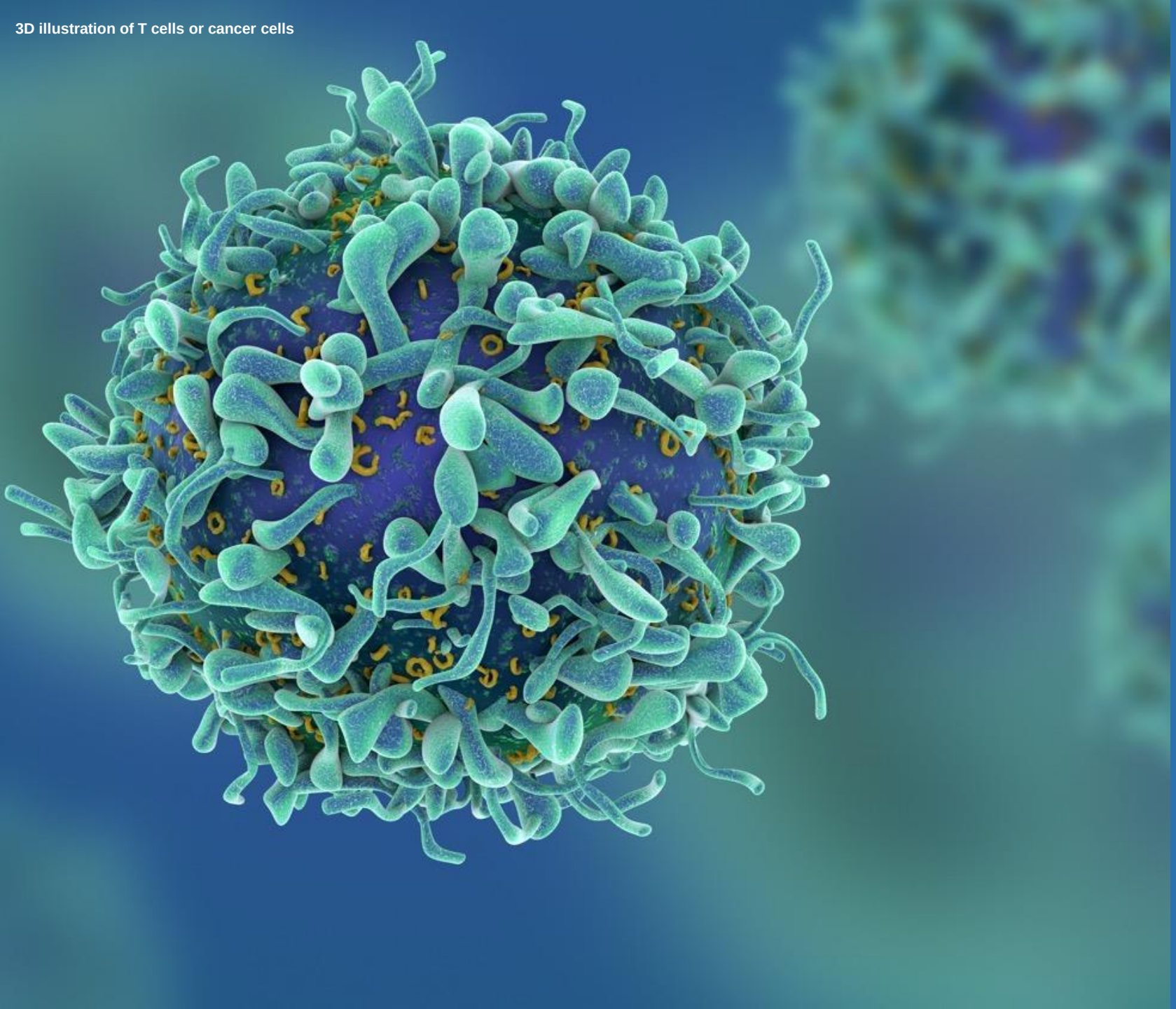

Mycoplasma Biology

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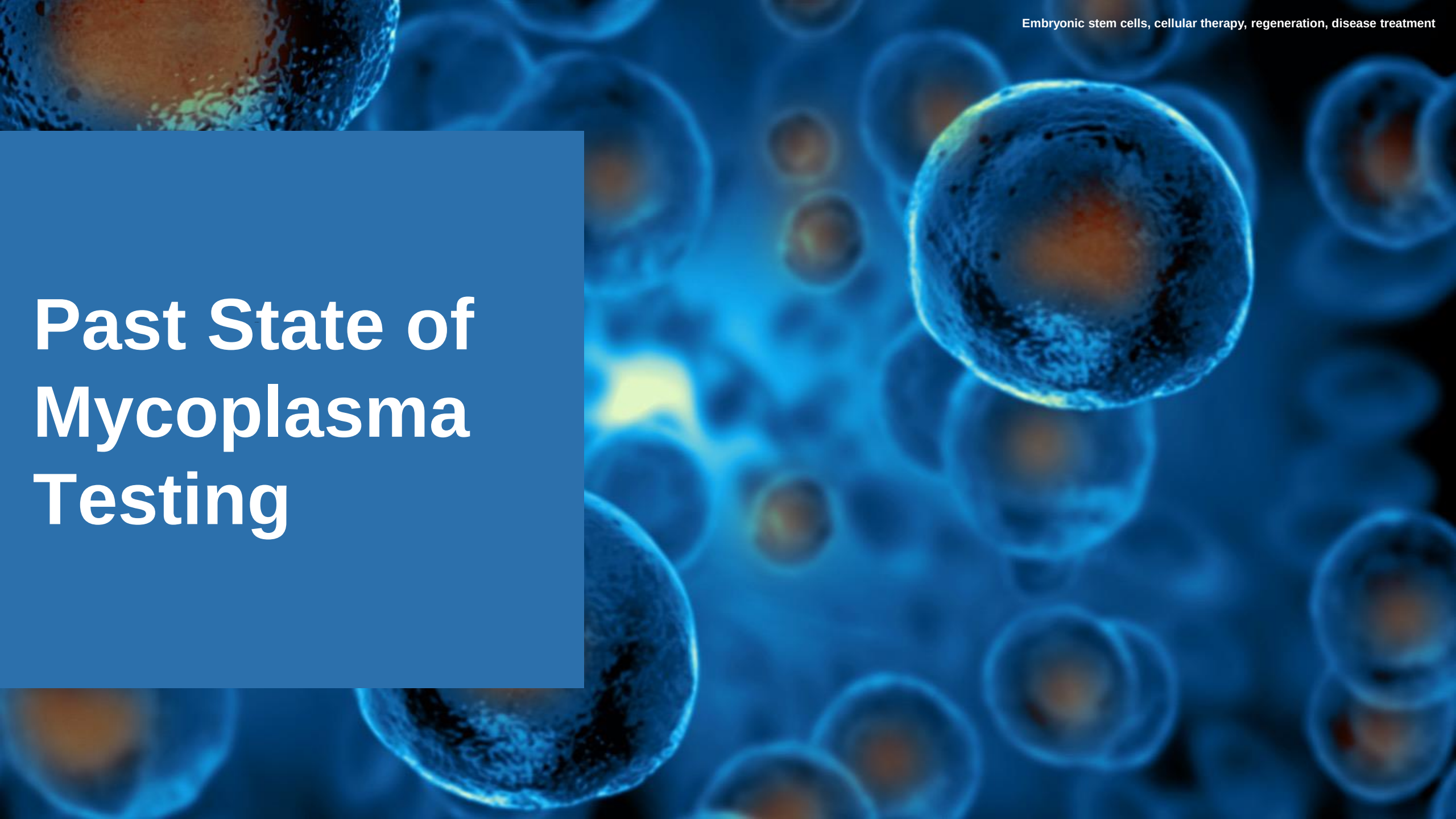
Mycoplasma

- Affect cell cultures negatively through cell death and altered host cell metabolism
- Difficult to detect microscopically
- Lack a cell wall
- Cannot be retained using a 0.2µm rated filter and may pass through 0.1µm filters
- A reported 15% of US cultures screened for Mycoplasma are infected[1]

[1]*Barile M.F., and Razin, S. (1993) Mycoplasmas in cell culture. In Rapid Diagnosis of Mycoplasmas (Kahane I., and Adoni, A., eds) pp. 155-193, Plenum Press, New York.*



Past State of Mycoplasma Testing

Agar/Broth and DNAF Host-Cell

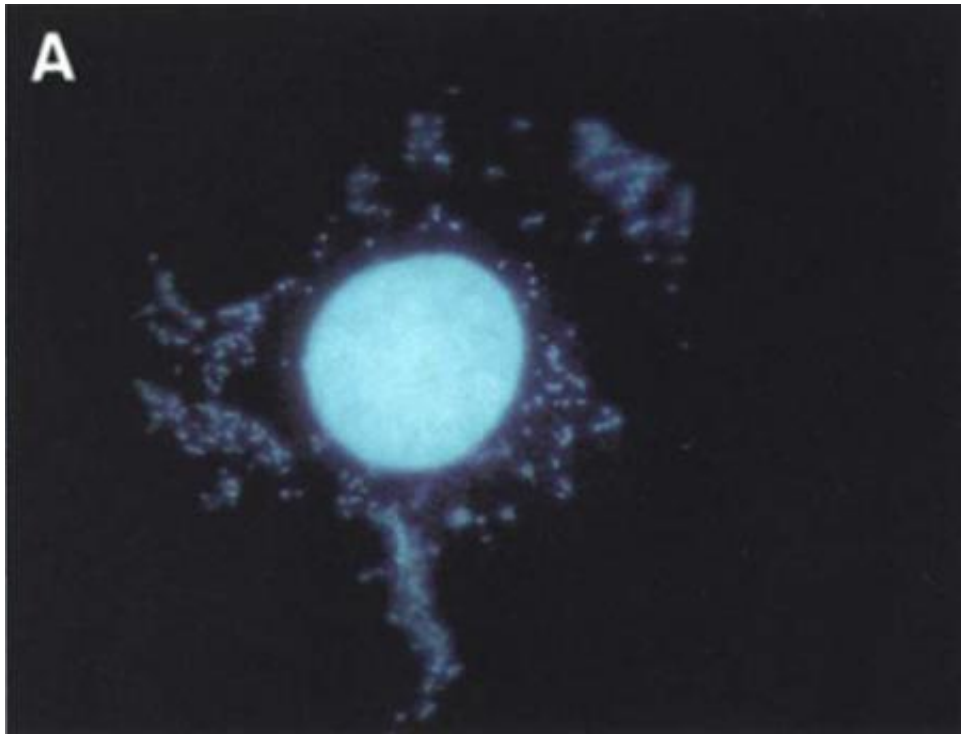
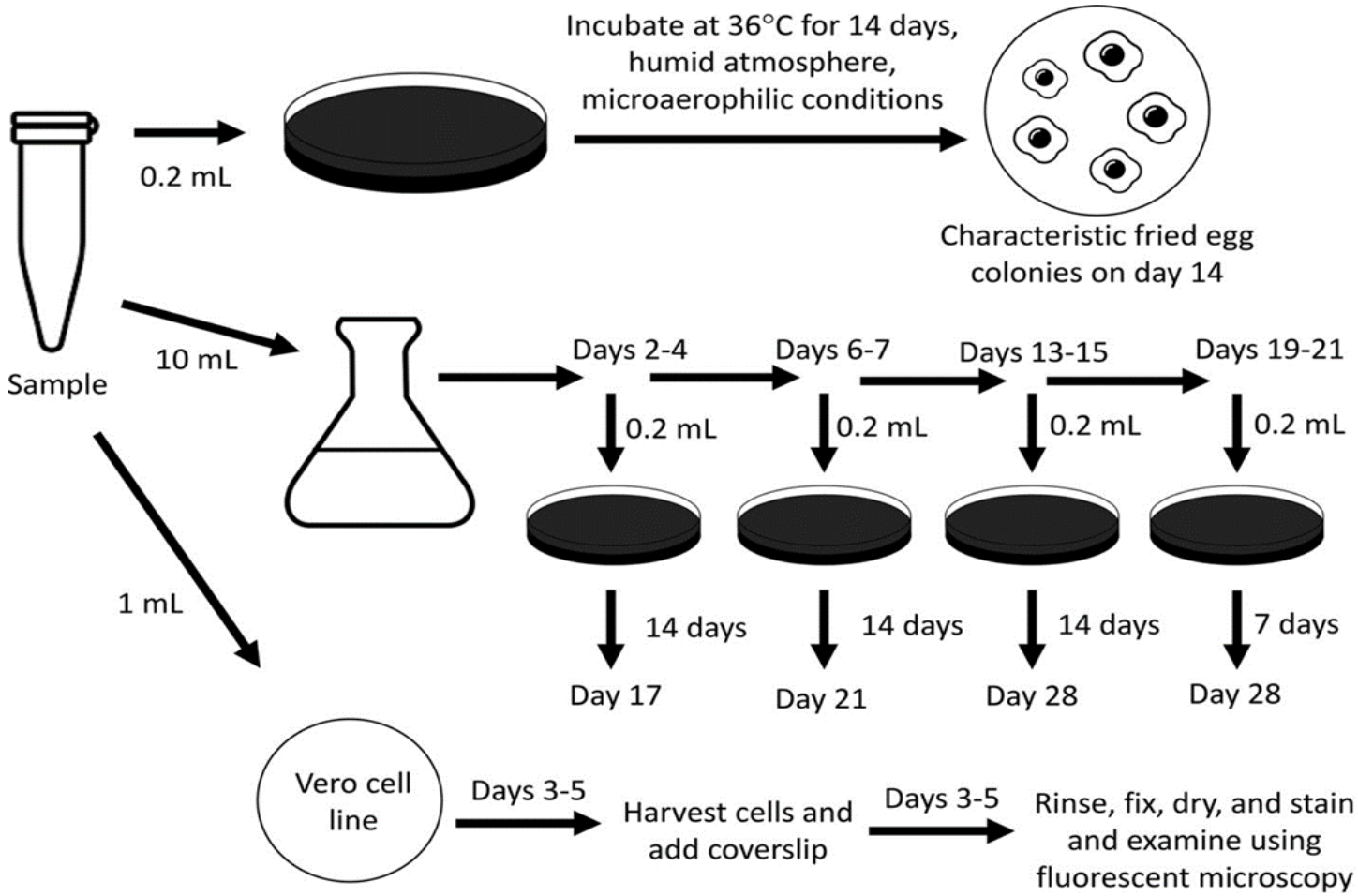


Figure A: Masover G.K., Becker F.A. (1998) Detection of Mycoplasmas in Cell Culture by Fluorescence Methods. In: Miles R., Nicholas R. (eds) Mycoplasma Protocols. Methods in Molecular Biology™, vol 104. Humana Press

Summary of methods required for USP <63> Mycoplasma testing



Method 1.
Agar cultivation for viable *Mycoplasma*

Method 2.
Broth cultivation for viable *Mycoplasma*

Method 3.
Indicator cell line for non-agar cultivable *Mycoplasma*

James E. T. Gebo, and Anna F. Lau J. Clin. Microbiol. 2020; doi:10.1128/JCM.01492-19

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Comparison of USP, EP, and JP

	USP Chapter 63	EP 2.6.7. Mycoplasmas	Japanese Pharmacopoiea (JP) XVII
Positive Controls:	When testing for Mycoplasmas Include in each test at least **two** known Mycoplasma species or strains (listed in Quality Control Test Strain Organisms) as positive controls, one of which should be a dextrose fermenter (i.e. **M. pneumomiae** or equivalent species and strain) and one of which should be an arginine hydrolyzer (i.e., **M. orale** or equivalent species and strain)	When testing for mycoplasmas in the product to be examined, at least **1** of the following species will be included as a positive control: – Acholeplasma laidlawii (vaccines for human and veterinary use where an antibiotic has been used during production); – Mycoplasma gallisepticum (where avian material has been used during production or where the vaccine is intended for use in poultry); – Mycoplasma **hyorhinis** (non-avian veterinary vaccines); – Mycoplasma **orale** (vaccines for human and veterinary use); – Mycoplasma **pneumoniae** (vaccines for human use) or other suitable species of d-glucose fermenter such as Mycoplasma fermentans; – Mycoplasma synoviae (where avian material has been used during production or where the vaccine is intended for use in poultry).	To demonstrate the capacity of the media to detect known mycoplasma, each testshould include control cultures of at least **two** known speciesor strains of mycoplasma, one of which should be a dextrosefermenter (i.e., **Mycoplasma pneumoniae** ATCC 15531, NBRC 14401 or equivalent species or strains) and one of which should be an arginine hydrolyser (i.e., **Mycoplasma orale** ATCC 23714, NBRC 14477 or equivalent species or strains).
Test Condition:	Inoculate no less than 10 mL of the test article/material per 100 mL of each liquid medium. Inoculate 0.2 mL of the test article/material on each plate of each solid medium. Incubate liquid media for **20–21** days. Incubate solid media for not less than **14** days, except those plates corresponding to the **20–21** day subculture, which are incubated for **7** days. Concurrently, incubate an uninoculated 100-mL portion of each liquid medium and agar plate, as a negative control. On days **2–4** after inoculation, subculture each liquid medium by inoculating 0.2 mL on at least 1 plate of each solid medium. Repeat the procedure between days **6 and 8**, again between days **13 and 15,** and again between days **19 and 21** of the test. **Observe** the liquid media every **2 or 3** days and if a color change occurs, subculture. If a liquid medium shows bacterial or fungal contamination, the test is invalid. The test is valid if at least 1 plate per medium and per inoculation day can be read. Include in the test positive controls prepared by inoculation of not more than 100 cfu of at least 1 test microorganism on agar medium or into broth medium. Where the test for Mycoplasmas is carried out regularly, it is recommended to use the test microorganisms in regular rotation. The test microorganisms used are those listed under Choice of Media. Incubate broths and plates in a humidi□ed atmosphere with **microaerophilic conditions (5%–10% CO).**	Incubate liquid media in tightly stoppered containers at 35-38 °C. Incubate solid media in microaerophilic conditions (nitrogen containing **5-10 per cent of carbon dioxide** and sufficient humidity to prevent desiccation of the agar surface) at 35-38 °C. Inoculate 10 mL of the product to be examined per 100 mL of each liquid medium. If it has been found that a significant pH change occurs upon the addition of the product to be examined, the liquid medium is restored to its original pH value by the addition of a solution of either sodium hydroxide or hydrochloric acid. Inoculate 0.2 mL of the product to be examined on each plate of each solid medium. Incubate liquid media for 20-21 days. Incubate **solid media** for not less than **14** days, except those corresponding to the 20-21 day subculture, which are incubated for 7 days. At the same time incubate an uninoculated 100 mL portion of each liquid medium and agar plates, as a negative control. On days **2-4** after inoculation, **subculture** each liquid medium by inoculating 0.2 mL on at least 1 plate of each solid medium. Repeat the procedure between the **6th and 8th** days, again between the **13th and 15th** days and again between the **19th and 21st** days of the test. **Observe the liquid media** every **2** or 3 days and if a colour change occurs, subculture. If a liquid medium shows bacterial or fungal contamination, the test is invalid.	3. Culture and Observation 1) Inoculate no less than 0.2 mL of test sample (cell suspension) in evenly distributed amounts over the surface of each of two or more agar plates. After the surfaces of the inoculated plates are dried, the plates should be incubated in an atmosphere of nitrogen containing 5 – 10% CO_2 and adequate humidity at 35 – 37C for no less than 14 days. 2) Inoculate no less than 10 mL of the test sample (cell suspension) into each of one or more vessels containing 100 mL of broth medium, and incubate at 35 – 37C. If the culture medium for the sample cells contains any growth-inhibiting factors, such as antibiotics, these factors should be removed. Refer to the Validation tests for growth inhibiting factors described in the Minimum Requirements for Biological Products for the detection of growth-inhibiting factors. 3) **Subculture** 0.2 mL of broth culture from each vessel on the **3**rd, **7**th, and **14**th days of incubation onto two or more agar plates. **Observe** the broth media every **2 or 3 days** and if a color change occurs, subculture. The plates should be incubated in nitrogen containing 5 – 10% carbon dioxide and adequate humidity at 35 – 37C for no less than 14 days. 4) **Examination of all plates** for mycoplasma coloniesshould be done microscopically on the **7th** and **14th** day at **100** **times magnification** or greater.
Result Intepretation	At the end of the prescribed incubation period, examine all inoculated solid media for the presence of Mycoplasma colonies. The product complies with the test if growth of typical Mycoplasma colonies has not occurred. The product does not comply with the test if growth of typical Mycoplasma colonies has occurred on any of the solid media. The test is invalid if 1 or more of the positive controls do not show growth of Mycoplasmas on at least 1 subculture plate. The test is invalid if 1 or more of the negative controls show growth of Mycoplasmas. If suspect colonies are observed, use a suitable validated method to determine whether they are due to Mycoplasmas.	At the end of the prescribed incubation period, examine all inoculated solid media microscopically for the presence of mycoplasma colonies. The product complies with the test if growth of typical mycoplasma colonies has not occurred. The product does not comply with the test if growth of typical mycoplasma colonies has occurred on any of the solid media. The test is invalid if 1 or more of the positive controls do not show growth of mycoplasmas on at least 1 subculture plate. The test is invalid if 1 or more of the negative controls show growth of mycoplasmas. If suspect colonies are observed, a suitable validated method may be used to determine whether they are due to mycoplasmas.	

USP: US Pharmacopoeia, EP: European Pharmacopoeia, JP: Japanese Pharmacopoeia

Monthly Testing Calendar (2x samples/week)

◄ Mar 2021 | **April 2021** | May 2021 ►

Sun	Mon	Tue	Wed	Thu	Fri	Sat
				1	2	3
4	5	6	7	8	9	10
11	12	13	14	15	16	17
18	19	20	21	22	23	24
25	26	27	28	29	30	

No Lab Tasks 1-2 Lab Tasks 3-4 Lab Tasks >4 Lab Tasks

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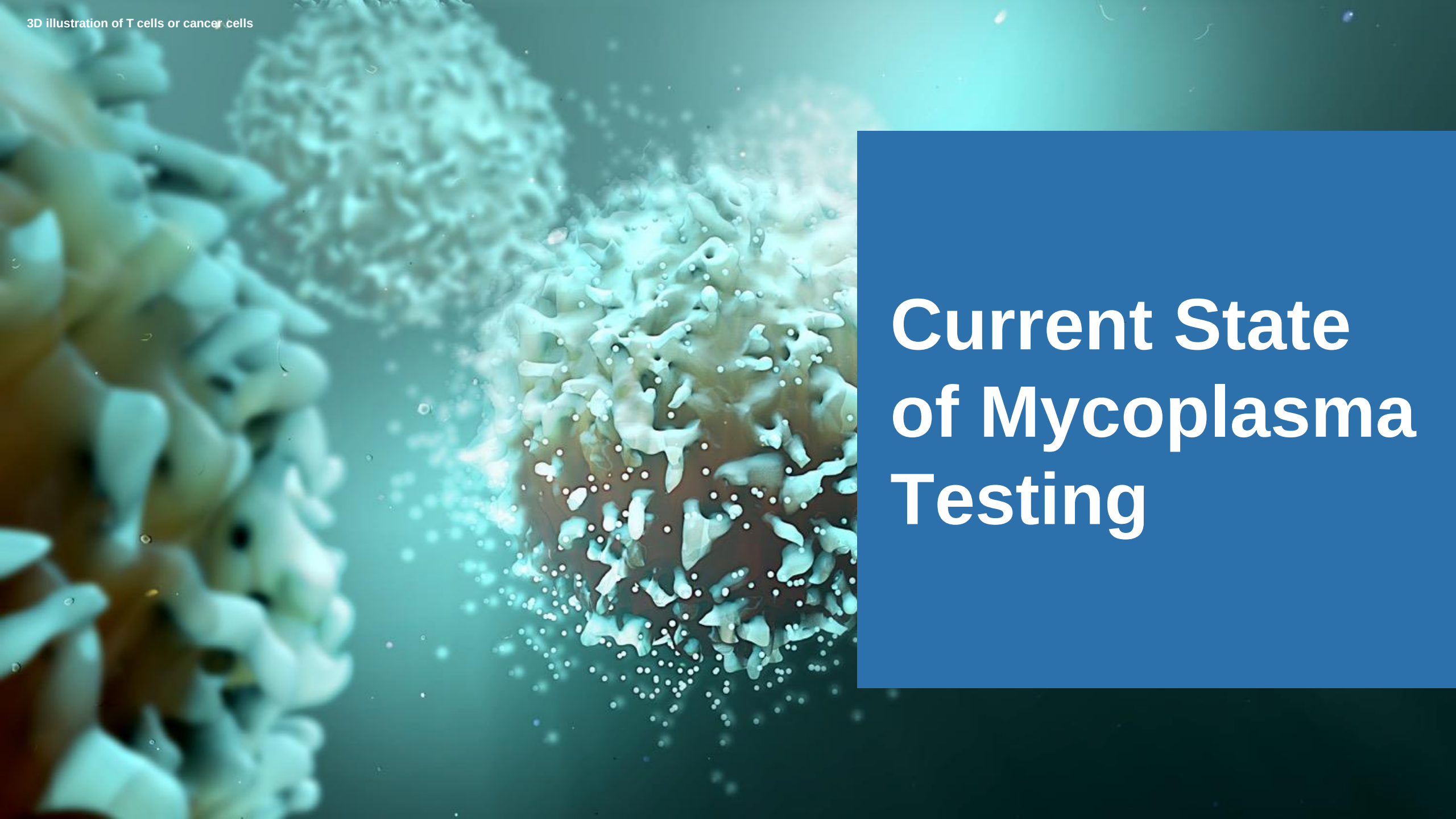
Monthly Testing Figures

Setups	• 5 setups, 10 samples
Agar	• 285 plates • 351 Observations
Broth	• 55 bottles of broth • 270 broth observations
Subculture	• 14 subculture events



3D illustration of T cells or cancer cells

Current State of Mycoplasma Testing

What are the Key Factors Driving Alternative Methods to Mycoplasma Testing?

Time

Culture test is 28 days

- CAR-T Vein to Vein Time
- Drug Substance Storage and Supply Chain

Cost

Labor Intensive

- Multiple FTE
- Manual Process

Reagents

- Specialized broth/agar
- Growth Promotion

Efficiency

Invalid Rates

- Multiple paths to user error
- Subjective observations—4 eye approach

Real-Time PCR with Semi-Automated Workflow

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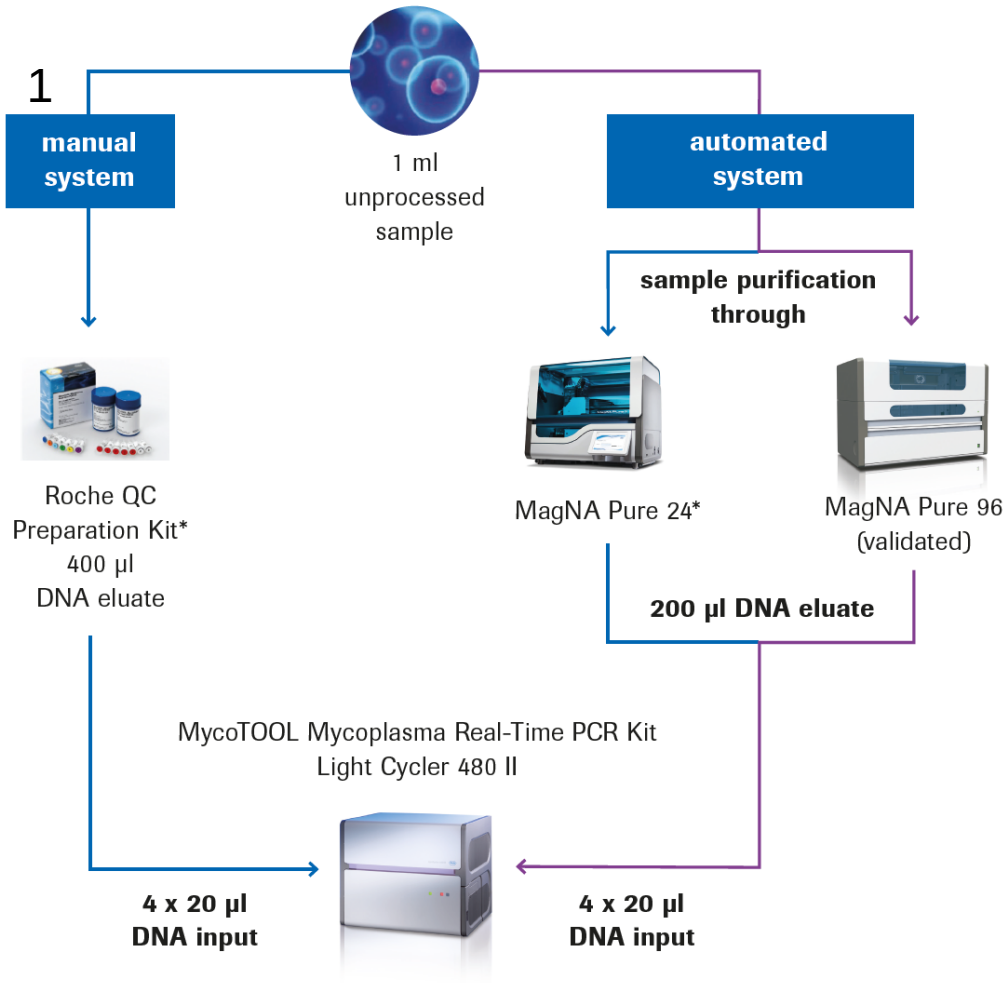

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Figure 3. One-hour workflow for the AutoMate *Express*™ system.

[1]https://custombiotech.roche.com/content/dam/internet/dia/custombiotech/custombiotech_com/en_GB/pdf/CustomBiotech_MycoTOOL_Validation_Study_Poster.pdf

[2]https://www.thermofisher.com/us/en/home/life-science/bioproduction/contaminant-and-impurity-testing/sample-prep-and-automation/automate-express-nucleic-acid-extraction-system-.html

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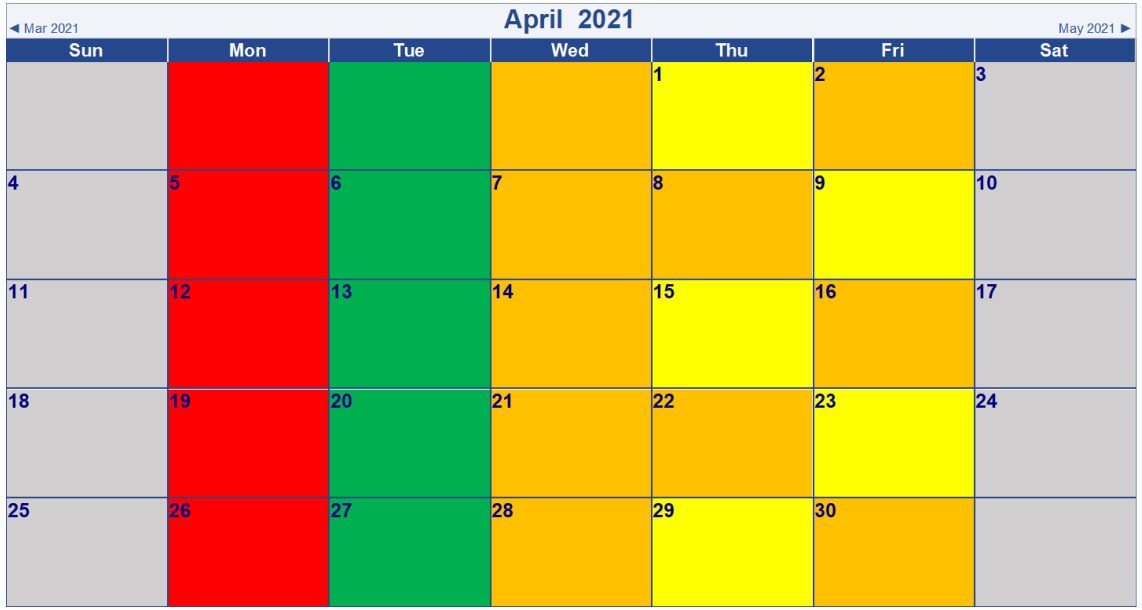
Cell Culture Based Testing vs. Real-Time PCR



Validation Guidelines

1. ICH Q2(R1)
   A. Limit tests for the control of impurities
2. USP 1223 and 1225
3. EP Section 2.6.7
4. 21 CFR 610.9(b)
5. PDA Technical Report No. 50
   A. Alternative Methods for Mycoplasma Testing

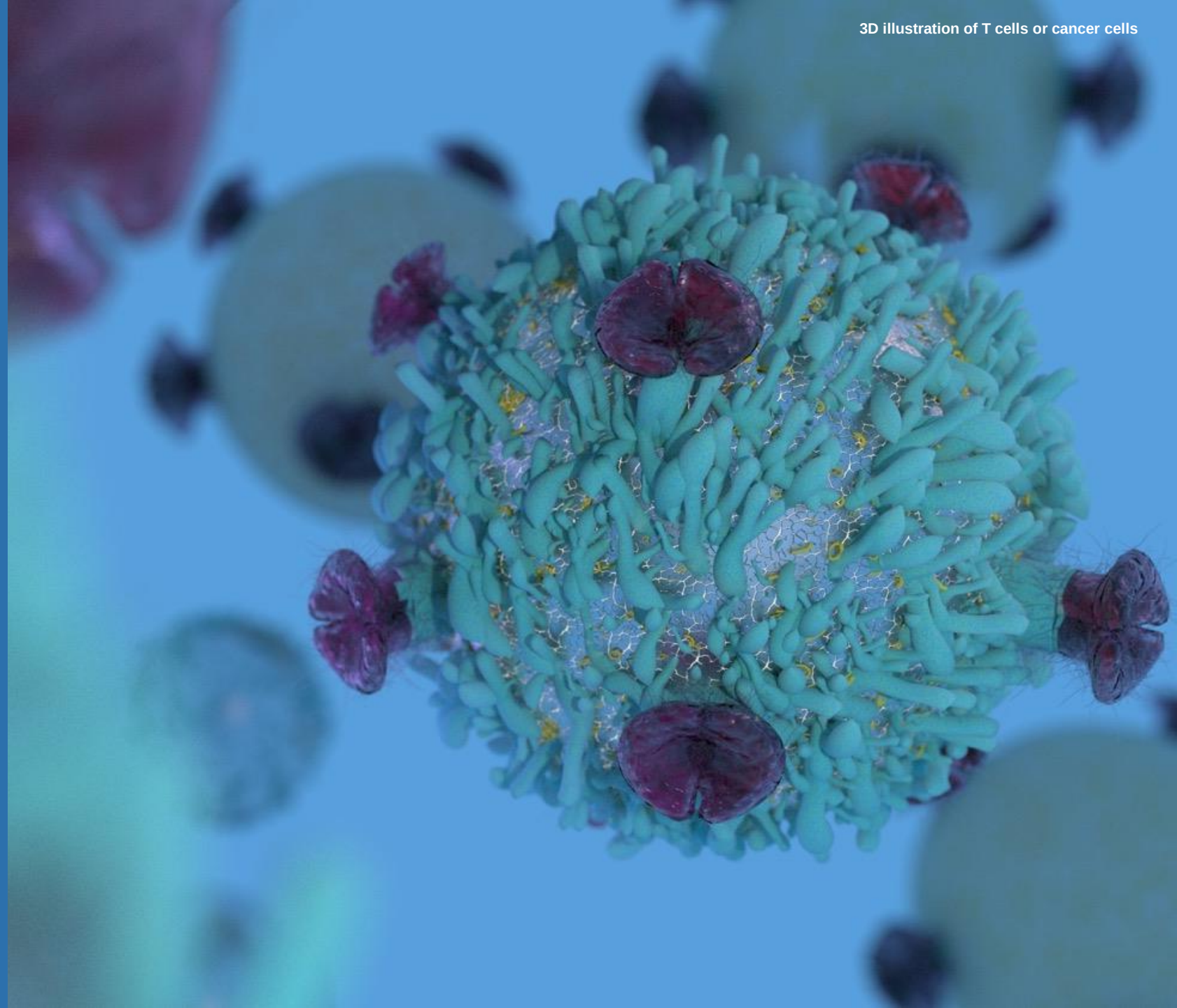
ICH: International Council for Harmonisation
CFR: Code of Federal Regulations
PDA: Paternal Device Association

Validation Considerations

- Biologics: Choose a matrix with a high cell titer for validation
  - Newer antibody-based therapeutic manufacturing have higher titers which may lead to difficulty in detecting Mycoplasma
  - General Method Validation → Product Specific Qualification for other products (limit of detection, specificity as outlined in the ICH guidelines)
- Cell and Gene Therapy
  - Use a BSL-2 level lab to perform validation activities in-house
  - If no BSL-2 level lab is available, use a CRO to develop method and co-validate activities using extracted Mycoplasma DNA from the CRO

[1]*Barile M.F., and Razin, S. (1993) Mycoplasmas in cell culture. In Rapid Diagnosis of Mycoplasmas (Kahane I., and Adoni, A., eds) pp. 155-193, Plenum Press, New York.*

Future State



3D illustration of T cells or cancer cells

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At-Line or In-Line Testing

Biofire Mycoplasma Testing

Pros

- Eliminates need of highly skilled QC analysts
- Can be performed on the manufacturing floor
- Highly automated

Cons

- Difficulty in batch testing
- Very closed environment can make investigation difficult
- Single Sourcing

1

[1]https://www.biomerieux-industry.com/pharma-healthcare/newsroom/media-news/2020-07-16-launch-biofire-mycoplasma-test-mycoplasma

Next Generation Sequencing

Genexus Thermofisher with automated liquid handler

Pros: Data integrity (DI) ready, automation ready with liquid handler
Cons: Cost, large equipment footprint, small user base

Illumina NextSeq 2000

Pros: Large user base, evaluated; evaluated/validated at several large biologics, robust bioinformatic pipeline
Cons: Cost, DI not ready for commercial release testing

Oxford Nanopore MiniION

Pros: Can sequence both RNA and DNA, small footprint, low initial cost

Cons: Long read accuracy, expensive consumables

New Technology Implementation: Considerations

- Develop a Business Case
  - Calculate Net Present Value
  - Utilize single equipment for multiple purposes
    - Example: Real-time PCR equipment for different types of release testing. Use the same equipment for Mycoplasma testing. Reduces the # of PM's, equipment maintenance costs
- Stakeholder Participation
  - End user considerations (Quality Control)
    - Ease of use, data integrity, LIMS integration, method execution, method analysis
- Consider steps for automation

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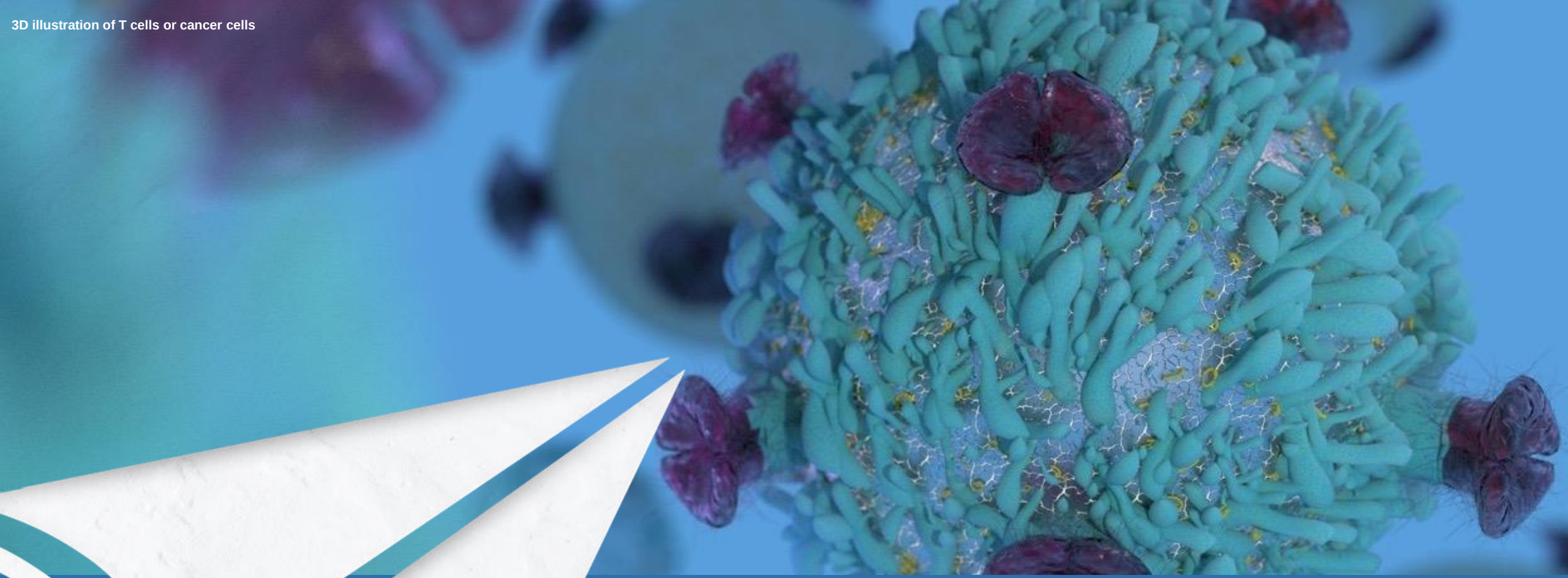

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Thank You

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