

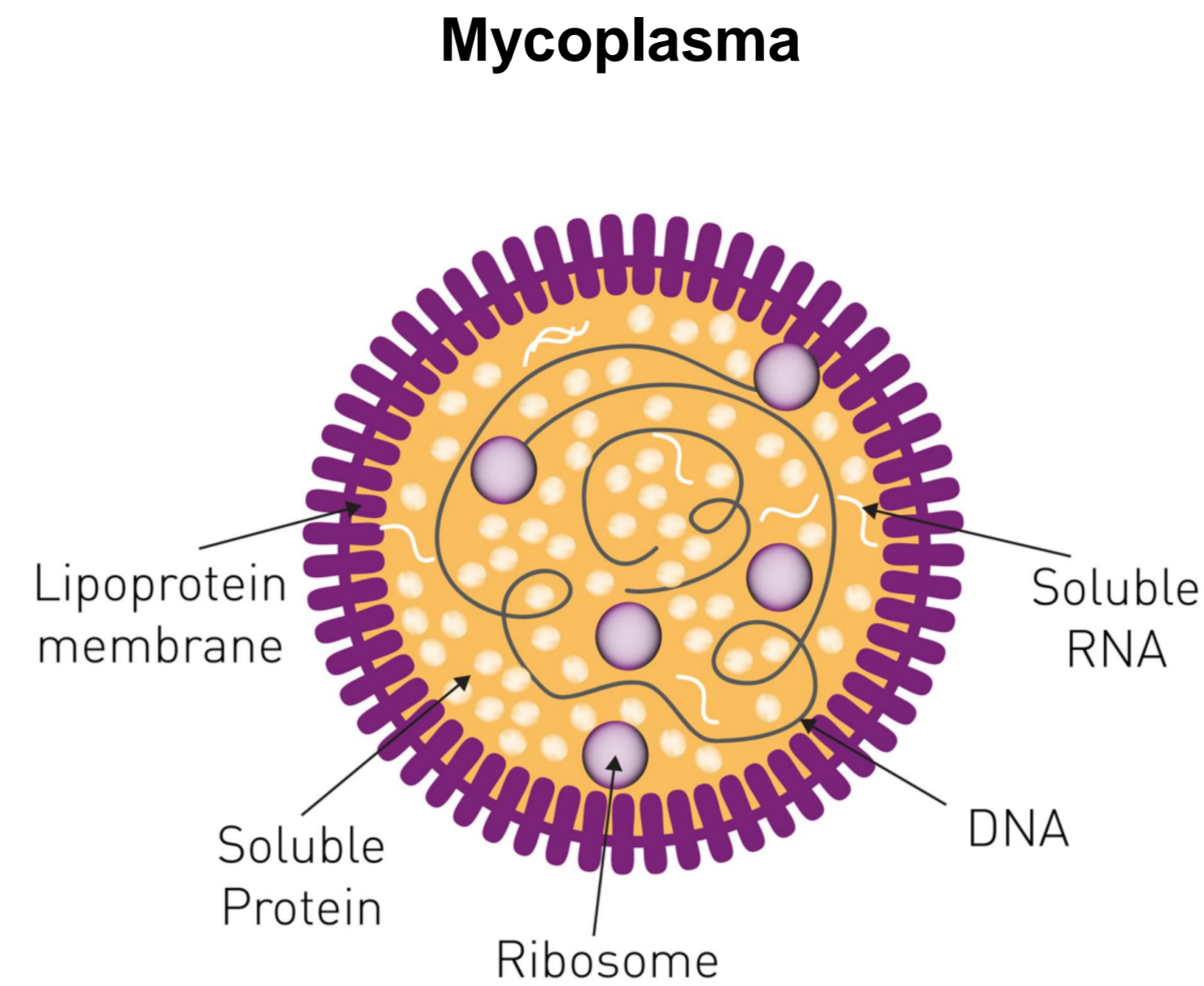
Mycoplasma Contamination Detection in Biopharmaceuticals

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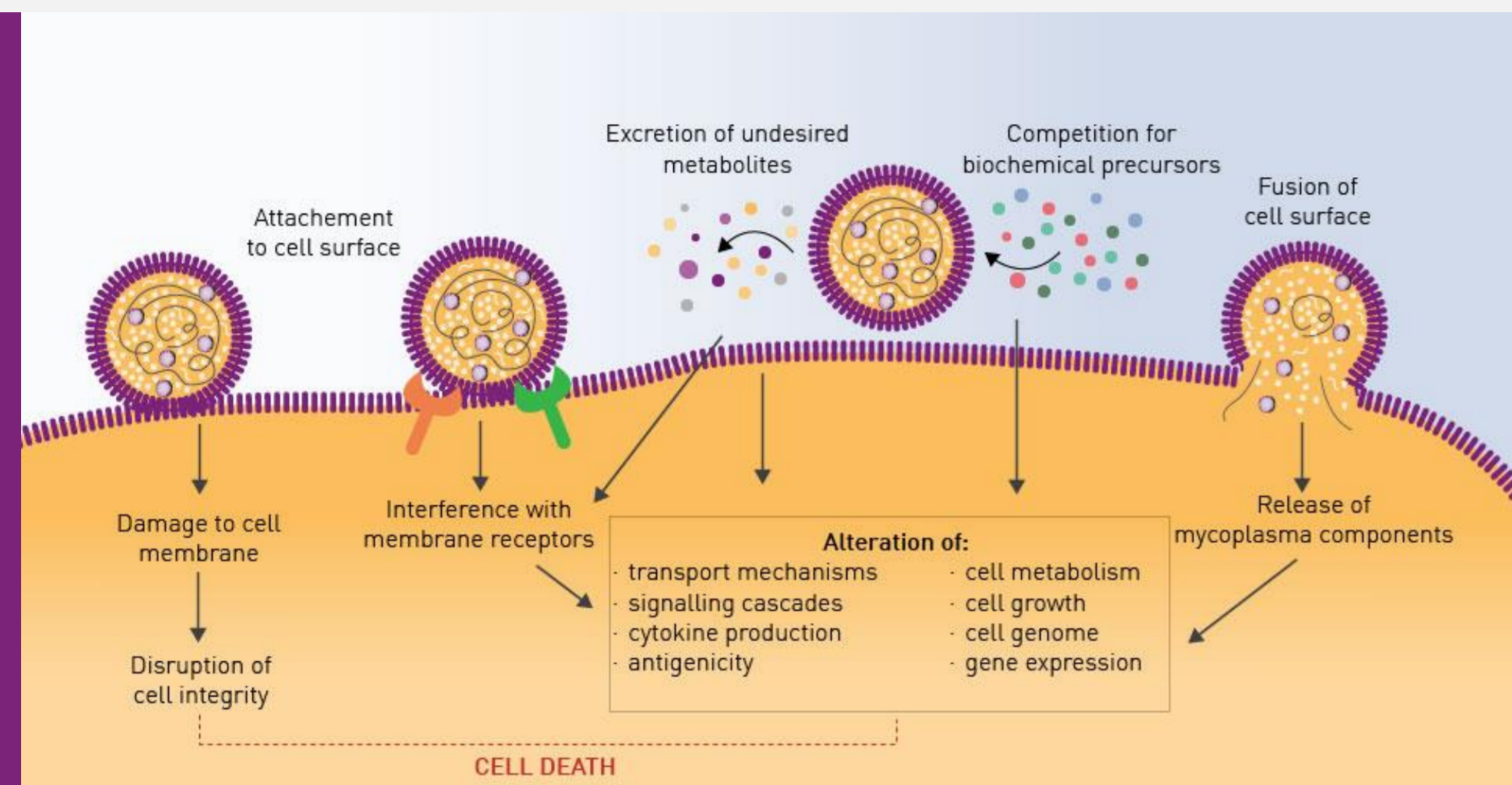
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Introduction

- Mycoplasmas are the smallest free-living organisms (~100nm) taxonomically ordered in class mollicutes.
- The unique features of lack of cell wall and plasma like form make them stand out from bacteria and other prokaryotes.
- The first mycoplasma was identified by Paster institute in 1898
- At present 190 species of mycoplasma are identified and are spread among human, animals, insects and plants.
- Among these species, only 8 species contribute to 95% of cell culture contamination.
- Hence manufacturer should ensure the safety of the patient by performing mycoplasma testing in the drug.



Mechanism of Mycoplasma contamination



Impact of Mycoplasma Contamination

- Patient safety
- Product loss
- Economic loss
- Unavailability of product
- Shut down of manufacturing facility
- Lowered quality of product
- Credibility Of the Company

Source of Mycoplasma Contamination

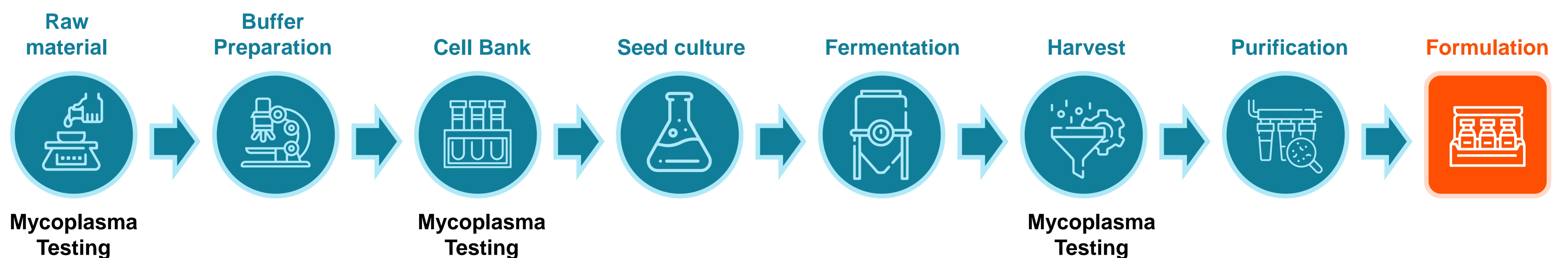
- Infected Cell Lines
- Contaminated raw materials (Media, Serum, Trypsin)
- Infected personnel
- Aerosol or droplets

Available Mycoplasma Testing Methods

Compendial Mycoplasma Testing and Features		
Agar and Broth Methods	Indicator Cell Line	NAT Based testing
Gold standard Test		
Detect Viable Mycoplasma		Detect both Viable and non-cultivable mycoplasma
Sensitive	Rapid	Specific, Sensitive and Rapid
Laborious	Detect non cultivable mycoplasma	Less Turnaround time
Time consuming (28 days)	Easy to perform	Cost effective and Easy to perform
Requirement of expertise	Requirement of expertise	Broad Detection spectrum
Does not detect non cultivable mycoplasma	Lack of mycoplasma specific DNA stain	High Throughput testing

Non Compendial Mycoplasma Testing and features
ELISA, Biochemical Assays and Staining method
Features: Rapid, Less sensitive and Cost effective

Potential Candidate for Mycoplasma Testing for Biopharmaceuticals



NAT Based Mycoplasma Detection at Syngene

- State of The Art Method
- Recommended by EP (2.6.7) and USP (63)
- Less Turnaround time
- Sensitive, Reproducible, specific and Rapid
- Employs qPCR-based method for the detection of Mycoplasma
- Syngene uses Mycoseq Mycoplasma detection Kit
- Mycoseq Mycoplasma detection kit targets 16s rRNA region of mycoplasma and detects over 100 species of mycoplasma including compendial mycoplasma species.
- This kit has been validated by the manufacturer
- Syngene had performed additional verification with *M. Orale*, *M.Fermentas*, *M. Pneumonia*, *A.Laidlawii*

Validation approach

Parameters Evaluated : LOD, Repeatability and Matrix interference

Parameter	No. Of Runs	No. of Analyst	Determination	Results
LOD	01	01	18	10 copies/mL of Mycoplasma DNA and 10 CFU/mL of Mycoplasma
Repeatability	02	02	36	Results were reproducible
Matrix Interference	02	01	36	Interfere observed in few cases of undiluted samples. No interference observed in 1:10 and 1:20 dilution

The results were interpreted in terms of Ct value and Tm value.

A Ct value of < 36.23 and a Tm value of > 75°C indicates a positive results.



Both Cells and Supernatant tested to detect free and cell associated mycoplasma

Extracted nucleic acid further tested with Undiluted, 1:10 and 1:20 dilution

Reference 1 EP 2.6.7. Mycoplasmas

- USP chapter: (63) Mycoplasma Tests
- Armstrong SE. et al., 2010. The scope of mycoplasma contamination within the biopharmaceutical industry. *Biologicals*. 38(2):211-3
- Donna KF. Et al., 2011 Historical Overview of Mycoplasma Testing for production of Biologics. *American Pharmaceutical Review* – Volume 14, Issue 4 may/June 2011
- MycoSEQ Mycoplasma Detection Kit, with Discriminatory Positive Control & sample preparation; manual