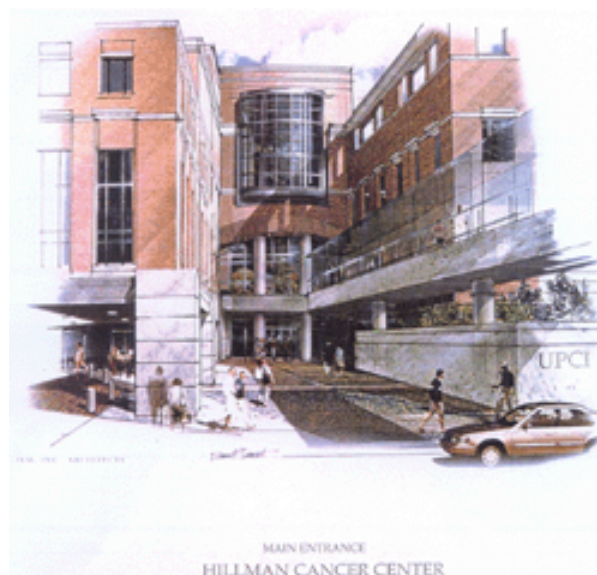


Rapid Release Testing



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Rapid Release Testing

- ↳ Overview
- ↳ IND requirements
- ↳ Regulations
- ↳ Methods
 - ↳ Endotoxin
 - ↳ Gram Stain
 - ↳ Mycoplasma
- ↳ Troubleshooting
- ↳ Documentation

Overview

- ↳ Rapid Release Testing is necessary:
 - ↳ Intraoperative procedures
 - ↳ Cultured products prior to distribution
- ↳ Drawbacks:
 - ↳ Product release may be delayed if test results are inconclusive
 - ↳ Can be expensive
 - ↳ The fastest tests may not be FDA approved

IND Requirements

- ↳ Will vary according to the type of procedure and FDA oversight
- ↳ Some examples that we have experience with:
 - ↳ Endotoxin testing
 - ↳ Mycoplasma testing
 - ↳ Gram Stain

FDA regulations

21 CFR 610

http://www.access.gpo.gov/nara/cfr/waisidx_03/21cfr610_03.html

- 21 CFR 610.1 General Safety
- 21 CFR 610.10 Potency
- 21 CFR 610.13 Purity
- 21 CFR 610.14 Identity
- 21 CFR 610.30 Test for Mycoplasma

Guidance for Industry: Sterile Drug Products Produced by Aseptic Processing

<http://www.fda.gov/cder/guidance/5882fnl.htm>

- Appendix 3, section B Aseptic processing of cellular therapy products and cell-derived products



Endotoxin

biology overview

- ↳ Found in Gram negative bacteria
- ↳ Causes both toxicity and immunogenicity responses (fever)
- ↳ Minute amounts of endotoxin are released as bacteria proliferate, but most remains intact in the cell wall until the bacteria lyses
- ↳ Endotoxins protect bacteria from attacks by immune systems
- ↳ Please refer to Dr. Kenneth Lodar's online Bacteriology text <http://textbookofbacteriology.net/endotoxin.html> for a detailed explanation of bacterial endotoxins (the lipopolysaccharide component of the lipid membrane)

Endotoxin

FDA approved methods

➤ Rabbit Pyrogen Test as described in 21 CFR 610.13 (impractical for rapid release!)

http://a257.g.akamaitech.net/7/257/2422/14mar20010800/edocket.access.gpo.gov/cfr_2003/aprqttr/21cfr610.13.htm

OR

➤ A validated Limulus amoebocyte lysate (LAL) Test (ancient document from 1987!)

<http://www.fda.gov/cber/gdlns/lal.pdf>



Endotoxin



- ↳ “Fast”

- ↳ Rabbit pyrogen test (3 hours)

- ↳ Faster

- ↳ Gel clot (60 minutes)

- ↳ Endpoint chromogenic (30-60 minutes)

- ↳ Kinetic turbidimetric or chromogenic (45-60 minutes)

- ↳ Fastest

- ↳ Endosafe PTS from Charles River (15 minutes)

Gram Stain

biology overview

- Gram staining is performed using a crystal violet stain and serial washes
- Bacterial cell walls contain a layer of murein
- Gram positive bacteria have a thick layer of murein and stain purple crystal violet
- Gram negative bacteria have a thin layer of murein and do not stain purple crystal violet
- Indicates presence of bacteria that are endotoxin negative
- <http://textbookofbacteriology.net/structure.html>

Gram Stain

FDA identified method

- Standard Microbiology Procedure in hospital-based Pathology labs
 - Crystal violet stain followed by washes with Iodine, Alcohol, and Safranin

Gram Stain

↳ STAT Gram Stain (15 minutes)

↳ Drawbacks:

- ↳ Messy with multiple washes
- ↳ Not automated
- ↳ Insensitive
- ↳ Subjective/technologist dependent
- ↳ Lack of controls
- ↳ Documentation

Other methods

↳ Fluorescent-based assay for gram pos or gram neg bacteria detection

↳ Endosafe PTS can identify if a bacterially contaminated product is Gram positive or Gram negative

Mycoplasma

biology overview

- ↳ Tiny bacteria that lack a cell wall, not classified as either Gram positive or negative
- ↳ Causative agents of tuberculosis and leprosy
- ↳ Intracellular parasite that causes cough, fever and weight loss
- ↳ Please refer to online text
<http://www.bact.wisc.edu/Microtextbook/index.php?name=Sections&req=viewarticle&artid=108&page=1>
for a synopsis of mycoplasmas.

Mycoplasma

FDA approved methods

- Home-brewed or Commercial Agar Media and Semi-solid Broth Tests as described in 21 CFR 610.30 (very impractical for rapid release!)

http://a257.g.akamaitech.net/7/257/2422/14mar20010800/edocket.access.gpo.gov/cfr_2003/aprqr/21cfr610.30.htm



Mycoplasma

↳ “Fast”

- ↳ Commercial Agar Media and Semi-solid Broth Tests (3-4 weeks)

↳ Faster

- ↳ Home-brewed Agar Media and Semi-solid Broth Tests (14 days)
- ↳ R-PCR (2 days)

↳ Fastest

- ↳ MycoAlert from Cambrex (20 minutes)

Troubleshooting

- ↪ Repeating a test (Avoid! Validate! Train!)
- ↪ Media and other reagents may interfere with the results of testing
 - ↪ Anticoagulants interfere with enzymatic reactions
 - ↪ Colored agents interfere with spectrophotometers
 - ↪ Cell concentration
- ↪ Corrective actions
 - ↪ Buffers
 - ↪ Dilutions
 - ↪ Technologist training
- ↪ Contact the Technical Service Representative for assistance

Documentation

- ↳ Form that records:
 - ↳ All of the pertinent data
 - ↳ Demographic info
 - ↳ Product lot
 - ↳ Reagent lot and expiration
 - ↳ Time of assay(s)
 - ↳ Results, including interpretation
 - ↳ Signature/date/time of personnel authorized to release the product.


PACT initiatives

- Endosafe PTS validation (Charles River)
- MycoAlert validation (Cambrex)

Resources

 www.fda.gov

 <http://www.rapidmicrobiology.com/>

 [http://www.biopharminternational.com/
biopharm/article/articleDetail.jsp?id=262
441&pageID=1&sk=&date=](http://www.biopharminternational.com/biopharm/article/articleDetail.jsp?id=262441&pageID=1&sk=&date=)

 <http://www.textbookofbacteriology.net/>