The Microbiology NETWORK

Microbiological Quality Control as Described in the Compendia

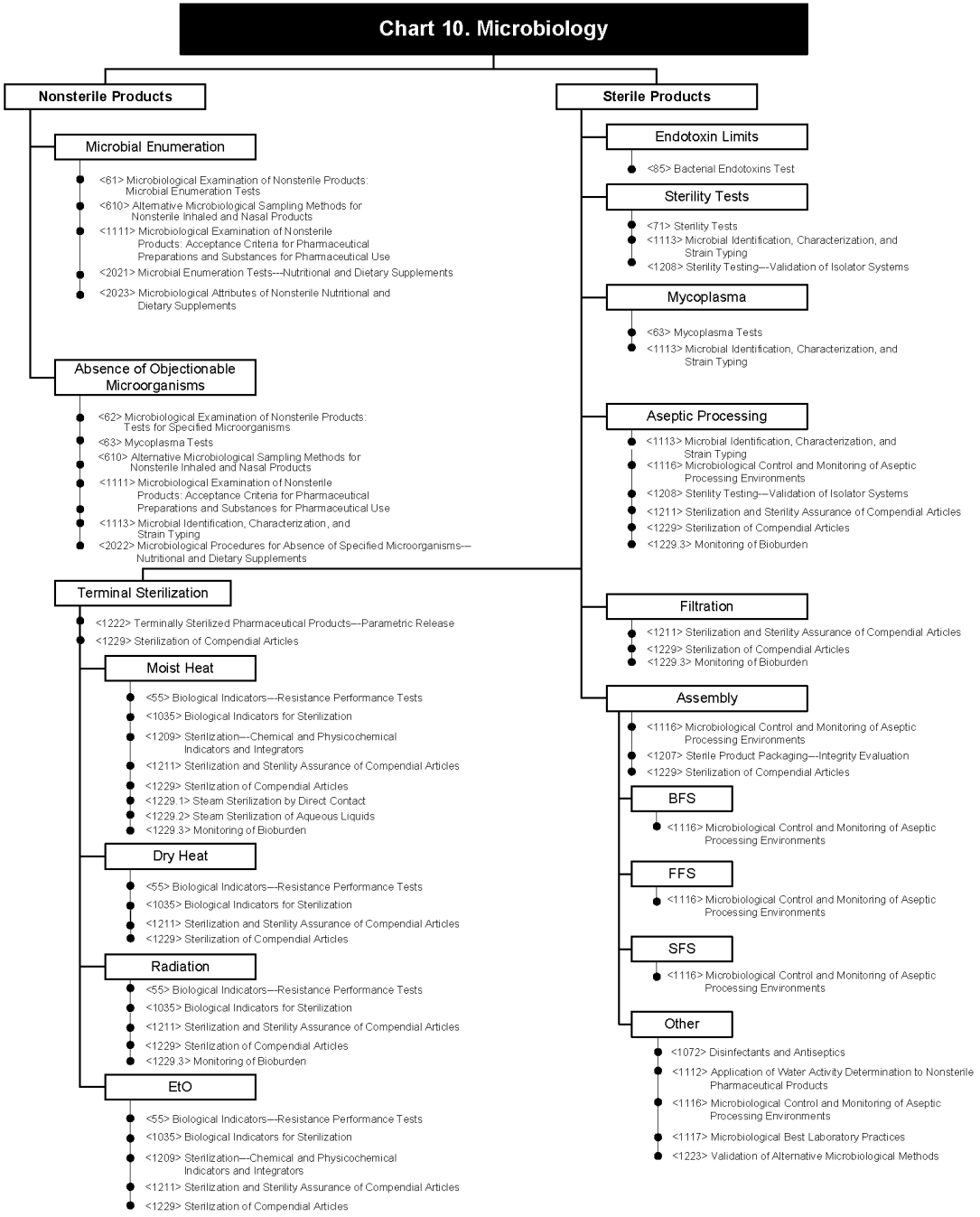
Scott Sutton, Ph.D.

www.microbiol.org

Microbiological Quality Control as Described in the Compendia

- <51> Antimicrobial Effectiveness Test
- <61>, <62>, <1111> Microbial Limits Tests
- <71> Sterility Testing
- <1117> Best Microbiological Laboratory Practices

Chart 10. Microbiology



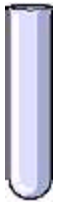
Antimicrobial Efficacy Test

USP <51>

- Designed to demonstrate the ability of a multidose product to withstand microbial challenge.
- High-level challenge, frequent sampling of the challenge suspension for survivors
- Completely rewritten for clarity in 2015
- Method Suitability included in test

Antimicrobial Efficacy Test

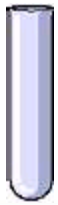
10^6 CFU/mL Each of five challenge microorganisms - 3 bacteria, 2 fungi



Incubate microbial suspension

Sample at 7 days, 14 days, 21 days, 28 days

10-fold serial dilutions



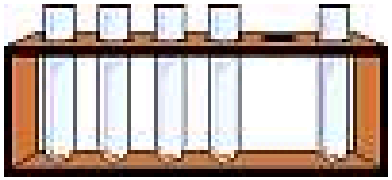


Plate in growth agar to count survivors

Determine Log Reduction
(log value of inoculum) - (log value of survivors at the time point)

USP Criteria for Passage

	Log_{10} Reduction			
	7 Day	14 Day	21 Day	28 Day
1A: Bacteria	1.0	3.0	--	NI
1A: Fungi	--	NI	--	NI
1B: Bacteria	--	2.0	--	NI
1B: Fungi	--	NI	--	NI
1C: Bacteria	--	1.0	--	NI
1C: Fungi	--	NI	--	NI
2: Bacteria	--	NI	--	NI
2: Fungi	--	NI	--	NI

AET Categories

Category	Product Description
1	Injections and other parenterals, otic, sterile nasal products, and opthalmics
2	Topical Products
3	Oral Products
4	Liquid Antacids

Scope of AET

- It can
  - Provide relative estimates of the biological activity of a preservative system in a particular formulation at a particular time.

- It cannot
  - Predict the preservative efficacy of the multidose finished product in all patients hands under all conditions.

Major Sources of Variability

- Preparation and handling of inocula
  - Source of culture
  - Growth conditions
    - Solid vs liquid
    - Length of time/Temperature incubated
  - Harvesting, buffer composition, storage conditions
- Recovery Conditions

  Determined in Method Suitability Study
- Plating conditions
  - Colony counting rules
  - Math

Proactive Documentation

Unique Microorganisms

FDA regulatory focus in recent years –

- *Bacillus cereus*
  Gram-positive, facultatively aerobic sporeformer. β-hemolytic and may be an emerging pathogen.
- *Burkholderia cepacia*
  Pseudomonad, clearly pathogenic to cystic fibrosis patients who can develop pneumonia.
  THE CONCERN IS THAT CF PATIENTS ARE EXPOSED TO PRODUCTS THAT MAY LEAD TO PNEUMONIA.

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Compendial Microbial Limits Tests

Internationally Harmonized

- USP <61> Enumeration, EP 2.6.12
- USP <62> Specified Organisms, EP Chapter 2.6.13
  - Test for *Staphylococcus aureus*
  - Test for *P. aeruginosa*
  - Test for *Salmonella spp*
  - Test for *Escherichia coli*
  - Test for Bile-tolerant Gram-negative Bacteria
  - Test for *Clostridia*
  - Test for *Candida albicans*
- USP <1111> Guidance on Microbial Quality, EP 5.1.4

USP <61> - Enumeration

- Sampling Plan
- Categories
- Methodology
  - Membrane Filtration
  - Plate Count: Pour Plate
  - Plate Count: Spread Plate
  - Most Probable Number (MPN)
- Method Suitability and Growth Promotion Requirements

<62> - Specific Tests

- Test for *Staphylococcus aureus*
- Test for *P. aeruginosa*
- Test for *Salmonella spp*
- Test for *Escherichia coli*
- Test for Bile-tolerant Gram-negative Bacteria
- Test for *Clostridia*
- Test for Candida *albicans*

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"Microbial Attributes" USP <1111> EP 5.1.4

- One page in USP, two tables:
  - Table 1 – Acceptance criteria for Microbial Quality of Nonsterile Dosage Forms
  - Table 2 – Acceptance Criteria of Microbiological Quality of Nonsterile Substances for Pharmaceutical Use (TAMC-10^3 CFU/g or mL, TYMC-10^2)
- Significance of other organisms should be evaluated

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Evaluation of Other Organisms

- Route of Administration
- Nature of the Product
- Method of Application
- Intended Recipient
- Use of Immunosuppressive Drugs
- Presence of Disease, Wounds, Organ Damage

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Sterility Testing

- Two separate tests
  - Membrane Filtration
  - Direct Transfer
- 20 Units, 2 media & temperatures
- Requires Growth
  - Incubation period - 14 days

Membrane Filtration

- Filter required amount of product through two filters
- Neutralize/Rinse
  - 3 100 mL volumes suggested
  - Formulations for dilution fluids suggested
- One filter into Soybean Casein Digest Broth (SCDB or TSB) – incubate at 20-25^{o}C for 14 days
- One filter into Fluid Thioglycollate Medium (FTM) – incubate at 30-35^{o}C for 14 days

Direct Inoculation

- Place required amount of product into sufficient recovery medium (with neutralizers?)
  - Soybean Casein Digest Broth (SCDB or TSB) – incubate at 20-25^{o}C for 14 days
  - Fluid Thioglycollate Medium (FTM) – incubate at 30-35^{o}C for 14 days

Product Requirements

- Minimum Quantity per Unit for Each Medium detailed in Table 2 of chapter
- Minimum Number of Units to be tested detailed in Table 3 of chapter

Method Suitability Test

Can we neutralize any antimicrobial properties of the medication?

Use specified challenge organisms

Use specified total amounts of products

Method Suitability Test for Each Challenge Organism

- Filter maximal amount of medication to be tested
- Filter 2 volumes (100 mL?) of diluting fluid
- Add third volume, inoculate with <100 CFU challenge organism
- Filter
- Show microbial growth from filter in relevant medium at relevant temperature in 5 days

Scope of Sterility Tests

- They can provide a recognized, standardized test.

- They cannot prove product sterility.
  - Limited sample size
  - Can only show what can grow

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USP <1117> Importance Aspects of Control

- Control of Media
- Control of Test Strains
- Control of Equipment
- Lab Lay-out and Operations
- Sample Handling
- Microbiological Media Incubation Times
- Training of Staff
- Laboratory Resources
- Control of Data and Documentation
- Interpretation of Results

Maintenance of Microbiological Cultures

- Must be handled carefully
- Confirm ID of culture from culture collection before use
- Resuscitate cultures as per manufacturer's instructions
- Use a seed lot technique, do not enter a master vial more than once or refreeze stock
- Track number of passages ("Any form of subculturing is considered to be a transfer/passage").

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Thank you for your attention

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ALWAYS IMPROVING QC

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Liz Kerrigan
Director of Standards, Sales & Marketing, ATCC
March 19, 2015

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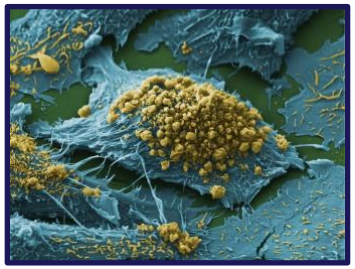
About ATCC

- Founded in 1925, ATCC is a non-profit organization with headquarters in Manassas, VA
- World's premiere biological materials resource and standards development organization
- ATCC collaborates with and supports the scientific community with industry-standard products and innovative solutions
- Broad range of biomaterials
  - Continuous cell lines, iPSCs, primary cells, and hTERT immortalized cells
  - Bacteria, fungi, yeasts, protists, and viruses
  - Microbial and tumor cell panels
  - Genomic and synthetic nucleic acids
  - Media, sera, and reagents

ATCC®

ATCC complete solutions for pharmaceutical QC

- Proficiency testing programs
  - PHARMASSURE – Test materials for chemical and microbiological analyses as well as sterility testing



- Mycoplasma quality control
  - Titered strains
  - Quantitative nucleic acids

- ATCC® Minis
  - ATCC Genuine Cultures® packaged in a convenient, single-use glycerol stock

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ATCC Genuine Cultures®

- **Microbial Portfolio**
  - 18,000 bacterial strains
  - 3,000 human and animal viruses
  - 50,000 yeast and fungal strains
  - 2,000 parasites
  - Nearly 1,000 ATCC Genuine Nucleics® from the collection
- **Brand Recognition**
  - Standard/reference cultures
  - Organizations and regulatory agencies specify ATCC cultures (USP, ISO, FDA, CLSI, USDA, ASTM, AOAC, and more)
  - Over 475 microbial cultures recommended as reference strains in microbial collection
  - Cultures are always authenticated

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Passage matters

USP clearly states that the working cultures used for testing should not be more than five passages from the ATCC reference culture.

The USP 37-NF32 <51> states:

> "The viable microorganisms used in the test must not be more than five passages removed from the original ATCC culture."

ATCC®

Microbial strain authentication

ATCC utilizes both classical and modern techniques

- Phenotypic analysis
- Genotypic & proteotypic analyses
- Functional analysis

No single method of identification is sufficient

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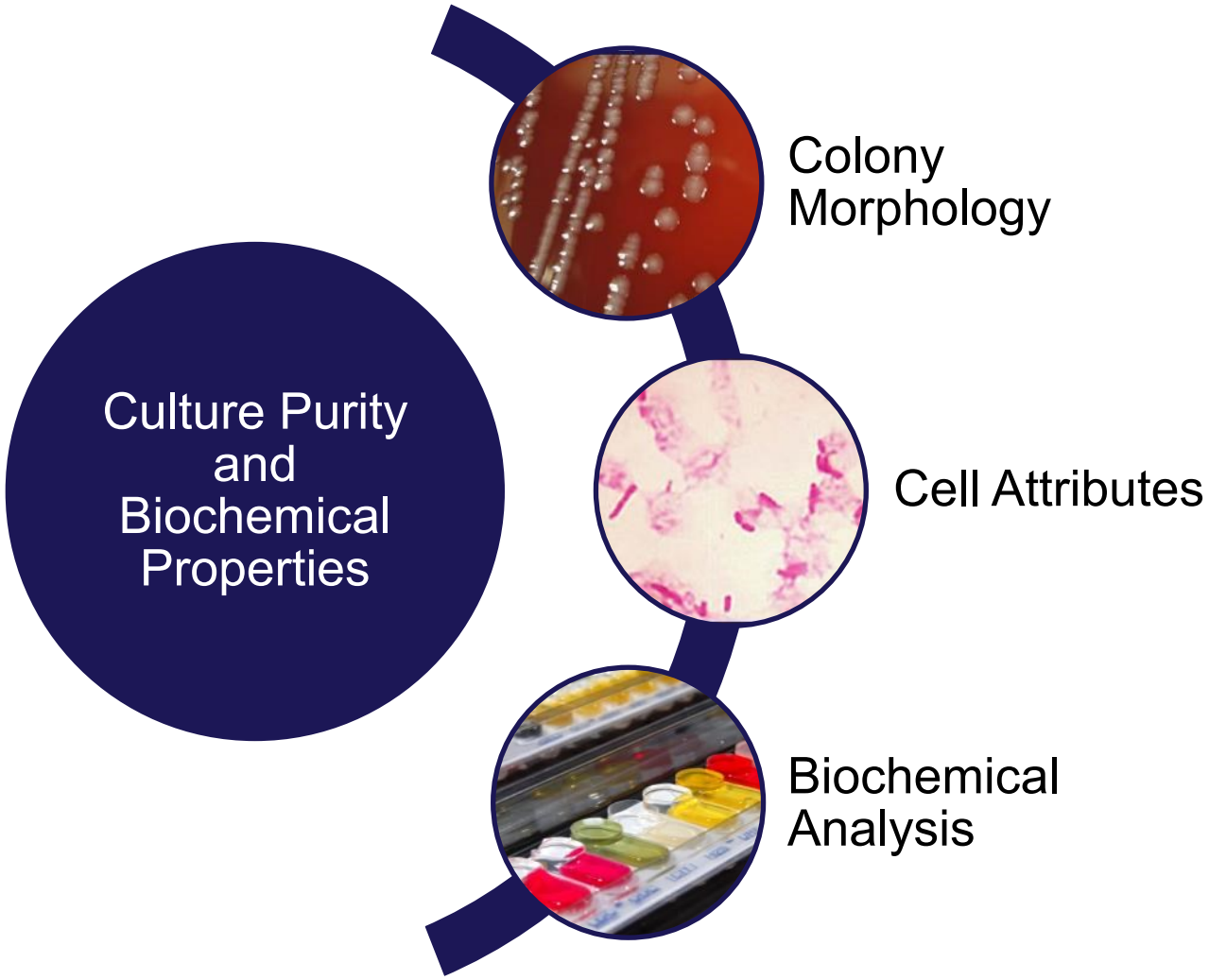
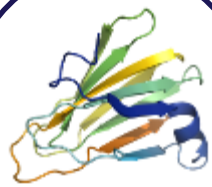
Phenotypic testing



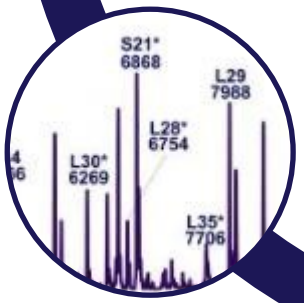
Genotypic & proteotypic testing

Sequencing

Toxinotyping

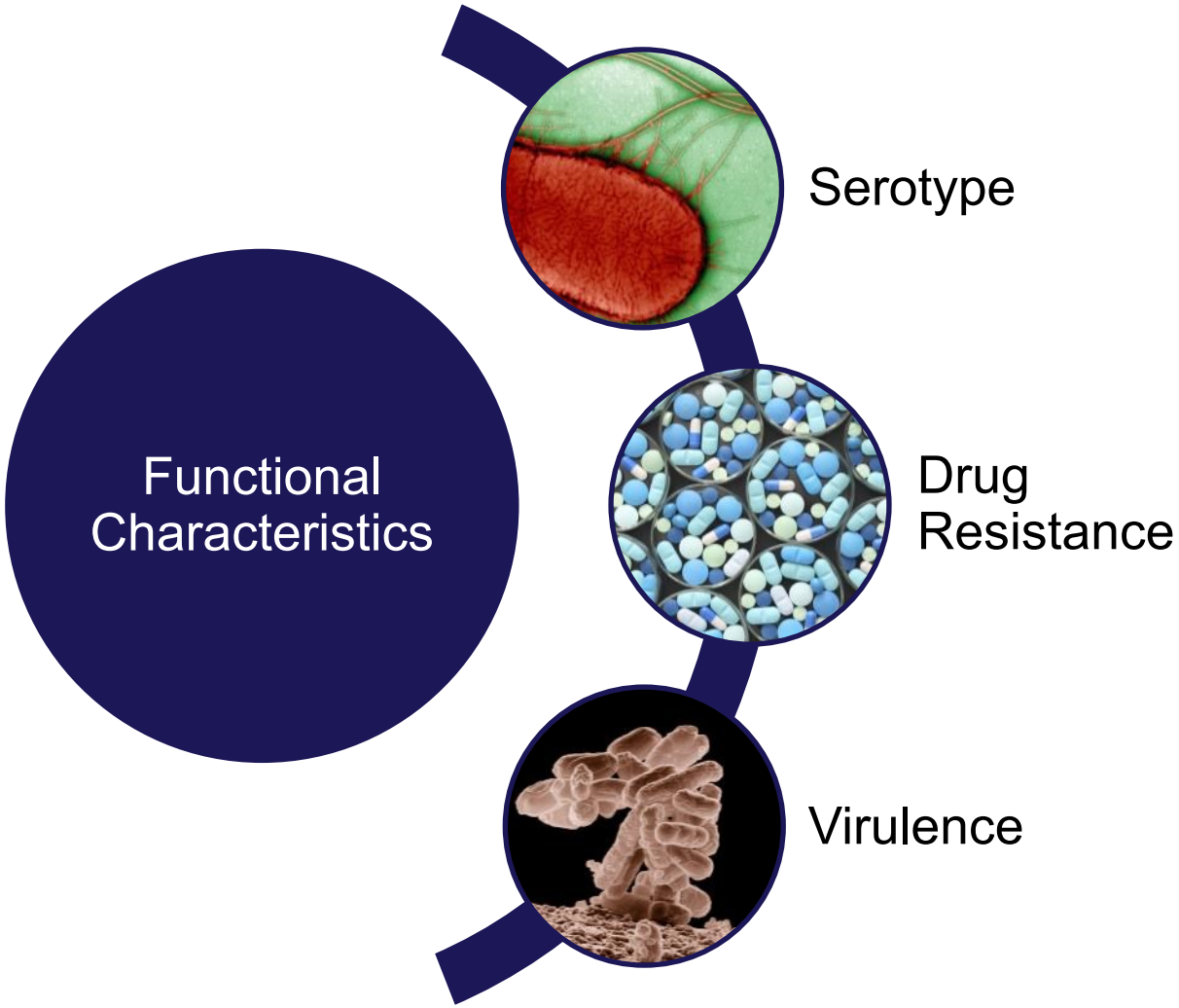


MALDI-TOF



Targeted Gene & Protein Sequencing

Functional testing



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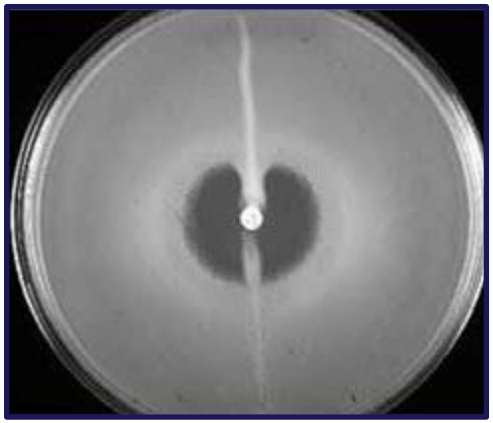
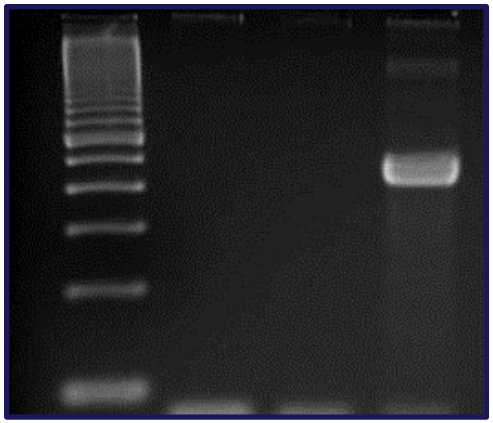
Verification of drug resistance

Modified Hodge Test



Recommended by CLSI and the CDC for the detection of carbapenemase production.

Endpoint PCR



Endpoint PCR used to detect the presence or absence of genes required for antibiotic production.

Antibiotic Profiling

VITEK used to analyze resistance to various antibiotic classes

Penicillins

Cephalosporins

Carbapenems

Quinolones

Aminoglycosides

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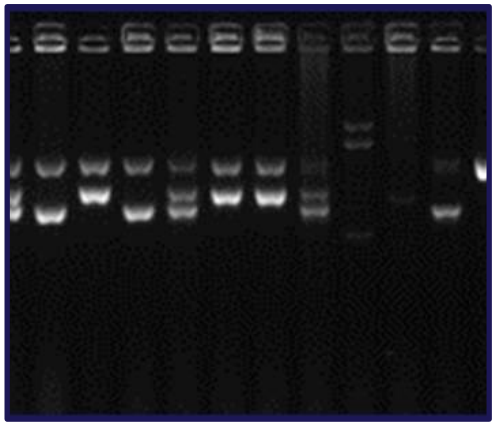
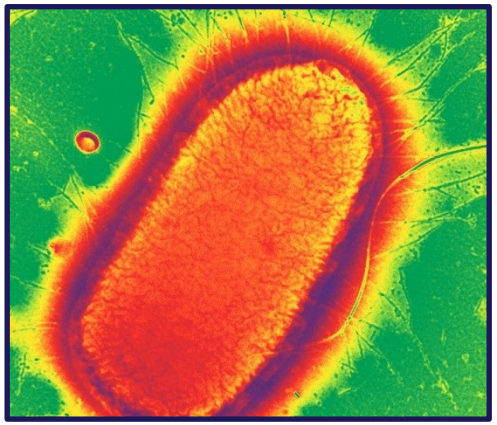
Verification of virulence

Endpoint PCR



Endpoint PCR used to detect the presence or absence of genes required for the production of toxins and other virulence factors.

Toxin Production



Detection of toxin production using an Enzyme Immunoassay (EIA)

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ATCC® Minis

BIG QUALITY
in a TINY TUBE

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Trusted ATCC Genuine Cultures® are offered in a convenient single-use “mini” format that saves you precious time and resources.

- Six pack of ready-to-use strains in glycerol stock
- Glass-free mini-cryovials with 2D barcode for easy storage
- Peel-off labels for fast and reliable recordkeeping

ATCC® Minis are authenticated and backed by ATCC polyphasic testing – ensuring the consistency and reliability that you have come to trust from ATCC Genuine Cultures®.

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ATCC® Minis USP QC organisms

2009: International USP <61> & US <62> Harmonization of US, Japan, EU Pharmacopeia[1]

Organism	ATCC® No.	USP 51 Antimicrobial	USP 61 Media	USP 62 Media
Pseudomonas aeruginosa	9027-MINI-PACK™	X	X	X
Staphylococcus aureus	6538-MINI-PACK™	X	X	X
Candida albicans	10231-MINI-PACK™	X	X	X
Aspergillus brasiliensis	16404-MINI-PACK™	X	X	
Escherichia coli	8739-MINI-PACK™	X		X
Bacillus subtilis	6633-MINI-PACK™		X	
Salmonella enterica	14028-MINI-PACK™			X
Clostridium sporogenes	11437-MINI-PACK™		X	

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[1]Sutton S, J Val Technol. 15 (3): 10, 2009 - ivtnetwork.com

ATCC® Minis general QC organisms

We are adding to the ATCC® Minis QC menu monthly
Visit our website regularly for updates: www.atcc.org

Organism	ATCC® No.	Organism	ATCC® No.
Escherichia coli	25922-MINI-PACK™	*Escherichia coli*	11775-MINI-PACK™
Staphylococcus aureus	25923-MINI-PACK™	*Staphylococcus epidermidis*	12228-MINI-PACK™
Pseudomonas aeruginosa	27853-MINI-PACK™	*Clostridium sporogenes*	19404-MINI-PACK™
Enterobacter aerogenes	13048-MINI-PACK™	*Streptococcus pyogenes*	19615-MINI-PACK™
Enterococcus faecalis	29212-MINI-PACK™	*Burkholderia cepacia*	25416-MINI-PACK™
Klebsiella pneumoniae	10031-MINI-PACK™	*Streptococcus pneumoniae*	49619-MINI-PACK™
Escherichia coli	11229-MINI-PACK™		

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Features & benefits

ATCC® Minis	QC Strains	Glycerol Stock	500 µL Tube	96-well Format
• Glass-free, convenient, easy-to-use “Mini” format that saves you precious time and resources	•The same trusted ATCC Genuine Cultures® you’ve come to know and trust	•The same ready-to-plate format many labs create in-house from ATCC strains	•Mini-cryovial takes less room than a standard cryovial •ATCC® Minis Working Rack helps you keep them upright	•Smaller storage space needed •ATCC® Minis Storage Box securely locks for safe keeping

6 Pack	Peel-off Label	2D Barcode	-80°C Storage	Labeled Exp.
•Convenient pack size allows you to plan ahead	•Apply to lab notebooks, plates, and flasks for proper labelling and tracking	•Easily ties into LIS/ LIMS for sample tracking	•The same storage many labs use for their lab-created glycerol stocks	•Required by many labs, and helps manage inventory

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Mini conveniences

- ATCC® Minis
  - Authenticated
  - Gold standard
  - Ready-to-plate
  - Single-use
  - Glass-free

ATCC Mini Accessories & Pack	
Storage Box	A lock tight storage solution for your ATCC® Minis. The ATCC® Minis Storage box with locking lid allows you to store ATCC® Minis in a convenient 96-well format that saves you precious freezer space.
Rack	A working rack to keep all your ATCC® Minis upright and ready-to-use. The 96-well format and low profile design allows you to easily work with and identify each of your ATCC® Minis.
Cap Tool	ATCC® Minis' little helper. The Cap Tool allows for aseptic opening of ATCC® Minis by fitting into the cap. Give it a twist and you're ready-to-plate.
QC Pack	ATCC has conveniently bundled the USP recommended QC organism ATCC® Minis 6 packs, with an overall cost savings of over 10% versus buying each individually.

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Thank you!

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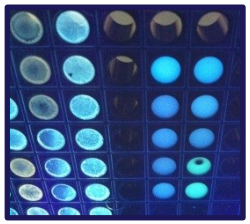
April 2, 2015
10:00 AM, 3:00 PM EST
James Clinton, Ph.D.
ATCC Transfection Reagents – Powerful Tools to Enable Genetic Manipulation



May 21, 2015
10:00 AM, 3:00 PM EST
Jodie Lee, M.S.
Seeing is Believing – Reporter-labeled Microbial Control Strains

Thank you for joining today!
Please send additional questions to tech@atcc.org

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