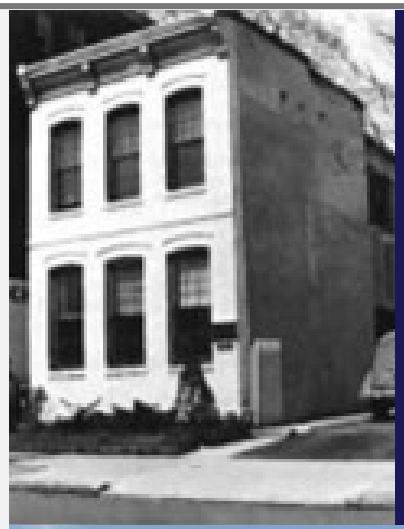
ATCC Microbiology – Best Practices for Stock Maintenance

Carol Horton
Marketing Manager, ATCC

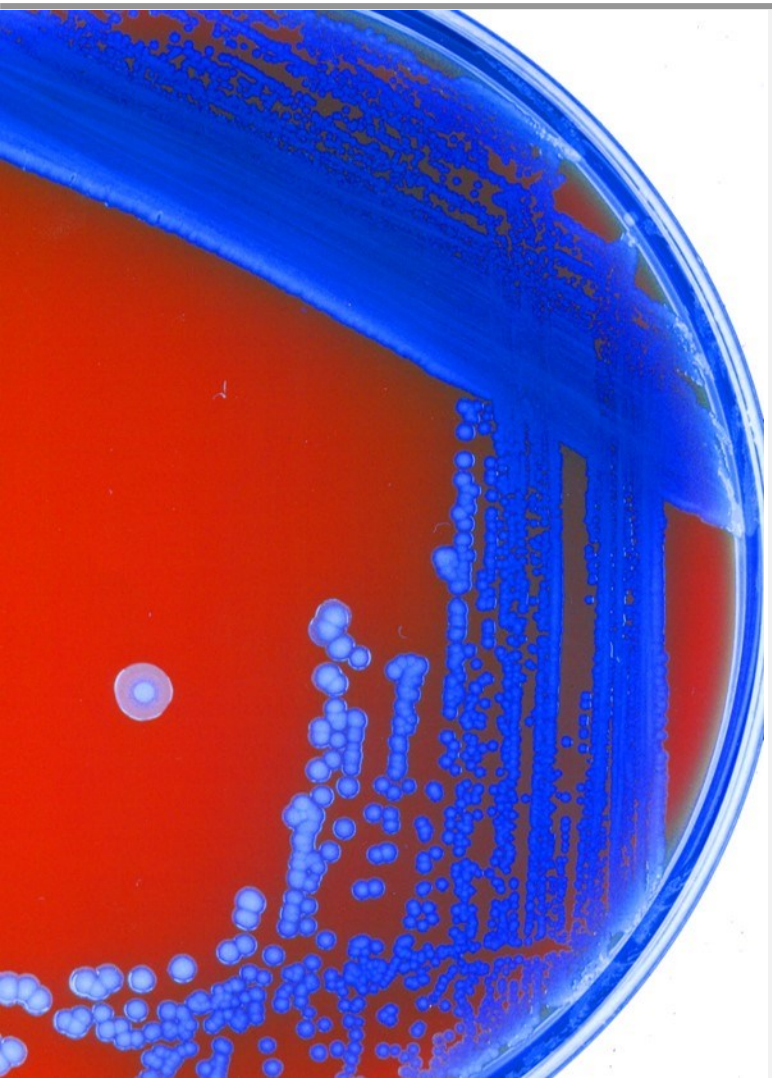
About ATCC

- 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 biological products and innovative solutions
- Strong team of 400+ employees; over one third with advanced degrees



Established partner to global researchers and scientists

Outline



- ATCC Microbiology workflow
- Passage matters
- Seed stock concept
- Principles of preservation and recovery
- Types of cultures and requirements
- Quality control
- Storage and safe-keeping

ATCC

ATCC Microbiology workflow

- Deposits come from every continent
- Newly identified species, outbreak strains, type strains, etc.

- Grow/expand deposit to a large enough scale to distribute
- Preserve according to the best method for the species
- Some cultures have to be maintained in continuous culture

- Confirm identity noted by the depositor
- Minimum test is purity, viability, and sequence
- Goal is two platforms to confirm the item

- Worldwide distribution
- Ship frozen/live materials

ATCC

Passage matters

Why is it important to minimize the number of passages?

- Reduce the chance of contamination
- Reduce/prevent genetic drift and mutations
- Minimize phenotypic variations

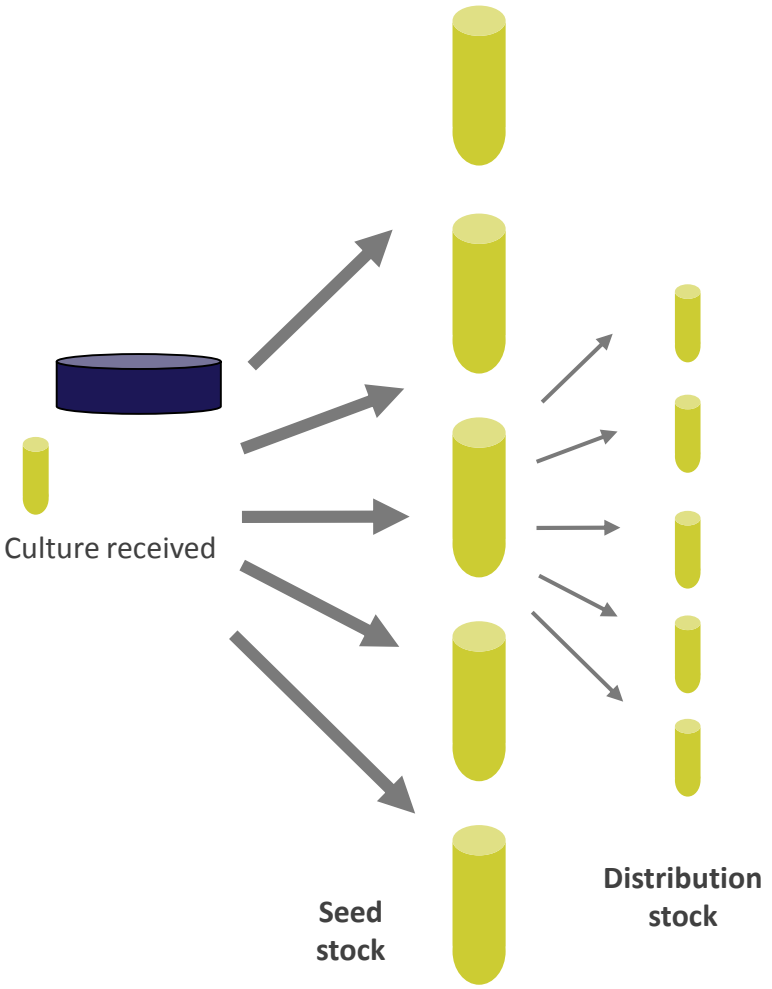
How many passages are acceptable?

Passage matters

USP <51> Antimicrobial Effectiveness Testing

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

Production



- Preserved cultures remain as close as possible to the original culture
- Seed stock is archived for future replenishment
- Distribution stock are used for distribution
- Authentication compares: Seed, Distribution, Initial culture

ATCC

Preservation defined

- Importance of preserving cultures:
  - Minimize passage
  - Maintain valuable stocks
- Most common methods:
  - Cryopreservation (freezing)
  - Lyophilization (freeze-drying)

Principles of freezing

- Ice crystals develop outside of the cells first, increasing the concentration of solutes & causing water to migrate out of the cells
- The addition of cryoprotectants can have different effects on this process:
  - Most cryoprotectants bind either the electrolytes, which increase in concentration during freezing, or the water molecules to delay freezing (e.g., glycerol)
  - Some cryoprotectants alter cell permeability (e.g., DMSO) to facilitate movement of water out of the cells
- The amount of water remaining inside the cells is critical:

Too much water = too many ice crystals

Too little water = damage from high intracellular osmolality

- In addition to using a cryoprotectant, the rate of cellular dehydration during the freezing process can be managed by using a -1°C/minute cooling rate

ATCC

Storage and recovery of frozen cultures

- Do not store frozen cultures in a freezer with a defrost cycle; this will expose the cultures to higher temperatures
- -130°C is the critical temperature for long-term storage of biological materials
- -80°C is sufficient for most bacteria and fungi for short-term storage (5 years or less)
- Thawing should be rapid at 37°C
- Initiate the culture in the medium recommended by ATCC or in the same growth medium used prior to freezing
- Incubate at optimum temperature & atmosphere

ATCC

Principles of freeze-drying

- Most cultures in the ATCC collection are lyophilized
- This method of preservation is still focused on managing both water & solvents, which are removed by sublimation
- Three stage approach:
  1. Freeze the material
  2. Primary drying – Removes most of the water and is achieved by applying just enough heat & pressure to vaporize (but not melt) the frozen liquid
  3. Secondary drying – Removes residual moisture by applying heat and low pressure to desorb any remaining bound water
- Results in stable, dehydrated material with a relatively indefinite shelf-life when stored appropriately
- Cryoprotectants vary based on the organism and method used (e.g., commercial, component, or manifold systems) – the most common cryoprotectant used in lyophilization is 20% skim milk

Storage and recovery of freeze-dried cultures

- Store freeze-dried cultures at 2-8°C
- Lower temperatures (-20°C or lower) will yield a longer shelf life
- Keep sealed, away from moisture and oxygen (i.e., free radical that will react with the dried material) and moisture
- Open vial and revive organisms
  - Instructions online at www.atcc.org
  - Use media recommended by ATCC
  - Incubate under appropriate conditions

ATCC

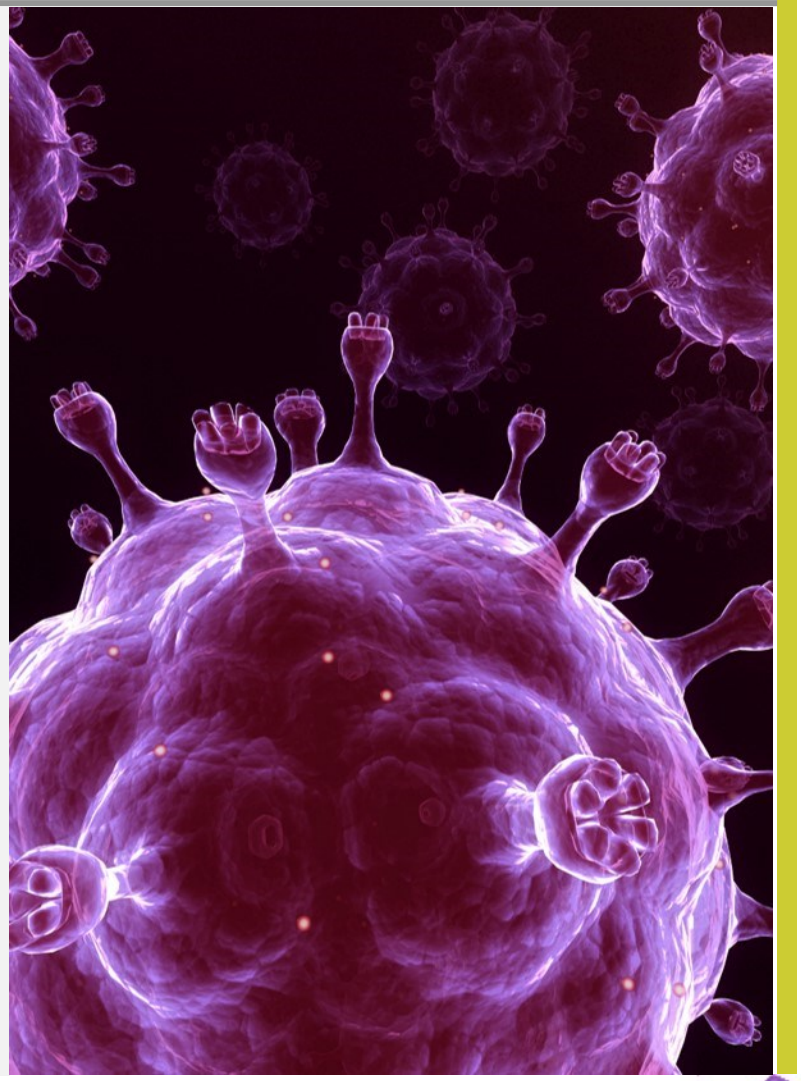
Bacteriology

- Most can be freeze-dried
- Almost all can be frozen

- Special cases:
  - Acidophiles (e.g., Thiobacillus acidophilus)
  - Anaerobes (e.g. methanogens)
  - Phages
  - Chlamydia and Rickettsia

Animal Virology

- Most viruses can be preserved using an uncontrolled method of freezing
- Cultures that are dependent upon the viability of their host cell should be preserved with a controlled method of freezing
- Viruses grown in eggs are typically frozen in the allantoic fluid or yolk sac



ATCC

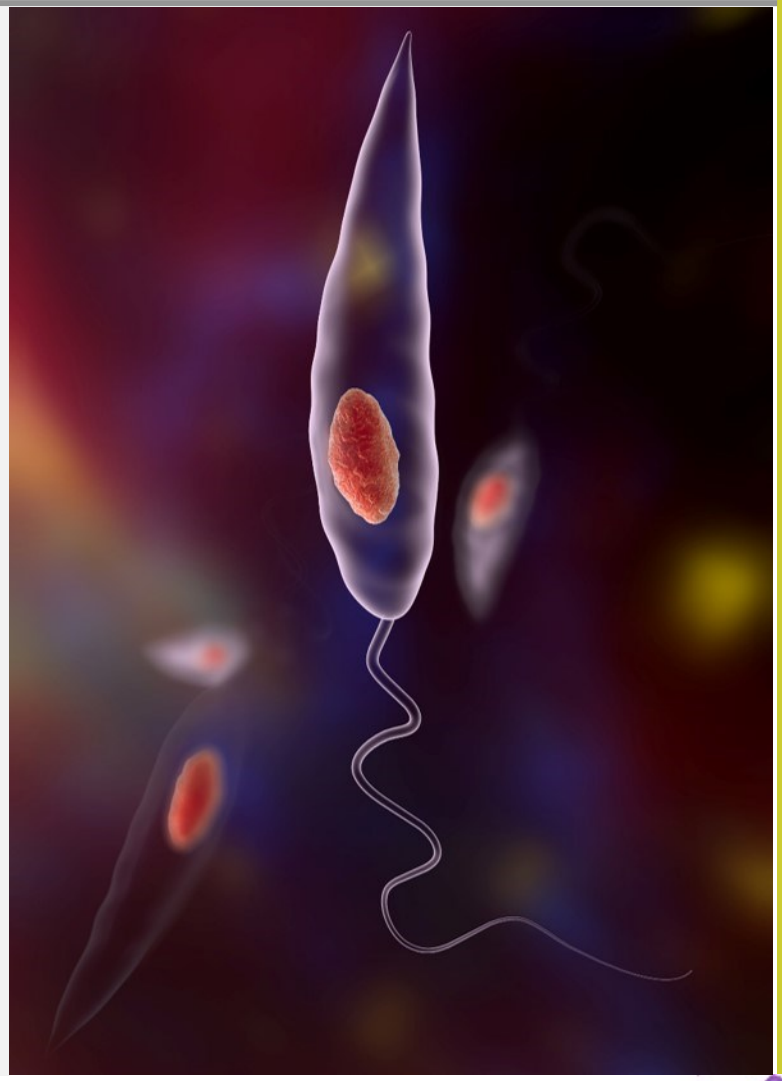
Mycology & Protistology

Mycology

- Yeasts can be preserved like bacteria
- The preservation method for filamentous fungi depends on whether the strain is sporulating or non-sporulating
- Slime molds can be frozen or freeze-dried

Protistology

- Over 100 protocols are used to preserve ATCC protozoa and algae
- Many strains are frozen, only a few can be freeze-dried or dried, and some are maintained as living cultures



ATCC

Factors that affect post-preservation viability

- Storage temperature was not maintained
  - Avoid refreezing even without complete thawing (i.e., avoid frost-free freezers)
  - Avoid thawing near-by materials when retrieving one ampoule from the freezer
- Age of the culture was too young or tool old
  - Best to harvest at late log or early stationary phase
- Culture or growth conditions
  - Aerated cultures are more permeable, allowing for more rapid water loss
  - Gram type (Gram-positive easier to freeze-dry) or fastidious culture

Microbial strain authentication

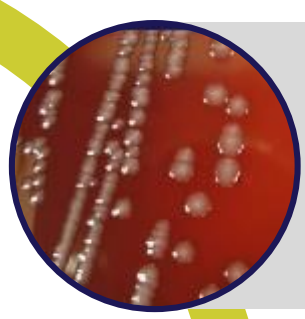
ATCC utilizes both classical and modern techniques

- Phenotypic analysis
- Genotypic analysis
- Proteotypic analysis
- Functional analysis

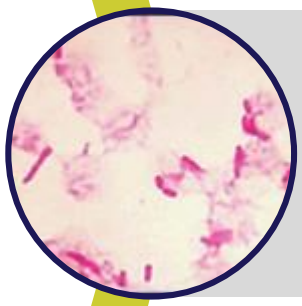
No single method of identification is sufficient

Phenotypic testing

Culture purity and biochemical properties



Colony morphology



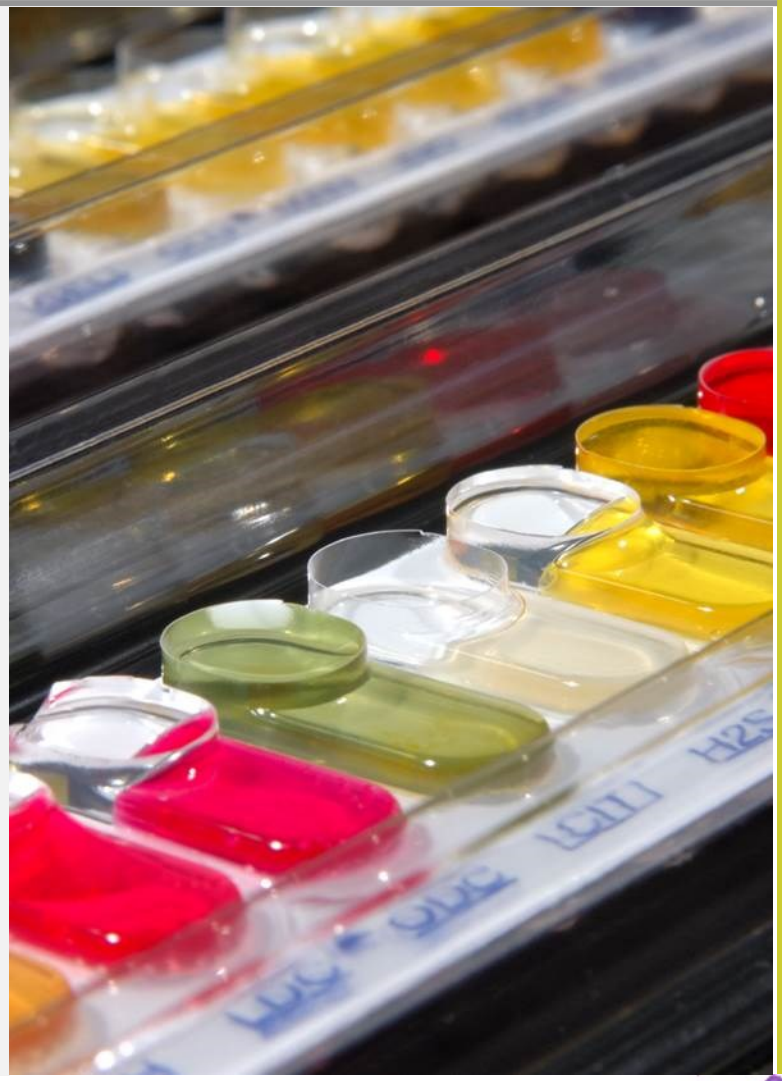
Cell attributes

Biochemical analysis

ATCC

Phenotypic testing platforms at ATCC

- API® ID
- Remel RapID™
- Biolog Microbial ID
- VITEK® 2
- VITEK ® 2/MS
- Serotyping
- Hundreds of biochemical tests



Genotypic testing

Sequencing

Toxinotyping

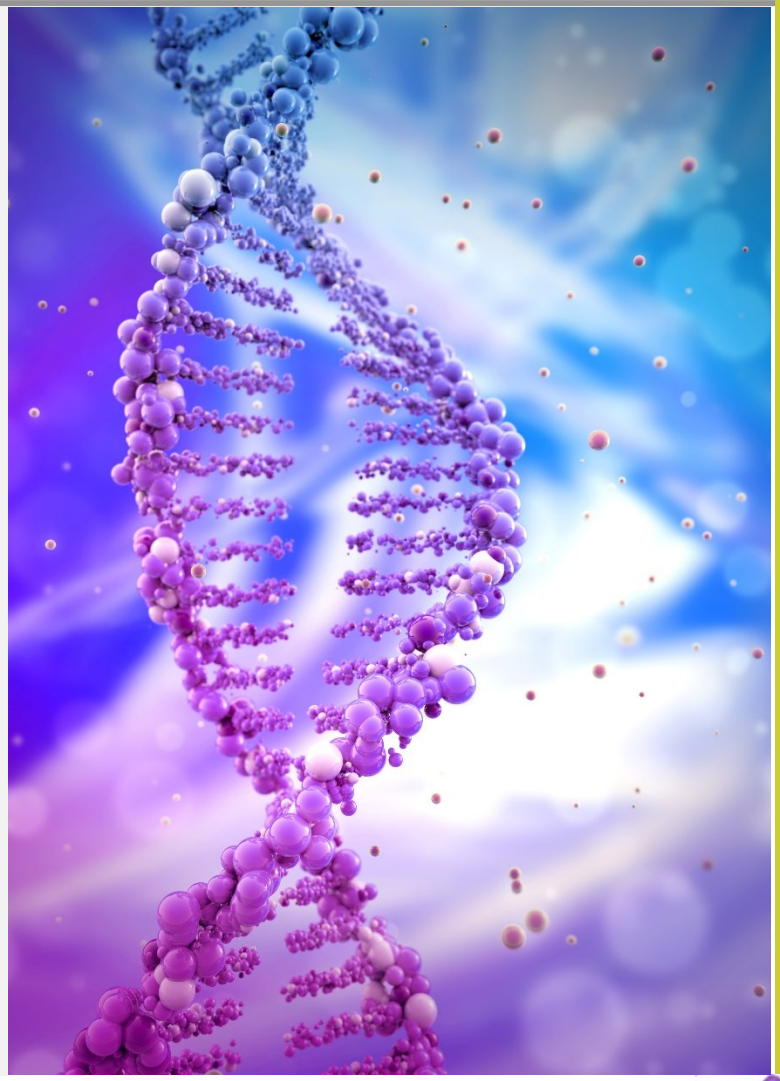
Ribotyping

Targeted gene sequencing

ATCC

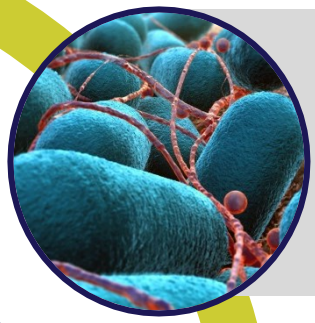
Genotypic testing platforms at ATCC

- ABI 3500 XL analyzers
  - MicroSeq® database
  - Public databases
  - Sequence provided by the depositor
- PCR
- DuPont™ RiboPrinter®
- Illumina® MiSeq



Functional testing

Functional characteristics



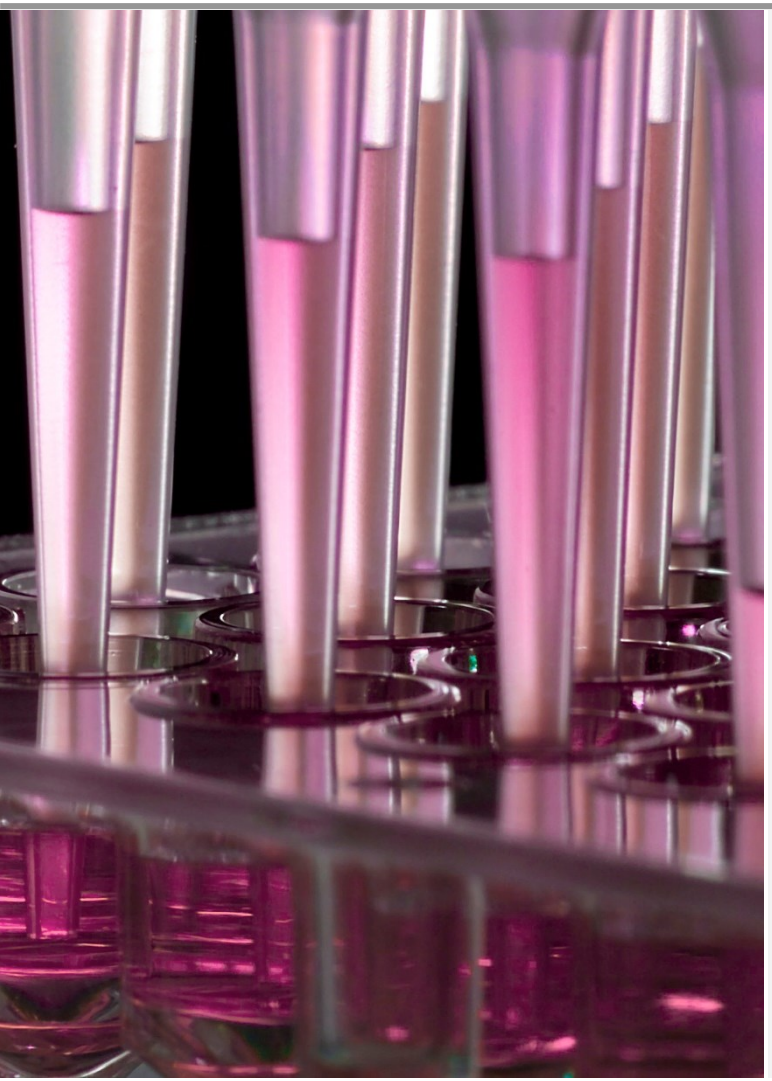
Serotype

Drug resistance

Virulence

ATCC

Functional testing platforms at ATCC

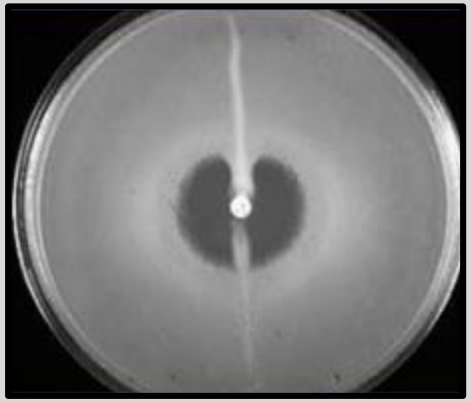


- AST
- Toxin assay
- IFA
- ELISA
- Cytotoxicity assay

ATCC

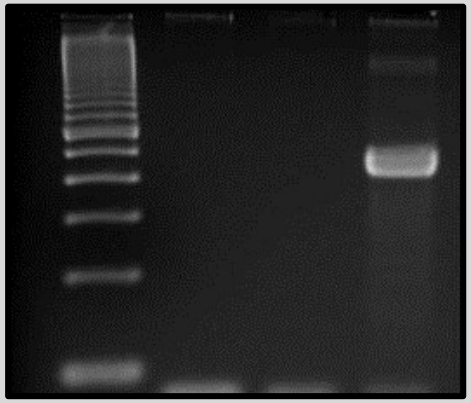
Verification of drug resistance

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

ATCC

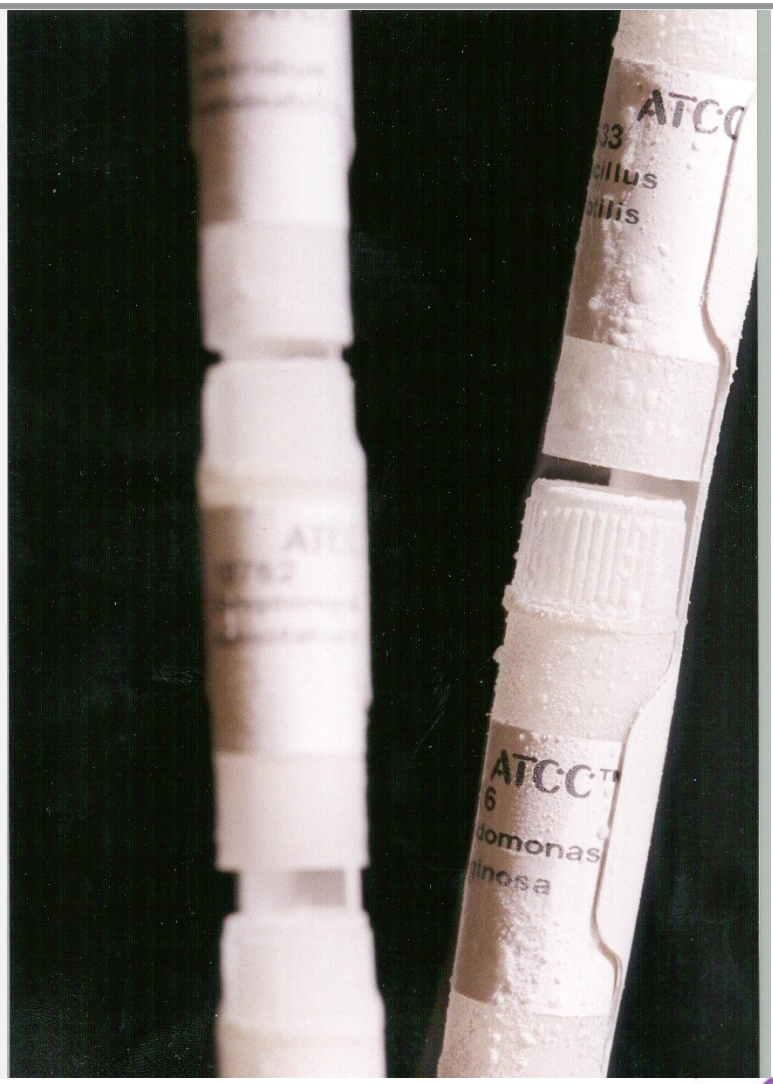
Safekeeping and security

- Keep good records
- Freezer alarms
- Monitor freezer temperature
- Call list for alarm response
- Spare freezers
- Backup storage (separate freezer or off-site)
- Dry ice cooling if necessary for -80°C freezers

ATCC

Storage temperature

- Storage temperature must be maintained at all times
- Warming and re-cooling, even in the absence of thawing, can be detrimental
- Avoid removing the entire inventory when retrieving one item
- -80°C is sufficient for most bacteria and fungi for short-term storage (5 years or less)
- -130°C is the critical temperature for biological materials
- -20°C is only adequate for freezing organisms when the stability has been established at this temperature



Summary points

Know your cultures and minimize passage	• Understand *in vitro* requirements of the organism • Use recommended media and atmospheric conditions • Track and minimize passage
Preserve valuable stocks using a method that best suits the organism	• Determine the optimal preservation method for the culture based on the organism's attributes and length of time in storage • Use a cryoprotectant that best suits the method of preservation
Verify stock identity when preparing MCB/WCB	• No one method is satisfactory for determining identity of an organism • Select phenotypic and genotypic methods of testing to validate authenticity – polyphasic approach
Maintain safekeeping and optimal storage	• Store materials under appropriate conditions • Monitor equipment and temperatures • Keep good records

ATCC

Disclaimers

© 2017 American Type Culture Collection. The ATCC trademark and trade name, and any other trademarks listed in this publication are trademarks owned by the American Type Culture Collection unless indicated otherwise. API® and VITEK® are registered trademarks of BioMérieux. RapID is a trademark of Thermo Fisher Scientific. RiboPrinter® and Dupont™ are registered trademarks or trademarks of E. I. du Pont de Nemours and Company. Illumina® is a registered trademark of Illumina, Inc. MicroSeq® is a registered trademark of Applied Biosystems.

Thank you for joining today!

Register for more ATCC "*Excellence in Research*" webinars, or watch recorded webinars, at www.atcc.org/webinars.

View our resources for microbiological quality control at www.atcc.org/QC.

Please email additional questions to: tech@atcc.org

ATCC