



Technical Bulletin

North Carolina State Laboratory of Public Health | Laboratory Improvement Unit

Review of Stock Culture Maintenance

Let's start out by making sure you understand the difference between a stock culture and a working culture. A stock culture is "a culture of a microorganism maintained solely to keep it viable for subculture into fresh medium". A working culture is defined as "a microorganism preparation derived from a reference stock culture used as a control on a regular day to day basis".

Why do I need these?

Control cultures are a necessary part of any quality assessment performed in a microbiology laboratory. Control organisms are tested simultaneously with patient samples and provide a positive and a negative reaction allowing the laboratory technician to report out accurate results. Check your package insert for the specified organisms the manufacturer recommends and document your results.

Where can I get these control cultures?

Quality control organisms are available from the American Type Culture Collection (ATCC®) or other commercial sources. The North Carolina State Lab of Public Health (NCSLPH) maintains a supply of the most frequently used control cultures that can be ordered by local health departments by filling out a request form located on the State Laboratory website: <http://slph.ncpublichealth.com/Forms/StockCultureOrderForm-2015.pdf>

What kind of media should I grow my control organisms on?

Control cultures should be grown on non-selective media such as blood agar plates or in a slant media. Non-selective media contains nutrients that will grow most of the organisms needed for stock cultures. Selective media such as GC-Lect® is **not** recommended for control organisms.

How do I inoculate my non-selective media plates?

It is important to inoculate the plate using a three or four quadrant method of streaking so you end up with isolated colonies. See example below:



How do I inoculate a broth tube?

To inoculate broth tubes, stab the sterile loop approximately halfway into the broth as illustrated below.



How long can I keep these organisms and how often should I subculture?

It is recommended that you obtain a new supply of organisms used regularly in your laboratory once a year because repeated sub culturing could lead to contamination and loss of viability. Follow

the instructions for reconstituting the lyophilized (freeze-dried) organisms. The stock organism is kept in the refrigerator at 2-8°C (tightly sealed) and is only used to subculture the working culture monthly or in case of contamination on the working culture. The working culture should be subbed from the stock culture every two weeks or at least monthly depending on the frequency of use and stored at 2-8°C. Below is a chart of the most common tests in the microbiology laboratory and the recommended control organisms.

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References:

CLSI M22-A3, Quality Control for Commercially Prepared Microbiological Culture Media; Approved Standard, 3rd ed. 2004.

<http://www.dictionary.com/browse/stock-culture>
<http://microbiologics.com/s.nl/c.915960/sc.11/category.78/ctype.KB/KB.98640/.f>

CLINICAL MICROBIOLOGY		
Test	Recommended QC Organisms	Expected Results
Gram Stain	<i>Escherichia coli</i> ATCC 25922	Gram negative rods
	<i>Staphylococcus aureus</i> ATCC 25923	Gram positive cocci
Oxidase	<i>Pseudomonas aeruginosa</i> ATCC 27853	Oxidase positive
	<i>Escherichia coli</i> ATCC 25922	Oxidase negative
ENVIRONMENTAL MICROBIOLOGY		
Colilert® or Colisure®	<i>Pseudomonas aeruginosa</i> ATCC 27853 or ATCC 10145	Negative for both total coliforms and fecal coliforms
	<i>Klebsiella pneumoniae</i> ATCC 31488	Positive for total coliforms and negative for fecal coliforms
	<i>Escherichia coli</i> ATCC 25922 or ATCC 11775	Positive for both total coliforms and fecal coliforms



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