

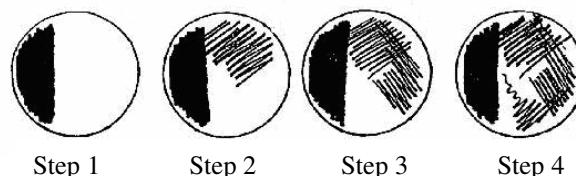
Quality control organisms are an integral part of any microbiology lab's quality assessment program. Media and reagent manufacturers will specify in their instructions which organisms should be used for quality control in order to obtain a positive reaction and which organism should be tested in order to obtain a negative reaction. Ensuring organism viability is very important in obtaining proper control results for media and reagents. Proper care and maintenance of these organisms is easy and inexpensive, and can prevent erroneous results that could result in your lab's inability to report accurate patient results.

The first consideration is what type of organism is required. The manufacturer's product insert will state the organism name and American Type Culture Collection (ATCC) number, if applicable. It is important to use the ATCC number prescribed to ensure proper results because different strains of the same organism can sometimes yield undesirable results. The ATCC organism listed has been tested and confirmed to perform according to manufacturer's standards.

Once the lab has acquired the proper organism, keeping it viable and pure is cost effective and will ensure reliable and accurate results. ATCC organisms vary in cost, and although some are not as expensive as others, cost does add up when purchasing organisms needlessly due to improper maintenance.

The key to proper care of the organism is to maintain both a stock culture and a working culture. Organisms should be cultured and maintained on non-selective medium such as blood agar or chocolate agar. Selective medium such as that used for *Neisseria gonorrhoeae* should not be used to maintain stock cultures. The stock culture can be kept refrigerated or at room temperature on an agar slant in a capped tube after growth has been established. The advantage to this method is that the tube stays tightly closed and contamination and drying of the agar are avoided. Tubed organisms should be sub-cultured periodically, depending on organism requirements, to ensure purity and viability. Refer to Clinical and Laboratory Standards Institute (CLSI) document M22-A3 or Microbiology reference books for further guidance on proper storage and sub-culturing requirements. Alternatively, a plate may be used, although the organism should be sub-cultured more often; i.e. no less than monthly to prevent drying of the medium. The stock culture is not used until the working culture dies or shows contamination, and then it is used only to establish a new working culture.

The working culture should be used for quality control and Gram staining. This culture can be set up on an agar slant and sub-cultured with each use or can be maintained on a plate. Plating is the preferred method, because it is easier to ensure culture purity. Streak the plates using the quadrant method illustrated below. Begin with a heavy inoculum in step 1, then streak out to three or four zones so that isolated colonies are present.



Plated organisms should be incubated for 24 hours or until good growth is established. Once colonies are present, plates should not be stored in the incubator, as the organisms will die. Instead, plates should be stored at room temperature or in the refrigerator, depending on the organism.

The CLSI document M22-A3 also offers guidance to the proper care and maintenance of quality control organisms. It states that tightly sealed slants used as stock cultures can be stored for ≤ 12 months at 2°C to 8°C . Molds and certain fastidious organisms such as *Neisseria gonorrhoeae* may require storage at 22°C to 35°C and may have a more limited survival time. Properly sealed and stored working cultures can be kept up to four weeks at 2°C to 8°C . Fresh working cultures should be prepared once per month. Over time, organisms can begin to mutate, so it is important to start a new stock culture at least once per year. Make sure that working cultures and stock cultures are properly labeled with the organism name, ATCC number, date, and technician's initials. It is also important to label them as either stock culture or working culture to ensure the integrity of the stock culture.

The Lab Improvement office at the North Carolina State Laboratory of Public Health maintains approximately 500 stock cultures. Although only a few have an ATCC number, they are available free of charge. Commonly requested organisms include those used by environmental labs for quality control of methods used to detect fecal coliforms in drinking water, as well as those commonly used in the clinical lab for gram stain, oxidase, and catalase quality control. For requests, contact Crystal Poppler at crystal.poppler@ncmail.net or 919/733-7186. Cultures cannot be delivered to post office boxes. Please allow up to two weeks for delivery.

RECOMMENDED ORGANISMS FOR MICROBIOLOGICAL QUALITY CONTROL

NC State Laboratory of Public Health – Laboratory Improvement

CLINICAL MICROBIOLOGY

Test	Recommended QC Organisms	Growth Requirements	Expected Results
Gram Stain	<i>Pseudomonas aeruginosa</i>	Blood agar plate; subculture weekly	Gram negative rods
	<i>Staph epidermidis</i>	Blood agar plate; subculture weekly	Gram positive cocci
Oxidase	<i>Pseudomonas aeruginosa</i>	Blood agar plate; subculture weekly	Oxidase positive
	<i>Staph epidermidis</i>	Blood agar plate; subculture weekly	Oxidase negative
Coagulase*	<i>Staph epidermidis</i>	Blood agar plate; subculture weekly	Coagulase negative
	<i>Staph aureus</i>	Blood agar plate; subculture weekly	Coagulase positive
Catalase*	<i>Strep species</i>	Blood agar plate; subculture weekly	Catalase negative
	<i>Staph epidermidis</i>	Blood agar plate; subculture weekly	Catalase positive

ENVIRONMENTAL MICROBIOLOGY (Water Bacti)

Test	Recommended QC Organisms	Growth Requirements	Expected Results
Colilert® or Colisure®	<i>Pseudomonas aeruginosa</i>	BAP; subculture weekly	Negative for both total coliforms and fecal coliforms
	<i>Klebsiella pneumoniae</i>	BAP; subculture weekly	Positive for total coliforms and negative for fecal coliforms
	<i>E. coli</i>	BAP; subculture weekly	Positive for both total coliforms and fecal coliforms

EDUCATIONAL MATERIALS AVAILABLE

Organism	Growth Requirements	Purpose
<i>Moraxella osloensis</i> : Lyophilized specimen or stained slide**	Chocolate agar, 3-5% CO ₂ environment; Subculture x3 weekly	To distinguish gram negative coccobacilli from gram negative diplococci
<i>Neisseria gonorrhoeae</i> : Lyophilized specimen or stained slide**	Chocolate agar, 3-5% CO ₂ environment; Subculture x3 weekly	To distinguish gram negative diplococci from other organisms

NOTE: Lyophilized specimens of *M. osloensis* and *N. gonorrhoeae* will grow on GC-Lect® media. However, **selective media is not recommended for stock culture maintenance.**

Other organisms available by request. For more information, contact Crystal Poppler at Lab Improvement at (919) 733-7186.

Notes: *test performed only in CLIA-certified high complexity labs

**growth requirements not applicable for stained slide

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References:

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

"Maintenance of Bacteria Strains", *Science in the Real World: Microbes in Action*, 1999.

MAINTAINING CULTURES, accessed June 28, 2007. <http://biology.fullerton.edu/biol302/302labf99/maintain.html>.

