

Quality matters: stock culture maintenance protocol

Key Words

Stock culture maintenance, microbiology, quality control organisms, storage, subculture, passage number, bacterial, fungal, reference strains, Thermo Scientific™ Culti-Loops™

Goal

To provide a stock culture maintenance protocol and relevant regulatory requirements for quality control organisms.

Background

Quality control organisms are a fundamental part of any quality assessment program for microbiology laboratories. Predominantly they are used for verifying performance of culture media, reagents, kits and systems to identify specified microorganisms, including a key role ensuring acceptable performance of susceptibility testing. Proper maintenance of these organisms is critical to achieving accurate control results for culture media and reagents. This document highlights industry guidelines and best practices to ensure compliance to various standards, at the same time as making optimum use of materials.

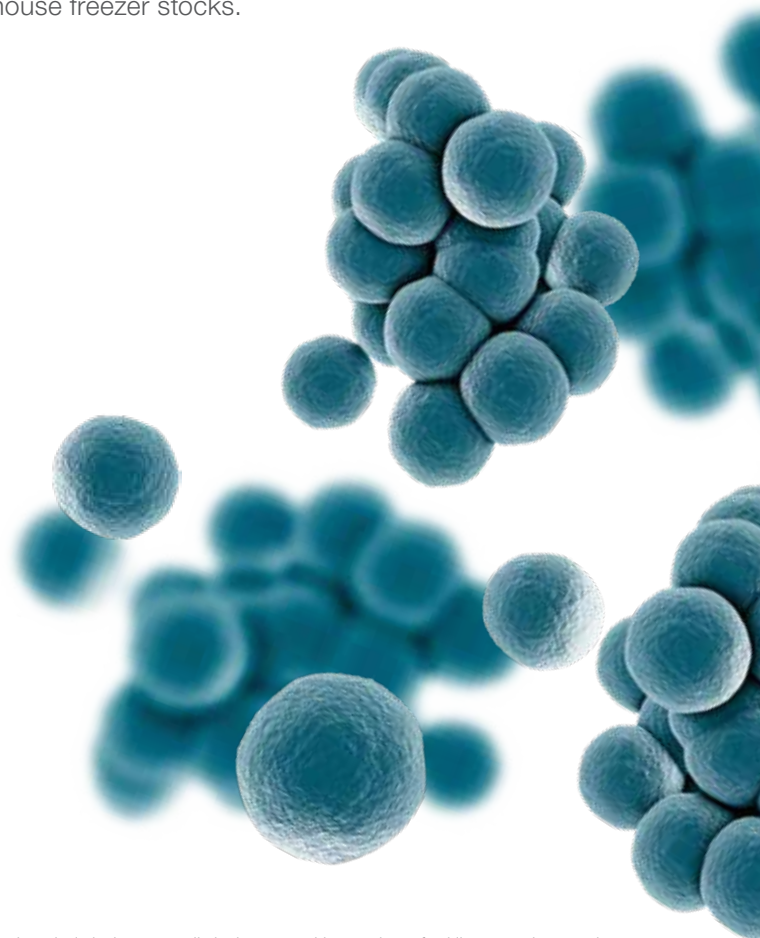


It is a general practice to sub-culture reference strains received from either culture collections or commercial suppliers such as Thermo Fisher Scientific.¹ Sub-culturing, also described as “passage”, means the transfer of viable microorganisms to a fresh culture medium.² There is a growing awareness of the importance to safeguard the purity and identity of cultures and sub-cultures. Regulatory guidance differs between advisory bodies on how many passages are allowed, however, there is overall agreement to limit the number of passages to a minimum. Over-passaging will increase the chance of variations, drift and contamination which should be avoided.

The Clinical & Laboratory Standards Institute (CLSI) M02 and M07 suggests 3 passages from the culture purchased from a reputable source.³ Best practice suggestion from both CLSI and EUCAST is to prepare fresh sub-cultures every week and use them for inoculating daily quality control cultures.⁴ A visual diagram outlining a recommended procedure for maintaining quality control stock and working cultures is shown on the following page. The diagram also complies with recommendations from ISO 11133:2014 for food, animal feed and water microbiology laboratories. The ISO 11133:2014 allows 3 passages from a reference stock received from commercial cultures similar to Culti-Loops Quality Control Organisms.⁵ A stock culture prepared from reference stock in a plate or a slant can be stored at room temperature or the fridge (2-8°C) for 4 weeks and is used to prepare working cultures. Working cultures must be renewed on a weekly basis and daily subcultures are prepared fresh from them.^{1,3,5} Some

strains may need new subcultures more frequently e.g. every two weeks for best results.⁶ It is also important to select a few colonies for preparing the subcultures to avoid selecting a mutant.⁴ For longer term freezer storage, use the stock cultures with appropriate cryoprotectants such as 10-15% glycerol with TSB. Storage must be at -70°C or below; ideally in vapor phase liquid nitrogen. It is also best practice to use all contents of a frozen vial once thawed and do not re-freeze any unused suspensions. Repeated freeze-thaw cycles damage chances of organism recovery and may alter characteristics. It is best practice to carry out some form of quality control tests to ensure purity and identity of the culture of the freezer stock.^{2,5} A careful labelling practice is essential for freezer stocks with details on passage numbers and other details for record keeping. Mislabelling could prove an expensive mistake when critical patient or product samples are at stake.

In the longer term, complete reliance on commercially-available cultures such as Culti-Loops Quality Control Organisms may prove to be a more cost-effective and risk-averse solution than preparing and maintaining in-house freezer stocks.



¹Due to the innate growth characteristics of some microorganisms, not all strains are suitable for stock culturing; these include, but are not limited to, anaerobic organisms, fastidious organisms, and organisms with plasmid mediated resistance used for susceptibility testing. Self-validation is required for stock cultures of these organism types.

²U.S. Pharmacopoeia [USP 35] (1117) Microbiological Best Laboratory Practices.

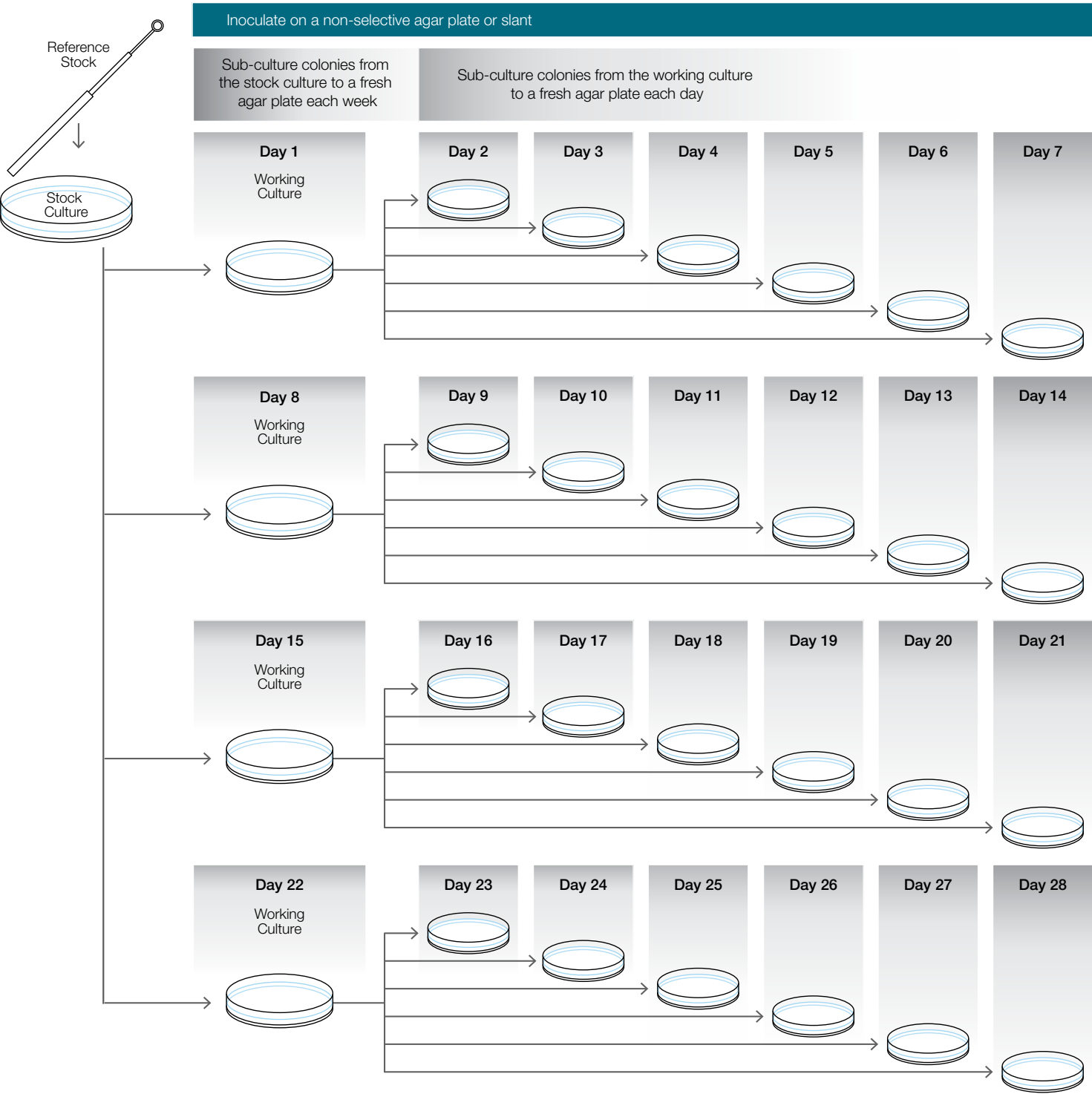
³Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Disk Susceptibility Tests: 13th ed. CLSI Standard M02 (ISBN 1-56238-835-5 [Electronic]). Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087 USA, 2018.

⁴EUCAST Disk Diffusion Method for Antimicrobial Susceptibility Testing - Version 6.0 (January 2017).

⁵ISO 11133:2014, Microbiology of food, animal feed and water - Preparation, production, storage and performance testing of culture media.

⁶Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; 9th ed. CLSI Standard M07-A9 ISBN 1-56238-784-7 (Electronic). Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, PA 19087 USA, 2012.

Stock Culture Maintenance



Culti-Loops Quality Control Organisms enable quick and safe preparation of ATCC® cultures for QC testing. They are ready-to-use bacteriological loops containing gel-stabilised micro-organisms. Each loop is individually packaged in a foil pouch and each pack contains 5 loops.

Below is a list of Culti-Loops Quality Control Organisms containing the recommended ATCC® strains for the EUCAST and CLSI test methods:

Culti-Loops Part Number	Description	ATCC® Strain	EUCAST		CLSI	
			Routine Testing	Extended Testing	Routine Testing	Extended Testing
R4601250	<i>Bacteroides fragilis</i>	25285™			✓	
R4601260	<i>Bacteroides thetaiotaomicron</i>	29741™			✓	
R4609498	<i>Campylobacter jejuni</i>	33560™	✓			
R4609452	<i>Clostridium difficile</i>	700057™			✓	
R4601951	<i>Eggerthella lenta</i>	43055™			✓	
R4607030	<i>Enterococcus faecalis</i>	29212™	✓		✓	✓
R4601996	<i>Enterococcus faecalis</i>	51299™	✓		✓	
R4607050	<i>Escherichia coli</i>	25922™	✓		✓	
R4601971	<i>Escherichia coli</i>	35218™			✓	
R4603810	<i>Haemophilus influenza</i>	10211™				✓
R4603830	<i>Haemophilus influenza</i>	49247™		✓	✓	
R4603806	<i>Haemophilus influenza</i>	49766™	✓		✓	
R4603074	<i>Klebsiella pneumoniae</i>	700603™		✓	✓	
R4609384	<i>Klebsiella pneumoniae</i>	BAA-1705™				✓
R4609385	<i>Klebsiella pneumoniae</i>	BAA-1706™				✓
R4609006	<i>Neisseria gonorrhoeae</i>	49226™			✓	
R4607060	<i>Pseudomonas aeruginosa</i>	27853™	✓		✓	
R4609389	<i>Staphylococcus aureus</i>	BAA-1708™			✓	
R4607010	<i>Staphylococcus aureus</i>	25923™			✓	
R4607011	<i>Staphylococcus aureus</i>	29213™	✓		✓	✓
R4609022	<i>Staphylococcus aureus</i>	43300™			✓	
R4606512	<i>Staphylococcus aureus</i>	BAA-976™				✓
R4606513	<i>Staphylococcus aureus</i>	BAA-977™				✓
R4609015	<i>Streptococcus pneumoniae</i>	49619™	✓		✓	



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Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing. 27th ed. CLSI supplement M100. Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087 USA, 2017

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Contact Information:

microbiology@thermofisher.com
USA +800 255 6730
International +44 (0) 1256 841144

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