



ACCREDITATION SCHEME FOR LABORATORIES

Guidance Note – C&B and ENV 003

Guidelines for Identification and Confirmation of Microorganisms

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1. Introduction

- 1.1. This document presents the guidelines for identification and confirmation of microorganisms in the Chemical & Biological and Environmental Testing fields.
- 1.2. This document shall be studied in conjunction with ISO/IEC 17025 General Requirements for the Competence of Testing and Calibration Laboratories, SAC-SINGLAS 002 Guidelines for the Application of ISO/IEC 17025:2017 and other C&B and ENV Series Technical Notes published by SAC-SINGLAS. For undated references, the latest edition of the referenced document (including any amendments) applies.

2. Definitions

- 2.1. Matrix/matrices refers to the component(s) of a sample. The matrix may contain complex inorganic and organic components which may interfere with the extraction, analysis and repeatability of the analysis. The matrices of foods, pharmaceutical products and traditional medicines can differ significantly and needs to be considered in the assessment of microbiological contamination levels in the sample.
- 2.2. Neutralisation refers to the inactivation of biologically inhibitory agents which may otherwise mask the presence of the organisms being tested for. Failure to neutralise these agents may prevent accurate assessment of levels of contaminants in the sample. The effectiveness of any neutralisation step should be validated by accredited/recognized methods
- 2.3. Biochemical test methods are methods that identify microorganisms by testing for the metabolic activity of the microorganisms or the enzymes that they produce. Examples include the Triple Sugar Iron test and the IMViC tests – Indole, Methyl Red, Voges Proskauer and Simmons Citrate Test. All biochemical tests, including rapid and/or automated tests should be performed and validated using accredited/recognized methods
- 2.4. Immunochemical test methods use the interaction of specific antibodies with unique structures on the cell surface of the microorganism or the proteins released by microorganisms to enable the identification of the microorganism. All immunochemical tests, should be performed and validated using accredited/recognized methods
- 2.5. Molecular methods identify microorganisms by analysis of unique sequences or patterns in the DNA of the microbial isolates. Molecular methods include those based on the Polymerase Chain Reaction (PCR), Pulse Field Gel Electrophoresis and Ribotyping technologies. All molecular methods should be performed and validated using accredited/recognized methods.
- 2.6. Quantitative assessments are methods for the determination of the numerical level of microbiological contamination in a sample. Examples of methods used for Quantitative assessments include the pour plate, spread plate and membrane filtration and MPN methods. All Quantitative assessments should be performed and validated using accredited/recognized methods.
- 2.7. Qualitative assessments are methods used to detect the presence of specific microbial contaminants. Qualitative assessments for low levels of contaminants may involve

resuscitation of microorganisms in non-selective broths, enrichment in selective broths followed by detection of the presence of the microorganism on selective and differential agar media. All Qualitative assessments should be performed and validated using accredited/recognized methods.

- 2.8. Selectivity (of culture media) refers to the degree to which culture media (used for enrichment and detection in Qualitative analysis) allow only the growth of the specific microorganisms being detected for.
- 2.9. Sensitivity (of culture media) refers to the degree to which culture media (used for enrichment and detection in Qualitative analysis) allow for the detection of a specified contaminant.
- 2.10. Growth Promotion Tests determine the suitability of culture media to culture selected microorganisms. The test involves challenging the medium with a small number of microorganisms to assure that the medium supports the growth of that microorganism. Example Growth Promotion Test are described in USP standards.

3. Sampling Requirements

- 3.1. The laboratory shall select the appropriate international accredited/recognised methods based on the category of samples to be tested. (i.e. Food and food products – FDA BAM, ISO or AOAC; Pharmaceutical products, nutraceutical, herbal or traditional medicine – USP, BP, JP or EP; Water – APHA, USEPA). Please refer to Annex 1 Table for appropriate recommended test methods.
- 3.2. Sampling must be carried out according to the requirements of the latest edition of the valid international accredited/recognised compendium of methods used (i.e. USP, BP, JP, EP, FDA BAM, APHA, AOAC etc.)
- 3.3. All samples to be tested must be kept at temperatures specified by appropriate approved methods
- 3.4. Sample preparation must be carried out according to the appropriate methods and based on the nature of the matrix (e.g. liquid, powdered, semisolid, solid, water soluble, non-water soluble, non-fatty, fatty, colloidal etc.).
- 3.5. The laboratory must use the volume/amount of sample according to the type of matrix and according to the selected method.
- 3.6. Samples that are suspected to have inhibitory or antimicrobial properties must be neutralised. Neutralization of inhibitory or antimicrobial properties must be verified through validation studies, if the sample is tested for the first time by the laboratory. Once validated the laboratory can follow the neutralisation SOP validated for the same type of samples in subsequent testing. Neutralisation can be through dilution of the antimicrobial properties and/or by adding appropriate neutralising agent(s) recommended by the method used. Re-validation must be performed when there has been a significant change in the character of the sample or when there has been a significant deviation in the precision of the results of the analysis.
- 3.7. Testing and confirmation must be carried out immediately after sample preparation steps to prevent death/inactivation of microorganisms due to environmental stress (e.g. osmotic stress due to dilution) which may affect the final results of the testing.

4. Testing and Confirmation

- 4.1. The laboratory shall select the appropriate methods, based on the matrices. (Please refer to Annex 1 Table). The selected methods shall be compliant with the latest valid edition of the valid international accredited/recognised compendium of methods, unless it is not appropriate or possible to do so. Where required, the laboratory shall clarify the testing specifications with their clients including whether quantitative assessment (detection with enumeration), qualitative assessment (detection without enumeration) or identification with confirmation of the isolates are required. It is mandatory to use internationally recognised biochemical, immunochemical or molecular methods for confirmation testing.
- 4.2. The laboratory should strictly use/prepare culture media as recommended by the selected method. Any deviation from the recommended culture media used should be validated for selectivity and sensitivity of test organisms. A Growth Promotion Test must be performed for each new batch of purchased ready-prepared medium, dehydrated medium, and medium prepared from components in the laboratory.
- 4.3. If the client's specification is for detection only, the laboratory should report positive results for any specific microbiological contaminant detected only as *presumptive positive*. The laboratory should also check with the clients if they wish to proceed with confirmation testing.
- 4.4. Identification and confirmation of specific microbiological contaminants should be carried out according to the appropriate methods. Different matrices have different recommended confirmation methods and the laboratory should not cross reference the confirmation test from another method that is not appropriate for the matrices.
- 4.5. If the selected test method does not have clear specifications of confirmation test methods to be used, the laboratory should carry out the confirmation tests using internationally recognised methods which may include biochemical test kits, molecular methods or immunological methods. The laboratory should validate the appropriateness of these confirmation methods.

Annex 1 – Recommended test methods for specific microorganisms

Microbes	Matrices	Reference Test Methods					
<i>Aeromonas</i>	Water/ Waste Water	APHA 9260L	NSM W9i1.3				
<i>Bacillus cereus</i>	Food/ Beverages	FDA-BAM Chapter 14	ISO 7932				
<i>Bacillus subtilis</i>	Food Water	BS EN ISO 7932					
<i>Campylobacter</i>	Food/ Poultry/ Beverages	FDA-BAM Chapter 7					
	Water	ISO 17995	APHA 9260G				
<i>Candida albicans</i>	Pharmaceutical products Medical Devices Biologics	US Pharmacopeia <62>	European Pharmacopeia, <2.6.13>	British Pharmacopeia Appendix XVI	Japanese Pharmacopeia <4.05>	Biochemical test kits	
	Beverages	US Pharmacopeia <62>					
	Cosmetics	FDA-BAM Chapter 23					
	Water	US Pharmacopeia <62>	European Pharmacopeia, <2.6.13>	British Pharmacopeia Appendix XVI	Japanese Pharmacopeia <4.05>		
<i>Clostridium perfringens</i>	Food	FDA-BAM Chapter 16	Compendium of Methods for the Microbiological Examination of Foods				

Microbes	Matrices	Reference Test Methods				
	Beverages	FDA-BAM Chapter 16	ISO 7937			
<i>Clostridium perfringens</i> (con't)	Water	NSM W5i3.1	ASTM 5916	ISO 6461 Part 1 & 2		
	Waste Water	NSM W5i3.1				
<i>Clostridium botulinum</i>	Food	FDA-BAM Chapter 17				
<i>Escherichia coli</i> O157:H7	Meat, Fruits, Vegetables	AOAC 2000.14	AOAC 996.09	AOAC Performance Tested Method 990701	Dynabeads® Method	US FDA BAM Chapter 4A
	Beverages	AOAC 2000.14				
<i>Escherichia coli</i>	Water	APHA 9221B/ F	APHA 9222 B/D/G	APHA 9223B	APHA 9225	ISO 9308-1
	Food	FDA-BAM Chapter 4	European Pharmacopeia	BS ISO 7251	British Pharmacopeia	US Pharmacopeia <62>
	Traditional Chinese Medicine/ Pharmaceutical products / Medical Devices	US Pharmacopeia <62>	Japanese Pharmacopeia 4.05	China Pharmacopeia	European Pharmacopeia	British Pharmacopeia Appendix XVI
<i>Legionella</i> spp.	Waste Water Cooling Tower Water / Fountain	ISO 11731	AS/NZS 3896	BS 6068-4.12		
	Spa water/ drinking water	ISO 11731-2	AS/NZS 3896			

Microbes	Matrices	Reference Test Methods					
<i>Listeria monocytogenes</i>	Food	FDA-BAM Chapter 10	AOAC PT 070202	VIDAS LMO2 method	ISO 11290-1		
	Environmentals	ISO 11290-1	Biochemical methods				
<i>Pseudomonas aeruginosa</i>	Food	ISO 13720	ISO 16266				
	Traditional Chinese Medicine	US Pharmacopeia <62>	British Pharmacopeia Appendix XVI	European Pharmacopeia 2.6.13			
	Water	ISO 16266	APHA 9213E	US Pharmacopeia <62>			
	Cosmetics/ Pharmaceutical products/ Medical Devices	FDA-BAM Chapter 23	European Pharmacopeia 2.6.13	US Pharmacopeia <62>	British Pharmacopeia Appendix XVI	Japanese Pharmacopeia <4.05>	
<i>Salmonella</i> spp.	Food	FDA-BAM Chapter 5	British Pharmacopeia	US Pharmacopeia <62>	AOAC 996.08	AOAC 2001.07	
		AOAC 2003.09	ISO 6579				
	Beverages	ISO 6579					
	Water/ Waste Water	APHA 9260D	APHA 9260B				
	TCM/ Pharma/ Med Devices	US Pharmacopeia <62>	European Pharmacopeia 2.6.13	British Pharmacopeia Appendix XVI	Japanese Pharmacopeia 4.05		
	Condemned offal	AOAC 2003.09					

Microbes	Matrices	Reference Test Methods				
<i>Shigella</i> spp.	Food	FDA-BAM Chapter 6	Compendium of Methods for the Microbiological Examination of Foods Chapter 38			
	Beverages	Compendium of Methods for the Microbiological Examination of Foods Chapter 38				
<i>Shigella</i> spp. (con't)	Water	APHA 9260E				
	Food, Feed, Swab on environmental samples and NHP faeces	ISO 21567				
<i>Staphylococcus aureus</i>	Food	FDA-BAM Chapter 12	AOAC 975.55	US Pharmacopeia <62>	British Pharmacopeia	European Pharmacopeia
	Water/ Waste	APHA 9213B				
	TCM	US Pharmacopeia <62>				
	Food & Animal feed	ISO 6888-1				
	Medical Devices/ Pharmaceutical products/ Biologics	European Pharmacopeia 2.6.13	US Pharmacopeia <62>	British Pharmacopeia Appendix XVI	Japanese Pharmacopeia 4.05	

Microbes	Matrices	Reference Test Methods	
<i>Vibrio cholerae</i> / <i>V. parahaemolyticus</i> / <i>V. vulnificus</i>	Food	FDA-BAM Chapter 9	Practical Food Microbiology, PHLS, Chapter 6.14
	Water	APHA 9260H	
<i>Yersinia enterocolitica</i>	Food	FDA-BAM Chapter 8	Practical Food Microbiology, PHLS, Chapter 6.17
	Swab on environmental samples, NHP faeces	ISO 10273	

Note : The list of microbial methods listed above is not exhaustive.