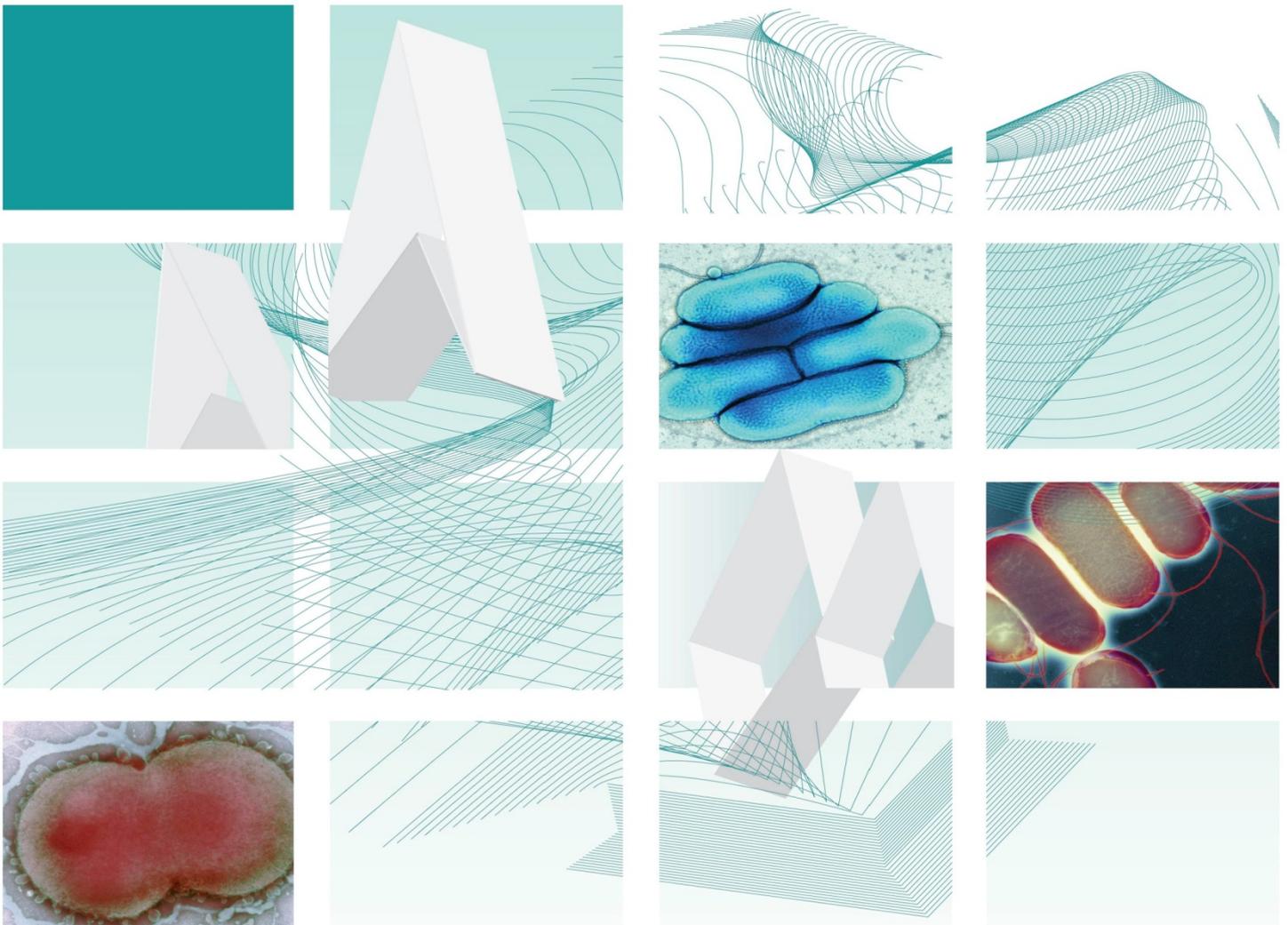




UK Standards for Microbiology Investigations

Porphyrin Synthesis (ALA) Test



Acknowledgments

UK Standards for Microbiology Investigations (SMIs) are developed under the auspices of Public Health England (PHE) working in partnership with the National Health Service (NHS), Public Health Wales and with the professional organisations whose logos are displayed below and listed on the website <https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories>. SMIs are developed, reviewed and revised by various working groups which are overseen by a steering committee (see <https://www.gov.uk/government/groups/standards-for-microbiology-investigations-steering-committee>).

The contributions of many individuals in clinical, specialist and reference laboratories who have provided information and comments during the development of this document are acknowledged. We are grateful to the Medical Editors for editing the medical content.

For further information please contact us at:

Standards Unit
Microbiology Services
Public Health England
61 Colindale Avenue
London NW9 5EQ

E-mail: standards@phe.gov.uk

Website: <https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories>

UK Standards for Microbiology Investigations are produced in association with:



Logos correct at time of publishing.

Contents

ACKNOWLEDGMENTS	2
CONTENTS	3
AMENDMENT TABLE	4
UK STANDARDS FOR MICROBIOLOGY INVESTIGATIONS: SCOPE AND PURPOSE.....	5
SCOPE OF DOCUMENT	8
INTRODUCTION	8
TECHNICAL INFORMATION/LIMITATIONS.....	8
1 SAFETY CONSIDERATIONS	9
2 REAGENTS AND EQUIPMENT	9
3 QUALITY CONTROL ORGANISMS	9
4 PROCEDURE AND RESULTS.....	9
APPENDIX: PORPHYRIN SYNTHESIS (ALA) TEST	11
REFERENCES	12



NICE has accredited the process used by Public Health England to produce Standards for Microbiology Investigations. Accreditation is valid for 5 years from July 2011. More information on accreditation can be viewed at www.nice.org.uk/accreditation.

For full details on our accreditation visit: www.nice.org.uk/accreditation.

Amendment Table

Each SMI method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from standards@phe.gov.uk.

New or revised documents should be controlled within the laboratory in accordance with the local quality management system.

Amendment No/Date.	6/06.01.15
Issue no. discarded.	2.3
Insert Issue no.	3
Section(s) involved	Amendment
Whole document.	Hyperlinks updated to gov.uk.
Page 2.	Updated logos added.
Introduction.	This section has been updated with information.
Technical information/Limitations.	This section has been updated and referenced.
Safety Considerations.	This section has been updated with referenced.
Reagents/Equipment.	Section updated and referenced.
Quality Control Organisms.	Positive control Non-X requiring <i>Haemophilus parainfluenzae</i> NCTC 7857 amended to <i>Haemophilus parainfluenzae</i> NCTC 10665.
Procedures and Results.	The procedures have been updated and referenced added.
Flowchart.	This flowchart has been amended and updated for easy guidance.
References.	Some references updated.

UK Standards for Microbiology Investigations[#]: Scope and Purpose

Users of SMIs

- SMIs are primarily intended as a general resource for practising professionals operating in the field of laboratory medicine and infection specialties in the UK.
- SMIs provide clinicians with information about the available test repertoire and the standard of laboratory services they should expect for the investigation of infection in their patients, as well as providing information that aids the electronic ordering of appropriate tests.
- SMIs provide commissioners of healthcare services with the appropriateness and standard of microbiology investigations they should be seeking as part of the clinical and public health care package for their population.

Background to SMIs

SMIs comprise a collection of recommended algorithms and procedures covering all stages of the investigative process in microbiology from the pre-analytical (clinical syndrome) stage to the analytical (laboratory testing) and post analytical (result interpretation and reporting) stages.

Syndromic algorithms are supported by more detailed documents containing advice on the investigation of specific diseases and infections. Guidance notes cover the clinical background, differential diagnosis, and appropriate investigation of particular clinical conditions. Quality guidance notes describe laboratory processes which underpin quality, for example assay validation.

Standardisation of the diagnostic process through the application of SMIs helps to assure the equivalence of investigation strategies in different laboratories across the UK and is essential for public health surveillance, research and development activities.

Equal Partnership Working

SMIs are developed in equal partnership with PHE, NHS, Royal College of Pathologists and professional societies.

The list of participating societies may be found at <https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories>.

Inclusion of a logo in an SMI indicates participation of the society in equal partnership and support for the objectives and process of preparing SMIs. Nominees of professional societies are members of the Steering Committee and Working Groups which develop SMIs. The views of nominees cannot be rigorously representative of the members of their nominating organisations nor the corporate views of their organisations. Nominees act as a conduit for two way reporting and dialogue. Representative views are sought through the consultation process.

SMIs are developed, reviewed and updated through a wide consultation process.

[#]Microbiology is used as a generic term to include the two GMC-recognised specialties of Medical Microbiology (which includes Bacteriology, Mycology and Parasitology) and Medical Virology.

Quality Assurance

NICE has accredited the process used by the SMI Working Groups to produce SMIs. The accreditation is applicable to all guidance produced since October 2009. The process for the development of SMIs is certified to ISO 9001:2008.

SMIs represent a good standard of practice to which all clinical and public health microbiology laboratories in the UK are expected to work. SMIs are NICE accredited and represent neither minimum standards of practice nor the highest level of complex laboratory investigation possible. In using SMIs, laboratories should take account of local requirements and undertake additional investigations where appropriate. SMIs help laboratories to meet accreditation requirements by promoting high quality practices which are auditable. SMIs also provide a reference point for method development.

The performance of SMIs depends on competent staff and appropriate quality reagents and equipment. Laboratories should ensure that all commercial and in-house tests have been validated and shown to be fit for purpose. Laboratories should participate in external quality assessment schemes and undertake relevant internal quality control procedures.

Patient and Public Involvement

The SMI Working Groups are committed to patient and public involvement in the development of SMIs. By involving the public, health professionals, scientists and voluntary organisations the resulting SMI will be robust and meet the needs of the user. An opportunity is given to members of the public to contribute to consultations through our open access website.

Information Governance and Equality

PHE is a Caldicott compliant organisation. It seeks to take every possible precaution to prevent unauthorised disclosure of patient details and to ensure that patient-related records are kept under secure conditions.

The development of SMIs are subject to PHE Equality objectives <https://www.gov.uk/government/organisations/public-health-england/about/equality-and-diversity>. The SMI Working Groups are committed to achieving the equality objectives by effective consultation with members of the public, partners, stakeholders and specialist interest groups.

Legal Statement

Whilst every care has been taken in the preparation of SMIs, PHE and any supporting organisation, shall, to the greatest extent possible under any applicable law, exclude liability for all losses, costs, claims, damages or expenses arising out of or connected with the use of an SMI or any information contained therein. If alterations are made to an SMI, it must be made clear where and by whom such changes have been made.

The evidence base and microbial taxonomy for the SMI is as complete as possible at the time of issue. Any omissions and new material will be considered at the next review. These standards can only be superseded by revisions of the standard, legislative action, or by NICE accredited guidance.

SMIs are Crown copyright which should be acknowledged where appropriate.

Suggested Citation for this Document

Public Health England. (2015). Porphyrin Synthesis (ALA) Test. UK Standards for Microbiology Investigations. TP 29 Issue 3. <https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories>

Scope of Document

The porphyrin synthesis test is used to identify haemin producing *Haemophilus* species. It avoids the problems of red cell carry over associated with tests for X and V dependence. The porphyrin test is considered to be the definitive method for the differentiation of *Haemophilus* species^{1,2}.

This SMI should be used in conjunction with other SMIs.

Introduction

Haemophilus species are not readily distinguishable by their colonial morphology or gram stain appearance. Oral flora grown from routine sputum cultures often contains organisms which resemble *Haemophilus* species. *H. influenzae*, which is the principal human pathogen, can be distinguished from other *Haemophilus* species and oral flora by determining the need for essential factors for growth, specifically Haemin (X factor) and Nicotinamide-adenine dinucleotide (NAD/V factor). *H. influenzae* requires both factors for growth where as some of the other species require only one. The requirement for one or both of the growth factors nicotinamide adenine dinucleotide (NAD or V factor) and haemin (X factor) is used to characterise *Haemophilus* species.

Strains which produce their own haemin possess the enzyme porphobilinogen synthase which can convert d-aminolaevulinic acid (ALA) to protoporphyrin and ultimately haemin.

This test demonstrates the ability of a bacterium supplied with d-aminolaevulinic acid to synthesise and excrete porphobilinogen and other porphyrins, indicating that they are not X dependent.

Technical Information/Limitations

False negative reactions may occur if the inoculum is insufficient or if the culture is greater than 24hr old. Cultures being tested must not be older than 24hr.

Inoculum must be heavy.

Fluorescence observations must be made in a darkened room to prevent false negative observations.

Oxidase positive and catalase positive bacteria commonly found in the oropharynx can make haem and haem precursors from ALA and yield false-positive results. Test only *Haemophilus* species with ALA¹.

1 Safety Considerations³⁻¹⁹

Refer to current guidance on the safe handling of all organisms and reagents documented in this SMI.

All work likely to generate aerosols must be performed in a microbiological safety cabinet.

The above guidance should be supplemented with local COSHH and risk assessments.

Compliance with postal and transport regulations is essential.

2 Reagents and Equipment

Discrete bacterial colonies growing on solid medium

Kovac's indole reagent²⁰

ALA enzyme substrate solution²¹;

Ingredients

d-aminolaevulinic acid Hydrochloride	2mol/L
Magnesium sulphate	0.8mol/L
Sodium phosphate buffer pH 6.9	0.1mol/L

Commercial reagents and disks are available. Follow manufacturer's instructions.

Small glass tubes

Bacteriological straight wire/loop or disposable alternative or disposable Pasteur pipette.

Ultra violet light (360nm)

3 Quality Control Organisms

Positive Control

Non-X requiring *Haemophilus parainfluenzae* NCTC 10665

Negative Control

X requiring *Haemophilus influenzae* NCTC 11931

Note: The reference strains are validated by NCTC for the test shown.

4 Procedure and Results^{20,21}

Method 1

- Distribute 0.5mL volumes of the enzyme substrate solution in small glass tubes.
- Add a large loopful of bacteria from a plate culture to a tube of the substrate and emulsify to produce a milky suspension. Test fresh subcultures of the quality control organisms alongside the test.

- Incubate for 4hr at 35-37°C.
- Observe the tubes under a Wood's lamp (UV 360nm) in a dark room

Interpretation

Positive

A brick-red fluorescence from either the bacterial deposit or the supernatant fluid in the tube indicates porphyrin synthesis and thus the absence of a requirement for X factor.

Negative

Absence of fluorescence indicates that the bacterium requires X factor for growth

Method 2

- Set up the test as in method 1 and incubate for 24hr at 35-37°C.
- Add 0.5mL of Kovac's indole reagent to the bacterial suspension after incubation. Shake the tube vigorously and allow the phases to separate.
- Observe the tubes for colour change.

Interpretation

Positive

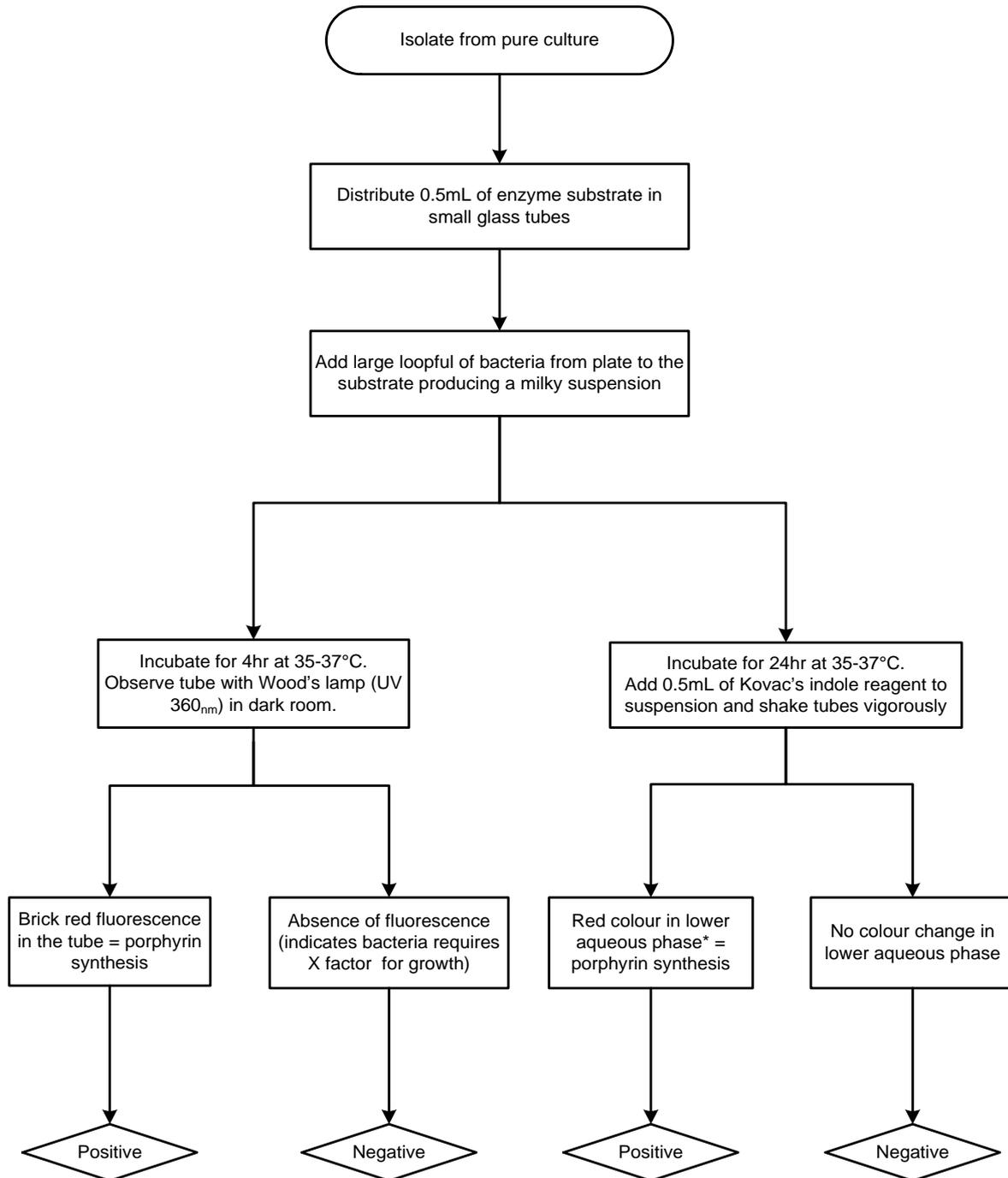
A red colour in the lower aqueous phase indicates porphyrin synthesis and the absence of a requirement for X factor.

Negative

No colour change either in the reagent layer.

Note: Kovac's Indole reagent also gives a red colour with indole production, but this will be seen only in the upper alcohol phase. Inoculate a tube without d-aminolaevulinic acid as a control for this.

Appendix: Porphyrin Synthesis (ALA) Test



Note:

Positive control *Haemophilus parainfluenzae* NCTC 10665
Negative control *Haemophilus influenzae* NCTC 11931

* Kovac's reagent also gives a red colour with indole production but only in upper alcohol phase. Inoculate control tube without d-aminolaevulinic acid

The flowchart is for guidance only.

References

1. Gadberry JL, Amos MA. Comparison of a new commercially prepared porphyrin test and the conventional satellite test for the identification of Haemophilus species that require the X factor. J Clin Microbiol 1986;23:637-9.
2. Kilian M. A rapid method for the differentiation of Haemophilus strains. The porphyrin test. Acta Pathol Microbiol Scand [B] Microbiol Immunol 1974;82:835-42.
3. European Parliament. UK Standards for Microbiology Investigations (SMIs) use the term "CE marked leak proof container" to describe containers bearing the CE marking used for the collection and transport of clinical specimens. The requirements for specimen containers are given in the EU *in vitro* Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1) which states: "The design must allow easy handling and, where necessary, reduce as far as possible contamination of, and leakage from, the device during use and, in the case of specimen receptacles, the risk of contamination of the specimen. The manufacturing processes must be appropriate for these purposes".
4. Official Journal of the European Communities. Directive 98/79/EC of the European Parliament and of the Council of 27 October 1998 on *in vitro* diagnostic medical devices. 7-12-1998. p. 1-37.
5. Health and Safety Executive. Safe use of pneumatic air tube transport systems for pathology specimens. 9/99.
6. Department for transport. Transport of Infectious Substances, 2011 Revision 5. 2011.
7. World Health Organization. Guidance on regulations for the Transport of Infectious Substances 2013-2014. 2012.
8. Home Office. Anti-terrorism, Crime and Security Act. 2001 (as amended).
9. Advisory Committee on Dangerous Pathogens. The Approved List of Biological Agents. Health and Safety Executive. 2013. p. 1-32
10. Advisory Committee on Dangerous Pathogens. Infections at work: Controlling the risks. Her Majesty's Stationery Office. 2003.
11. Advisory Committee on Dangerous Pathogens. Biological agents: Managing the risks in laboratories and healthcare premises. Health and Safety Executive. 2005.
12. Advisory Committee on Dangerous Pathogens. Biological Agents: Managing the Risks in Laboratories and Healthcare Premises. Appendix 1.2 Transport of Infectious Substances - Revision. Health and Safety Executive. 2008.
13. Centers for Disease Control and Prevention. Guidelines for Safe Work Practices in Human and Animal Medical Diagnostic Laboratories. MMWR Surveill Summ 2012;61:1-102.
14. Health and Safety Executive. Control of Substances Hazardous to Health Regulations. The Control of Substances Hazardous to Health Regulations 2002. 5th ed. HSE Books; 2002.
15. Health and Safety Executive. Five Steps to Risk Assessment: A Step by Step Guide to a Safer and Healthier Workplace. HSE Books. 2002.
16. Health and Safety Executive. A Guide to Risk Assessment Requirements: Common Provisions in Health and Safety Law. HSE Books. 2002.

17. Health Services Advisory Committee. Safe Working and the Prevention of Infection in Clinical Laboratories and Similar Facilities. HSE Books. 2003.
18. British Standards Institution (BSI). BS EN12469 - Biotechnology - performance criteria for microbiological safety cabinets. 2000.
19. British Standards Institution (BSI). BS 5726:2005 - Microbiological safety cabinets. Information to be supplied by the purchaser and to the vendor and to the installer, and siting and use of cabinets. Recommendations and guidance. 24-3-2005. p. 1-14
20. Barrow GI, Feltham RKA. Appendix C; Characterisation Tests. Cowan and Steel's Manual for the Identification of Medical Bacteria. 3rd ed. Cambridge: Cambridge University Press; 1999. p. 235-6.
21. MacFaddin J. Porphyrin- d- Aminolevulinic Acid (ALA) Test. Biochemical tests for Identification of Medical Bacteria. 3rd ed. Philadelphia: Lippincott Wilkins and Williams; 2000. p. 403-6.