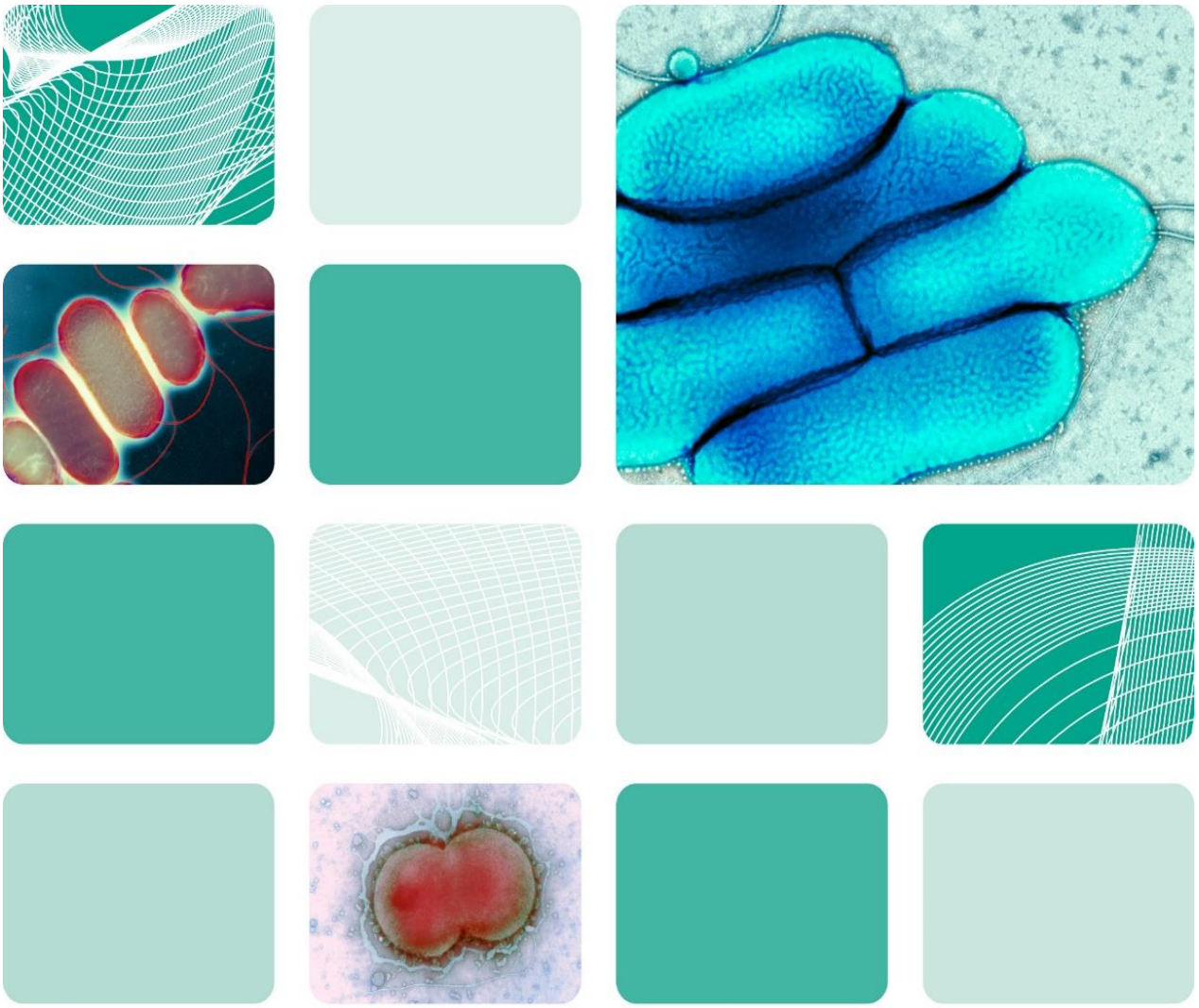
UK Health Security Agency

UK Standards for Microbiology Investigations

ONPG (β-Galactosidase) test



Issued by the Standards Unit, UK Standards for Microbiological Investigations, UKHSA

Acknowledgments

UK Standards for Microbiology Investigations (UK SMIs) are developed under the auspices of UKHSA working in partnership with the partner organisations whose logos are displayed below and listed on the UK SMI website. UK SMIs are developed, reviewed and revised by various working groups which are overseen by a 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.

UK SMIs are produced in association with:

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Contents

Amendment table

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

Any alterations to this document should be controlled in accordance with the local document control process.

Amendment number/date	8/28.02.25
Issue number discarded	4
Insert issue number	4.1
Section(s) involved	**Amendment**
Whole document.	**This is an administrative point change.** **The content of this UK SMI document has not changed.** **The last scientific and clinical review was conducted on 03/12/2018** Hyperlinks throughout document updated to Royal College of Pathologists website. Public Health England replaced with UK Health Security Agency throughout the document, including the updated Royal Coat of Arms Partner organisation logos updated. Broken links to devolved administrations replaced. References to NICE accreditation removed. Scope and Purpose replaced with General and Scientific information to align with current UK SMI template.

Amendment number/date	7/03.12.18
Issue number discarded	3
Insert issue number	4
Anticipated next review date*	03.12.21
Section(s) involved	**Amendment**

Whole document.	Document updated. Technical limitations updated with subheadings. References updated with grades.

*Reviews can be extended up to 5 years where appropriate

1 General information

View general information related to UK SMIs.

2 Scientific information

View scientific information related to UK SMIs.

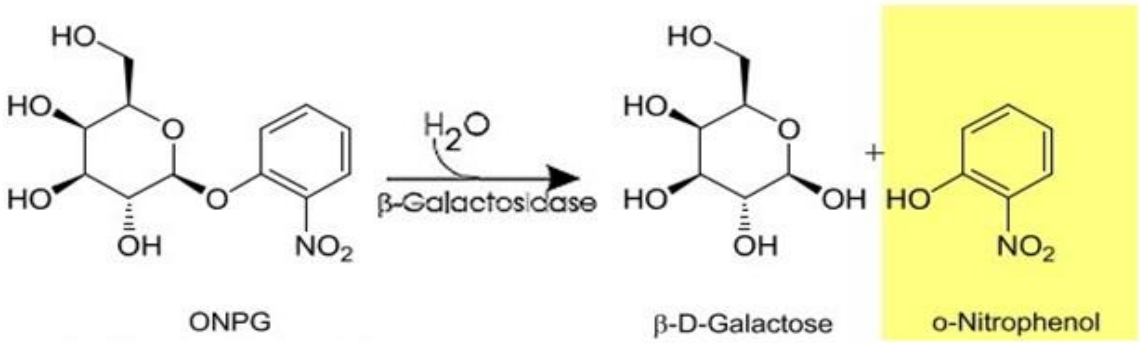
3 Scope of document

This document covers the procedure for ONPG test. The test is important in differentiating among the Enterobacteriaceae which are commonly classified according to their ability to ferment lactose[1]. It is also used to differentiate *Neisseria lactamica* from other fastidious *Neisseria* species.

This UK SMI should be used in conjunction with other UK SMIs.

4 Introduction

The ONPG (o-nitrophenyl-β-D-galactopyranoside) test is used to determine the presence or absence of the enzyme β-galactosidase in an organism[2]. The presence of two enzymes, permease and β-galactosidase, are required to demonstrate lactose fermentation. Permease allows the lactose to enter the bacterial cell. In lactose-fermenting bacteria the breakdown of lactose to glucose and galactose involves the enzyme beta-galactosidase[3]. True lactose non-fermenters do not possess either of these enzymes. Late lactose fermenting organisms do not have permease, but do possess β-galactosidase. ONPG is similar in structure to lactose. If β-galactosidase is present, the colourless ONPG is split in to galactose and o-nitrophenol, a yellow compound[4,5]. The reaction is shown as follows:



(Colourless) (Colourless) (Yellow)

Note: "ONPG" (also known as "2-Nitrophenyl β-D-galactopyranoside") is a Chemical analog of the sugar lactose and is hydrolysed by the enzyme lactase. Like β-galactosidase, lactase breaks lactose down into galactose and glucose.

5 Technical information/limitations

5.1 Growth media

The test should be performed, where possible, from a non-selective medium. If the test is performed from selective agar, a purity plate must be included to check for purity of the organism. Organisms that have grown on glucose containing media show less reactivity than those grown on lactose containing media. Glucose inhibits β-galactosidase.

5.2 Pigmentation in organisms

The test cannot be performed on organisms containing a yellow pigment or other coloured pigmentation as it makes it difficult to read the test[5].

5.3 Interpretation of results

The ONPG solution must be correctly buffered to prevent false negative and false positive reactions.

A heavy inoculum is necessary to obtain a high concentration of enzyme.

Discard the substrate if it looks yellow prior to inoculation.

6 Safety considerations[6-23]

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

7 Reagents and equipment

Discrete bacterial colonies growing on solid medium.

ONPG broth (alternatively, commercially available prepared ONPG discs may be used according to the manufacturer's instructions).

Bacteriological straight wire/loop (preferably nichrome) or disposable alternative.

8 Quality control organisms

For Enterobacteriaceae,

Positive control:

Escherichia coli	NCTC 10418 or NCTC 12241

Negative control:

Proteus mirabilis	NCTC 10975

For *Neisseria* species,

Positive control:

Neisseria lactamica	NCTC 10617

Negative control:

Neisseria gonorrhoeae	NCTC 8375

Note: These strains are validated by NCTC to give this result.

9 Procedure and results

- a loopful of test organism from a culture plate or slant should be sufficient. Include the positive and negative controls with every batch of tests
- inoculate tubes containing ONPG reagent and incubate at 35-37°C for up to 24hr
- examine for yellow colour after 4hr and for up to 24hr

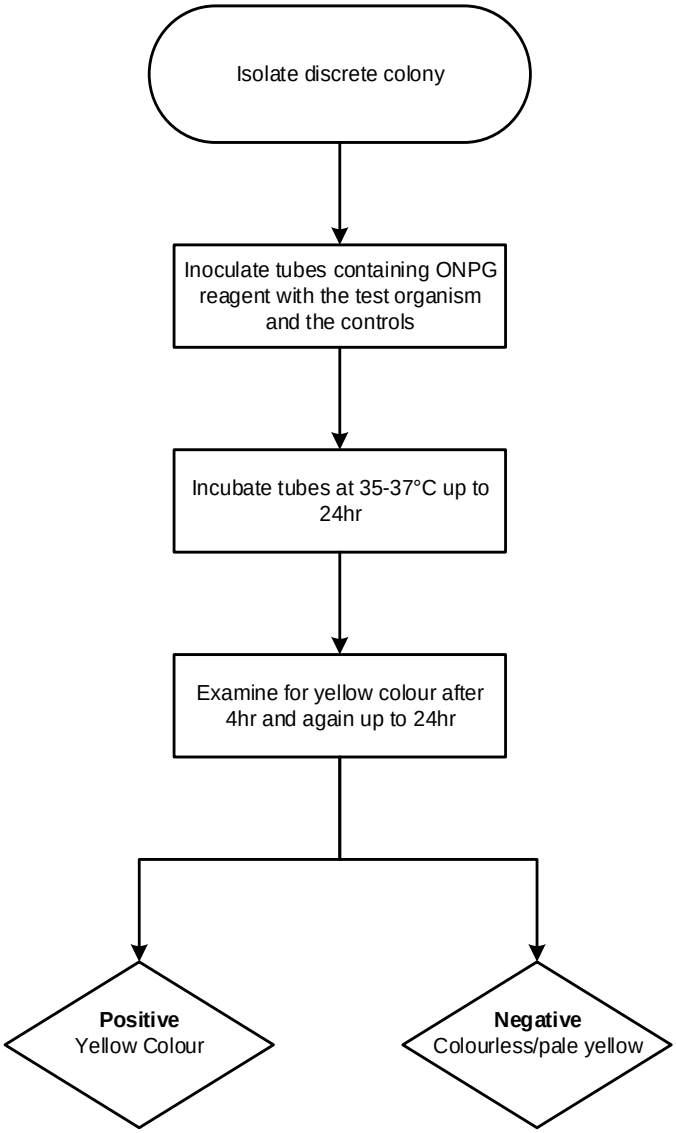
Positive result:

Yellow colour (indicates lactose fermenter).

Negative result:

Colourless/pale yellow (indicates lactose non-fermenter).

Algorithm: ONPG (β-Galactosidase) test



Note:

For Enterobacteriaceae
Positive control: *Escherichia coli* NCTC 10418 or NCTC 12241

Negative control: *Proteus mirabilis* NCTC 10975

For *Neisseria* species
Positive control: *Neisseria lactamica* NCTC 10617

Negative control: *Neisseria gonorrhoeae* NCTC 8375

References

An explanation of the reference assessment used is available in the scientific information section on the UK SMI website.

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