

NATIONAL STANDARD METHOD

UNDER REVIEW

ONPG (β -GALACTOSIDASE) TEST

BSOP TP 24

Issued by Standards Unit, Evaluations and Standards Laboratory
Specialist and Reference Microbiology Division

Association of Medical Microbiologists
Association of Medical Microbiologists
Association of Medical Microbiologists



ONPG (β -GALACTOSIDASE) TEST

Issue No: 1.1 Issue date: 03.05.05 Issued by: Standards Unit, Evaluations and Standards Laboratory Page 1 of 7

Reference no: BSOP TP 24i1.1

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STATUS OF NATIONAL STANDARD METHODS

National Standard Methods, which include standard operating procedures (SOPs), algorithms and guidance notes, promote high quality practices and help to assure the comparability of diagnostic information obtained in different laboratories. This in turn facilitates standardisation of surveillance underpinned by research, development and audit and promotes public health and patient confidence in their healthcare services. The methods are well referenced and represent a good minimum standard for clinical and public health microbiology. However, in using National Standard Methods, laboratories should take account of local requirements and may need to undertake additional investigations. The methods also provide a reference point for method development.

National Standard Methods are developed, reviewed and updated through an open and wide consultation process where the views of all participants are considered and the resulting documents reflect the majority agreement of contributors.

Representatives of several professional organisations, including those whose logos appear on the front cover, are members of the working groups which develop National Standard Methods. Inclusion of an organisation's logo on the front cover implies support for the objectives and process of preparing standard methods. The representatives participate in the development of the National Standard Methods but their views are not necessarily those of the entire organisation of which they are a member. The current list of participating organisations can be obtained by emailing standards@hpa.org.uk.

The performance of standard methods depends on the quality of reagents, equipment, commercial and in-house test procedures. Laboratories should ensure that these have been validated and shown to be fit for purpose. Internal and external quality assurance procedures should also be in place.

Whereas every care has been taken in the preparation of this publication, the Health Protection Agency or any supporting organisation cannot be responsible for the accuracy of any statement or representation made or the consequences arising from the use of or alteration to any information contained in it. These procedures are intended solely as a general resource for practising professionals in the field, operating in the UK, and specialist advice should be obtained where necessary. If you make any changes to this publication, it must be made clear where changes have been made to the original document. The Health Protection Agency (HPA) should at all times be acknowledged.

The HPA is an independent organisation dedicated to protecting people's health. It brings together the expertise formerly in a number of official organisations. More information about the HPA can be found at www.hpa.org.uk.

The HPA aims to be a fully 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 conditions¹.

More details can be found on the website at www.evaluations-standards.org.uk. Contributions to the development of the documents can be made by contacting standards@hpa.org.uk.

Please note the references are now formatted using Reference Manager software. If you alter or delete text without Reference Manager installed on your computer, the references will not be updated automatically.

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ONPG (β -GALACTOSIDASE) TEST

Issue No: 1.1 Issue date: 03.05.05 Issued by: Standards Unit, Evaluations and Standards Laboratory Page 2 of 7

Reference no: BSOP TP 24i1.1

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INDEX

STATUS OF NATIONAL STANDARD METHODS	2
INDEX.....	3
AMENDMENT PROCEDURE	4
INTRODUCTION	5
TEST PRINCIPLE.....	5
1.0 SAFETY CONSIDERATIONS.....	5
2.0 REAGENTS AND EQUIPMENT	5
2.1 REAGENTS	5
3.0 METHOD/PROCEDURE AND RESULTS	6
4.0 PRECAUTIONS/LIMITATIONS OF PROCEDURE	6
REFERENCES	7

ONPG (β-GALACTOSIDASE) TEST

Issue No: 1.1 Issue date: 03.05.05 Issued by: Standards Unit, Evaluations and Standards Laboratory Page 3 of 7

Reference no: BSOP TP 24i1.1

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AMENDMENT PROCEDURE

Controlled document reference	BSOP TP 24
Controlled document title	Standard Operating Procedure for ONPG (β -galactosidase) test

Each National Standard Method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from standards@hpa.org.uk.

On issue of revised or new pages each controlled document should be updated by the copyholder in the laboratory.

Amendment Number/ Date	Issue no. Discarded	Insert Issue no.	Page	Section(s) involved	Amendment
1/ 03.05.05	1	1.1	1	Front page	Redesigned
			2	Status of document	Reworded
			4	Amendment page	Redesigned

ONPG (β -GALACTOSIDASE) TEST

Issue No: 1.1 Issue date: 03.05.05 Issued by: Standards Unit, Evaluations and Standards Laboratory Page 4 of 7

Reference no: BSOP TP 24i1.1

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STANDARD OPERATING PROCEDURE FOR THE ONPG (β -GALACTOSIDASE) TEST

INTRODUCTION

The test is important in differentiating the Enterobacteriaceae which are commonly classified according to their ability to ferment lactose. It is also used to differentiate *Neisseria lactamica* from other fastidious *Neisseria* species.

TEST PRINCIPLE

The ONPG (o-nitrophenyl- β -D-galactopyranoside) test is used to determine the presence or absence of the enzyme β -galactosidase in an organism². The presence of two enzymes, permease and β -galactosidase are required to demonstrate lactose fermentation. True lactose non-fermenters do not possess either of these enzymes. Late lactose fermenting organisms do not have permease but do possess β -galactosidase, which hydrolyses lactose to form galactose and glucose. ONPG is similar in structure to lactose. If β -galactosidase is present, the colourless ONPG is split into galactose and o-nitrophenol, a yellow compound³.

1.0 SAFETY CONSIDERATIONS⁴⁻⁹

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

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

2.0 REAGENTS AND EQUIPMENT¹⁰

Discrete bacterial colonies growing on solid medium

2.1 REAGENTS

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

Quality Control Organisms

Positive control *Serratia marcescens* NCTC 11935 or *Neisseria lactamica* NCTC 10617

Negative control *Providencia rettgeri* NCTC 7475 or *Neisseria gonorrhoeae* NCTC 8375

ONPG (β -GALACTOSIDASE) TEST

Issue No: 1.1 Issue date: 03.05.05 Issued by: Standards Unit, Evaluations and Standards Laboratory Page 5 of 7

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3.0 METHOD/PROCEDURE AND RESULTS

Include positive and negative controls with every batch of tests

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

Inoculate tubes containing ONPG reagent and incubate at 35-37°C for up to 24h

Examine for yellow colour after 4h and for up to 24h

Positive result yellow colour (indicates lactose fermenter)

Negative result colourless/pale yellow (indicates lactose non-fermenter)

4.0 PRECAUTIONS/LIMITATIONS OF PROCEDURE

The phosphate crystallises out during storage. Warm to 37°C to re-dissolve prior to use

Discard the substrate if it looks yellow prior to inoculation

Organisms grown on glucose containing medium show less reactivity than those grown on lactose containing media. Glucose inhibits β -galactosidase

The test cannot be performed on organisms containing a yellow pigment

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

ONPG (β -GALACTOSIDASE) TEST

Issue No: 1.1 Issue date: 03.05.05 Issued by: Standards Unit, Evaluations and Standards Laboratory Page 6 of 7

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