



# **Guide to determining the equivalence of food microbiology test methods**

## **Part 3: Confirmation tests**



This Australian Standard® was prepared by Committee FT-035, Food Microbiology. It was approved on behalf of the Council of Standards Australia on 9 December 2014. This Standard was published on 3 February 2015.

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This Standard was issued in draft form for comment as DR AS 4659.3:2014.

Standards Australia wishes to acknowledge the participation of the expert individuals that contributed to the development of this Standard through their representation on the Committee and through the public comment period.

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Australian Standard<sup>®</sup>

**Guide to determining the equivalence of  
food microbiology test methods**

**Part 3: Confirmation tests**

First published as AS/NZS 4659.3:1999.

Second edition redesignated and published as AS 4659.3:2015.

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Published by SAI Global Limited under licence from Standards Australia Limited, GPO Box 476, Sydney, NSW 2001, Australia

ISBN 978 1 74342 962 4

## PREFACE

This Standard was prepared by the Standards Australia Committee FT-035, Food Microbiology, to supersede AS/NZS 4659.3:1999, incorporating Amendment No. 1 (February 2002).

After consulting with stakeholders in both countries, Standards Australia and Standards New Zealand decided to develop this Standard as an Australian Standard rather than an Australian/New Zealand Standard.

The objectives of this revision are—

- (a) to provide guidance on determining the equivalence of microbiological test methods;
- (b) to explain how to determine whether an alternate method for confirmation tests using a pure culture, will yield a result equivalent to an Australian Standard method;
- (c) to update the references; and
- (d) to incorporate minor technical variations to emphasize the scope and limitations of the Standard.

This Standard is one of a series of guides covering determination of the equivalence of food microbiology test methods. The series now comprises the following:

AS	
4659	Guide to determining the equivalence of food microbiology test methods
4659.1	Part 1: Qualitative tests
4659.2	Part 2: Quantitative tests
4659.3	Part 3: Confirmation tests

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## FOREWORD

An alternate method will be considered to be equivalent if it is shown to yield results that are not different to those obtained using the Australian Standard method.

Reference cultures specified in the relevant part of AS 5013, need to be included when performing an alternate method as specified in the relevant part of AS 5013, even after equivalence of the alternate method has been determined according to this Standard.

A minimum of 20 isolates of the target organism and 30 isolates of negative control organisms are to be tested by the alternate method and the Australian Standard method. The number of positive results is recorded and analysis performed. An equivalent number of negative control organisms are also tested. If the particular test requires a purification and/or isolation step, then it is essential that this be included in the study design.

It is not intended that this Standard be applied retrospectively to existing laboratory validation studies, nor that it replace the validation of methods performed under the auspices of organizations such as, but not limited to the Association of Official Analytical Chemists International (AOAC-International) and Association Française de Normalisation (AFNOR); or those methods validated according to ISO 16140:2003, *Microbiology of food and animal feeding stuffs—Protocol for the validation of alternative methods*.

This Standard for the determination of equivalence is intended for individual laboratories wishing to demonstrate performance of procedures that are alternatives to Australian Standard methods. It allows an equivalence determination to be performed in a single laboratory. The result of following the procedures in this Standard is the production of a report which will in some specific situations allow a laboratory and its clients to determine or agree on whether an alternative method is suitable as a substitute for a Standard method.

STANDARDS AUSTRALIA

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**Australian Standard**

**Guide to determining the equivalence of food microbiology test methods**

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Part 3: Confirmation tests

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## 1 SCOPE

This Standard sets out a protocol that may be used to determine whether an alternate method will yield a result equivalent to an Australian Standard, food microbiology method (AS 5013 series), for confirmation tests using a pure culture.

As this Standard deals with pure cultures, the matrix will have no bearing on the validation of a particular test kit.

## 2 REFERENCED DOCUMENTS

The following documents are referred to in this Standard:

AS  
5013      Food microbiology (series)

## 3 DEFINITIONS

For the purpose of this Standard the definitions below apply:

### 3.1 Alternate method

The method for which equivalence is to be determined.

### 3.2 Equivalent

A determination according to this Standard, that two methods (the reference and alternate methods) give results that are not statistically different when confirming cultures.

### 3.3 False negative

When the alternate method yields a negative result the reference method yields a positive result.

### 3.4 False positive

When the alternate method yields a positive result but the reference method yields a negative result.

NOTE: A 'false positive' may be a true positive which was not detected by the reference method. Such occurrences need to be carefully verified. For the purpose of this Standard they will be considered false positives because the guide is aimed at demonstrating the equivalence of the two methods.

### 3.5 Reference method

The Australian Standard method against which the alternate method will be compared.

### 3.6 Target organism

The genus, species, antigenically, toxicologically or physiologically defined group of organisms which the reference method is designed to detect.

## 4 PROCEDURE

### 4.1 Define the equivalence determination

The equivalence determination should be defined in terms of the following:

- (a) The target organism's genus, species, serotype, etc.
- (b) The alternate method—precisely defined by reference to a publication, manufacturer's instructions and any optional procedures employed or deviation from the published method.
- (c) The reference method—including the specification of any optional steps.
- (d) The steps of the methods that are to be compared.

### 4.2 Define the conditions of the equivalence determination

The conditions of the equivalence determination should be defined in terms of the following:

- (a) The laboratory where testing is performed.
- (b) Controls observed by the laboratory during testing, for example, controls on the environment of the laboratory, prevention of cross contamination, controls on media and reagents, calibration of equipment, etc. where these factors are considered critical to the success of either method.
- (c) The staff performing the tests (experience, qualifications, etc.).
- (d) The starting and finishing dates of the tests.
- (e) The batch numbers of media, reagents, etc. used.

NOTE: This information is defined for the purpose of reporting on the equivalence determination and recording factors which may have some bearing on the results obtained. These factors do not necessarily affect the veracity of the study.

### 4.3 Select test organisms

Fifty strains should be selected. These are to include diversity in the strain selected. A minimum of 20 positive isolates and 20 closely related negative isolates should be selected. In addition, a minimum of 10 non-related strains should be tested.

The reference cultures prescribed in the Australia Standard method should be included. The other strains may be selected from the following list (in order of preference):

- (a) Strains of the target organism isolated by the laboratory.
- (b) Strains of the target organism isolated by reference laboratories or industry sources as representative.
- (c) Strains of the target organism held by culture collections.

The identity of the strains chosen should be determined by appropriate means (e.g. biochemical, physiological, serological, molecular) before performing further work. Consultation with a reference laboratory or an expert may be necessary. The source and identity of the strains should be recorded.

NOTE: It is possible, by the selection of test strains, to bias the results of the study. Care should be taken to select representative strains which do not possess characteristics which are likely to lead to biased results being obtained.

### 4.4 Preparation of inoculum

The test strain inoculum should be such that at least equivalence in sensitivity to the reference method may be demonstrated. The strains chosen should be prepared as prescribed by the method being studied.

## 4.5 Testing

The reference method should be performed in parallel with the testing of the alternate method by the same operator or group of operators who are proficient in both methods under study.

All results should be taken to the final stages of confirmation.

## 4.6 Results

### 4.6.1 Reporting of the results

The results should be collated in the following table:

<b>INOCULATED TESTS</b>		
<b>Reference method</b>	<b>Alternate method</b>	
	<b>Positive</b>	<b>Negative</b>
Positive	<i>A</i>	<i>B</i>
Negative	<i>C</i>	<i>D</i>

### 4.6.2 Calculation

The following statistics should be calculated:

$$\text{False positive} = \frac{C}{C+D} \quad \text{False negative} = \frac{B}{A+B}$$

$$\text{Sensitivity} = \frac{A}{A+B} \times 100$$

$$\text{Specificity} = \frac{D}{C+D} \times 100$$

## 4.7 Acceptance criteria

The alternate method will only be accepted if  $B + C = 0$ .

NOTE: If  $B$  or  $C$  is greater than zero then equivalence of the alternate method has not been adequately demonstrated. An explanation should be sought for the discrepancy. It may lie in the choice of test organism, the performance of a critical step in the alternate method or some other factor. Once the reason is determined, the procedure may be repeated, after redefining the test organisms where necessary, to fulfil the requirement that  $B = 0$ .

## 5 REPORT

The report should contain the following information:

- (a) All details necessary for identification of sample types.
- (b) Reference to this Standard (i.e. AS 4659.3) and other appropriate Australian Standards.
- (c) Reference to the alternative method.
- (d) Each step of the method above reported in full.
- (e) The results obtained.
- (f) Date of testing.
- (g) Any circumstances that may have influenced the result.

NOTES

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ISBN 978 1 74342 962 4

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