AS 4659.2:2015

Australian STANDARD

Guide to determining the equivalence of food microbiology test methods

Part 2: Quantitative tests

STANDARDS Australia

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- Australian Food and Grocery Council
- Australian Institute of Food Science and Technology
- Australian Society for Microbiology
- CSIRO
- Dairy Industry Association of Australia
- Department of Agriculture (Australian Government)
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- Food Technology Association of Australia
- Meat and Livestock Australia
- National Association of Testing Authorities Australia
- National Measurement Institute
- Queensland Health Forensic and Scientific Services
- University of Melbourne

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Australian Standard®

Guide to determining the equivalence of food microbiology test methods

Part 2: Quantitative tests

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PREFACE

This Standard was prepared by the Standards Australia Committee FT-035, Food Microbiology, to supersede AS/NZS 4659.2:1999.

After consultation 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 quantitative tests will yield a result equivalent to an Australian Standard method;

(c) to update 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

CONTENTS

Page

FOREWORD

An alternate method will be considered to be equivalent if it is shown to yield results that are not statistically different to those obtained using the Australian Standard method. A method can only be considered to be validated for the matrix in which the tests were performed.

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.

Quantitative methods—for each of the media, reagents or equipment specified in the alternate method five different samples per matrix type will be tested by the reference method and the alternate method. The results are recorded and statistical analysis performed.

NOTE: The purpose of using five samples is an attempt to encompass the variation that may be found within the matrix.

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 an Australian Standard method.

STANDARDS AUSTRALIA

Australian Standard

Guide to determining the equivalence of food microbiology test methods

Part 2: Quantitative tests

1 SCOPE

This Standard sets out a protocol that may be used to determine whether an alternate enumeration method will yield an equivalent result to an Australian Standard food microbiology method (AS 5013 series).

NOTES:

1 An example of such methods is the standard plate count or enumeration of coagulase positive staphylococci.

2 This Standard is not intended to replace the requirement for full validation for methods that have not previously been validated, for example, under the auspices of AOAC International, AFNOR, etc, or for the determination of equivalence of a standard method used with a matrix outside the scope of the methods initial validation.

2 REFERENCED DOCUMENTS

The following documents are referred to in this Standard:

AS	
5013	Food microbiology (series)
5013.12.1	Method 12.1: Microbiology of food and animal feeding stuffs—Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species)—Technique using Baird-Parker agar medium

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 testing defined types of food samples.

3.3 Matrix

The food sample in which the target organism is to be detected.

3.4 Presumptive

The result given by the alternate method at the point where a decision may be made as to whether or not the matrix may contain the target organism. The method continues to confirm whether the presumptive result is in fact a positive result.

3.5 Reference culture

The culture designated in the appropriate Australian Standard method.

3.6 Reference method

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

3.7 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 matrix under examination; the food and the characteristics that define it—the matrix should be defined in terms of characteristics such as pH, solids level, season of production, brand name, water activity, presence and composition of preservatives etc. depending upon the nature of the matrix.

NOTE: When determining equivalence of an alternate method the laboratory should select the matrices to be included in the evaluation from those within the scope of the standard method. This Standard is not intended for the validation of matrices outside of the scope of either the alternate method or the standard method. Reference should be made to standard texts and the literature to support the choices of matrices. The choice should take into account the range of samples tested by the laboratory and the effects that the natural variation in significant parameters may have on the detection of the target organism.

(c) The alternate method—precisely defined by reference to a publication, manufacturer's instructions and any optional procedures employed or deviation from the published method.

(d) The reference method—including the specification of any optional steps.

For example, the validation of the use of XYZ agar as an alternative to Baird Parker agar in the AS 5013.12.1 method for the enumeration of coagulase positive staphylococci, from custards containing spices and flavours and a pH greater than 4.5.

(e) Steps of methods which are to be compared, for example, the alternate method may completely replace the reference method or only a section of the reference method.

(f) The range of colony forming units for which the alternate method is deemed equivalent.

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 or its applicability in other laboratories.

4.3 Select test organisms

4.3.1 *For non-selective methods*

All samples should be naturally contaminated samples.

4.3.2 *For selective quantitative methods*

A minimum of five strains of the target organism should be selected. The reference culture prescribed as a positive control should be one of the five strains. The other strains may be selected from the following list (in order of preference):

(a) Strains of the target organism isolated by the laboratory from previous samples of the matrix under examination.

(b) Strains of the target organism isolated by reference laboratories or industry sources as representative of the strains found in the type of matrix under examination.

(c) Strains of the target organism traceable to culture collections.

NOTE: Commercial preparations of known concentrations may be used.

The strains selected should encompass the variation that may be expected to be found within the target organism in the matrix under examination.

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 a biased result being obtained.

4.4 Select samples of the matrix

Five typical samples of the matrix should be selected. They should represent as far as possible the range of variation found within the defined matrix. The choice of samples will depend upon the matrix, but may consist of batches with high or low background contamination, samples with different pH values, fat, protein or moisture levels. The aim of the selection is to choose samples which will represent the range of variation expected to be found in the defined matrix. Care should be taken to select samples with characteristics which are expected to yield homogeneous results in the test being validated. Naturally contaminated samples, if available, should be used instead of artificially contaminated samples. Care should be taken to determine the identity, level and homogeneity of contamination.

The significant characteristics of the product should be measured and the results recorded.

4.5 Preparation (selective quantitative method)

For artificially inoculated samples, the strains chosen should be prepared for use by culturing the strains of the test organism in a non-selective medium (e.g. tryptone soya agar, nutrient agar) to early stationary phase (e.g. 18 hour culture) at the optimum growth temperature. A cocktail of strains may be prepared by combining approximately equal numbers of each strain grown as a pure culture. The cocktail of strains should be added to each matrix sample at two levels:

(a) Approximately 10 times the lower limit of detection of the reference method.

(b) Approximately within one $\log_{10}$ of the upper limit of detection of the alternate method or the highest expected level of the target organism.

The number of target organisms added to a sample is determined using a non-selective medium (e.g. tryptone soya agar), incubated at the optimum temperature for the target organism and for an appropriate time to reach the start of the stationary phase.

NOTE: For example:

(a) If the reference method is capable of detecting 100 target organisms per gram of the matrix then ~ 1000 cells of the selected test organism should be added per gram of the matrix.

(b) If the highest expected level of organisms in the product is 1×10^6 cfu/g, then the second inoculation level should be ~ 1×10^5 to ~ 1×10^7 cfu/g.

(c) The number of colony forming units of the test organism may be verified by a nonselective method such as a most probable number or by plate count of a sufficient volume of the suspension to obtain an accurate count.

Serial dilutions of the cultures of the test organism should be made to enable enumeration of the test organism and inoculation of the broth to which the matrix sample is added.

NOTE: Commercially available preparations of known concentrations of the test organism may be used.

The method of preparing the strains for inoculation into the matrix, the method of inoculating the matrix, the method used to determine the level added, and the level added should be recorded.

4.6 Testing

4.6.1 *General*

The alternate method should be performed according to the published method or the manufacturer's instructions. If not specified in the instructions for the alternate method, prepare samples and dilutions in accordance with the instructions given for that product in the appropriate Australian Standard. 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.

Wherever possible, the same sample should be used for the reference and alternate methods.

Calibration of the test equipment should be performed according to the manufacturer's instructions.

4.6.2 *Non-selective quantitative methods*

A total of 10 replicates of each matrix sample should be tested using both the alternate method and reference method. See Table 1.

TABLE 1

NON-SELECTIVE TESTING

Matrix samples (see Clause 4.4)	Replicates																			
	1		2		3		4		5		6		7		8		9		10	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
1																				
2																				
3																				
4																				
5																				

LEGEND
A = Reference method
B = Alternate method

4.6.3 *Selective quantitative methods*

Ten replicates should be inoculated with a cocktail of five strains of the test organism; five replicates at the low level and five at the higher level as described in Clause 4.5. See Table 2.

NOTE: It is preferable to perform tests with only one matrix sample on a single day so that potentially significant variables, outside the control of the validating laboratory, can be allowed to act, more closely simulating the conditions found during routine use of the methods.

All tests should be performed in their entirety, recording both presumptive (if applicable) and final results.

TABLE 2

SELECTIVE TESTING

Matrix samples (see Clause 4.4)	Replicates																			
	Low										High									
	1		2		3		4		5		6		7		8		9		10	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
1																				
2																				
3																				
4																				
5																				

LEGEND
A = Reference method
B = Alternate method

5 ANALYSIS OF RESULTS

A t-test should be performed as follows:

The $\log_{10}$ of all counts should be taken before performing the following calculations. Let x_i be $\log_{10}$ of the i_{th} reading, out of n, using the standard method and y_i the corresponding i_{th} reading using the alternate method.

Let $d_i = x_i - y_i$ (the difference between the $\log_{10}$ counts using each method)

where

$\overline{d}$ = The mean of all d_i

$t = \overline{d} / se\left(\overline{d}\right)$

where

$$\overline{d} = \sum_{i=1}^{n} d_i / n$$

and

$$se\left(\overline{d}\right) = \left[\sum_{i=1}^{n} \left(d_i - \overline{d}\right)^2 / \left(n\left(n-1\right)\right)\right]^{\frac{1}{2}}$$

t is significant if its value is less than −1.982 or greater than 1.982 (for 100 samples). The t-test tables should be checked for appropriate values for t for the number of samples tested.

A significant value for t suggests that there was a significant difference between the Standard and alternate methods i.e. the alternate method is rejected.

Also, d_i (vertical axis) vs. $[x_i + y_i]$ should be plotted. There should be a uniform scatter about a horizontal line with zero intercept, and deviations from this uniform scatter should be easy to spot. The plot should be examined for trends of more or less scattering as x_i increases. Approximate confidence limit lines (also horizontal) can be added to aid interpretation.

NOTE: There are a number of other statistical techniques (e.g. non-parametric techniques) that may be helpful in describing the relationship between the results produced by the two methods. Computer-based statistical methods may be used.

6 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 4659.2) and other appropriate Australian Standards.

(c) Reference to the alternate method.

(d) The range of counts, food matrix and season of production (where applicable) for which the validation tests have been performed.

(e) The results obtained.

(f) Date of testing.

(g) Any circumstances that may have influenced the result.

NOTES

NOTES

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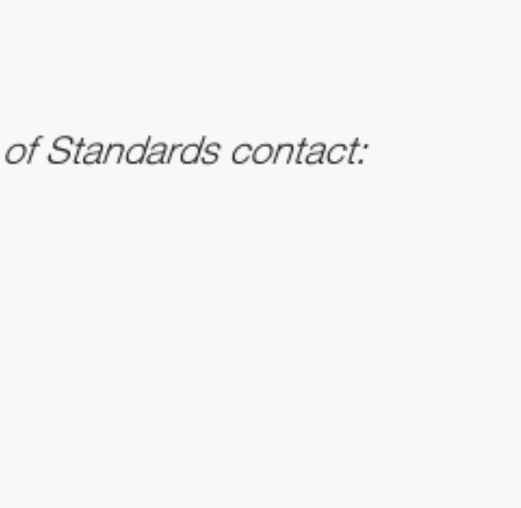

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