Public Health England (PHE) Certified Reference Materials for Microbiology

Certificate of Analysis

This certificate should not be parted from the PHE certified reference material with batch number: *1*315-13

Uncertified data and **guidance for use** can be found at the end of this certificate.

4003

1. Public Health England Reference Material (RM) Manufacturer

This certificate is designed and the reference material values and uncertainties are determined in accordance with:

- *ISO Guide 31:2000 'Reference materials – contents of certificates and labels';*
- *ISO Guide 34:2009 'General requirements for the competence of reference materials producers' and*
- *ISO Guide 35:2006 'Reference materials – general and statistical principles for certification'.*

2. Description of Reference Material

Bacteria, yeasts or moulds in a pure culture preserved in a tablet format (LENTICULE® disc) with a self-indicating silica gel desiccant.

The micro-organisms provided in LENTICULE disc format are classified as Hazard Group 2 according to the UK Advisory Committee on Dangerous Pathogens using the *'Approved List of Biological Agents'* http://www.hse.gov.uk/pubns/misc208.pdf. A Group 2 organism may cause human disease and may be a hazard to laboratory workers, but is unlikely to spread to the community. These products are dispatched under the Dangerous Goods Classification: UN 3373 Biological substances, Category B (commodity code 300190 99 00.)

Refer to the Safety Data Sheet for further information.

The silica gel self-indicating inserts are not classified as dangerous material.

Catalogue Number:	**CRM09001L**
Batch number:	***1*315-13**
Starting Material:	**NCTC 9001 – *Escherichia coli* (Freeze-dried micro-organism in a glass ampoule)**
Starting Material Batch:	**NCTC 9001 Batch 22**

Produced by: Food and Environmental Proficiency testing Unit, Public Health England, 61 Colindale Avenue, Colindale, London, NW9 5EQ. Tel: +44 (0) 20 8327 7719, Fax: 44 (0) 20 8200 8264, Email: foodeqa@phe.gov.uk

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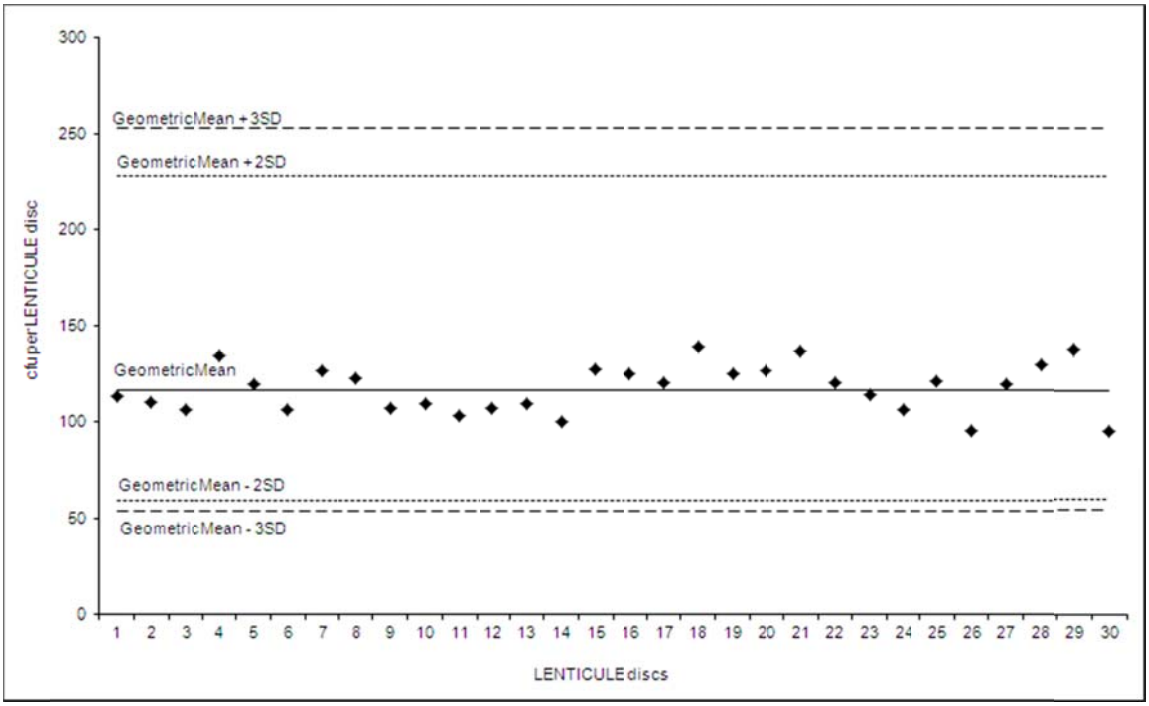
Tel: +44 (0) 1980 612512, Email: culturecollections@phe.gov.uk, Website: www.phe-culturecollections.org.uk

3. Certified Values and Uncertainties

Organism name:	***Escherichia coli***
NCTC number:	**9001**
Conditions:	**Columbia blood agar/aerobic/30°C/48h**
Geometric Mean Value:	**$1.2x10^2$ colony forming units (cfu)**
Geometric Mean +3SD	**$2.5x10^2$ colony forming units (cfu)**
Geometric Mean +2SD	**$2.3x10^2$ colony forming units (cfu)**
Geometric Mean -2SD	**59 colony forming units (cfu)**
Geometric Mean -3SD	**53 colony forming units (cfu)**
Expanded uncertainty (log_{10} value):	**0.017 (95% CI; coverage factor = 2)**
Expected range:	**59 - $2.3x10^2$ cfu per LENTICULE disc**

4. FEPTU Quality Control (QC) Data for Geometric Mean Value

QC Batch Size:	**45 LENTICULE discs**
Date of Testing:	**4 December 2013**
Conditions:	**Columbia blood agar/aerobic/30°C/48h**



The counts were undertaken by surface inoculation and incubation as described above. This is in accordance with the technique described in the Food and Environmental Proficiency Testing Unit (FEPTU) Method Manual for Food Microbiology Section 12: Method for the Enumeration of Micro-organisms – Colony Count Technique at 22ºC and 30ºC (aerobic colony counts) which is based on ISO 4833: Part 2. Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of microorganisms – colony count at 30ºC by the surface plating technique.

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5. Traceability to NCTC culture by Independent Means

This strain is traceable to seed stock for *Escherichia coli* NCTC 9001, **batch NCTC 22** and is **manufactured from one sub-culture** ensuring that the characteristics are retained. This strain has been verified by the PHE's Applied and Functional Genomics (AFG) Unit at Microbiology Services (Colindale) using fluorescent amplified fragment length polymorphism (FAFLP) analysis, and is traceable back to the NCTC seed stock (refer to page 1 for Starting Material Batch details). The AFG Unit is accredited by Clinical Pathology Accreditation (UK) Ltd (CPA) and is audited by FEPTU to *ISO/IEC 17025:2005 - General requirements for the competence of testing and calibration laboratories.*

***Reference**: Desai, M; Russell J.E; and Gharbia S. (2006) Comparison of the genetic stability of paired reference strains preserved by freeze-drying and on LENTICULE discs with fluorescent amplified fragment length polymorphism.* FEMS Microbiology Letters *257:1 158.*

6. Instructions for Correct Storage of this Reference Material

Storage:
On receipt store the plastic vial containing the LENTICULE discs unopened at **–20 ± 5ºC.**

7. General Instructions for Intended Uses of this Reference Material

Remove the plastic vials from the freezer and allow the LENTICULE disc to reach ambient temperature (5 - 10 minutes) before use.

Once removed from the freezer, do not refreeze the LENTICULE disc. Use within one hour of transfer to ambient temperature.

Intended Uses:
i) Quality control of culture media
ii) Microbiology process control for internal quality control
iii) Spiking samples for internal quality control

***Refer also to the uncertified data and guidance for use at the end of this certificate*.**

i) Quality Control of Culture Media
If the LENTICULE discs are to be used for media other than those stipulated for the geometric mean value(s) you are advised to calibrate the LENTICULE discs for the conditions in your own laboratory. You are advised to use a minimum of 20 LENTICULE discs for calibration. If you need a spreadsheet to help with your calibrations please e-mail your request to: culturecollections.technical@phe.gov.uk.

Direct Inoculation – Solid Media
Invert the plastic container over the centre of the agar plate until the LENTICULE disc drops directly onto the agar. Allow the LENTICULE disc to rehydrate for approximately

10 minutes (until a drop of liquid forms) then spread the resultant drop over the entire plate. Incubate under routine conditions for the organism and count the number of colonies obtained.

Inoculation Through Liquid Media
Invert the plastic container until the LENTICULE disc drops into the liquid medium (e.g. 1mL, 10mL, and 100mL). Diluents such as nutrient broth, buffered peptone water, peptone saline (MRD), Page's saline or 1/40 Ringers solution are recommended. Allow the liquid culture to stand for approximately for 10 minutes to rehydrate the LENTICULE disc, and then mix thoroughly. Inoculate the agar plates using the routine enumeration methods. Incubate under routine conditions and count the number of colonies obtained.

Quality Control of Liquid Media Invert the plastic container until the LENTICULE disc drops into an appropriate amount of the broth medium (e.g. 10mL, 225mL) allow the liquid culture to stand for approximately 10 minutes to rehydrate, and then mix thoroughly. Incubate and sub-culture in accordance with your routine procedures.

ii) Process Controls

Invert the plastic container until the LENTICULE disc drops into an appropriate amount of the primary broth (e.g. buffered peptone water) for 10 minutes to rehydrate, and then mix thoroughly. Continue in accordance with your routine procedures, preparing dilutions as appropriate. Incubate and sub-culture in accordance with your routine procedures.

iii) Spiked Samples Confirm that the sample to be spiked does not contain the RM organism. Weigh/measure an appropriate sample size and prepare the sample in the diluent used routinely for the relevant sample type (e.g. buffered peptone water).

Invert the plastic container until the LENTICULE disc drops into the sample, e.g. homogenised food/broth medium (e.g. 10mL, 225mL) or water sample for 10 minutes to rehydrate, and then mix thoroughly. Process in accordance with your routine procedures for the target organism.

8. Hazardous Information

Refer to the Safety Data Sheet for PHE Reference Materials in LENTICULE disc format.

9. Homogeneity

This reference material was prepared in accordance with FEPTU Standard Operating Procedures that have been shown to produce a homogeneous product. A sample size of 45 LENTICULE discs was used to confirm batch homogeneity.

10. Quality Standard Documentation

Accredited by the United Kingdom Accreditation Service (UKAS) to *ISO Guide 34:2009 'General requirements for the competence of reference material producers'* through assessment against this Guide and the relevant requirements of *ISO/IEC 17025:2005*.

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11. Expiration and Shelf-life

Date of Certification: **9 January 2014**

Expiry Date: **4 December 2014**

Certificate Number: FEPTU710/06/01/14/*1*315-13

Certificate Prepared by: Heena Shah

Certificate Approved on behalf of PHE by:

Nita Patel (Head of FEPTU) **Date** 9 January 2014

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Uncertified Data and Guidance for Use

For general information about PHE certified reference materials (CRMs) go to: www/phe-culturecollections.org.uk

These CRMs are designed for use as controls in food and water microbiology testing laboratories and most of the products have multiple uses.

The CRMs may be inoculated into real or simulated food and/or water matrices to provide process controls for traditional and molecular methods. CRMs with lower levels are particularly useful for determining the sensitivity of a detection method or the filtration methods used for water testing.

Some of the lower level CRMs are useful for quality control of culture media. However, the certified values provided for any CRM batch are specific for those conditions indicated in Section 4 of the certificate. Results may be different under conditions other than those indicated in Section 4. Table 1 on the following page provides guidance for consideration when using PHE CRMs.

The data in Table 1 is not certified data and may require validation in the testing laboratories that use these materials. It is provided for guidance when selecting the CRM that will be most likely to fit the purpose for which it is required. The data in Table 1 was derived from studies undertaken in the PHE Food and Environmental Proficiency Testing Unit (FEPTU).

For further information regarding uncertified data and guidance for use contact: culturecollections.technical@phe.gov.uk

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Table 1: Guidance for use of PHE certified reference materials under conditions other those used to determine the certified values

Organism name	NCTC no.	Culture medium	Incubation conditions (temp /time)	Potential difference from certified data
Bacillus cereus	7464	PCA	30°C/48 h	30-35% reduction compared with CBA
Bacillus cereus	7464	PEMBA	30°C/48 h	30-35% reduction compared with CBA
Bacillus cereus	7464	MYP agar	30°C/48 h	35-40% reduction compared with CBA
Cronobacter sakazakii	11467	PCA	30°C/48 h	20% reduction compared with CBA
Escherichia coli	9001	PCA	30°C/48 h	15-40% reduction compared with CBA
Escherichia coli	9001	Brilliance *E.coli*	37°C/24 h	20-45% reduction compared with CBA
Escherichia coli	9001	TTC agar	37°C/24 h	20-45% reduction compared with CBA
Escherichia coli	9001	TBX agar 37°C/24 h	37°C/24 h	55-80% reduction compared with CBA
Escherichia coli	9001	VRBG agar	37°C/24 h	55-90% reduction compared with CBA
Escherichia coli	9001	MacConkey agar	37°C/24 h	70-95% reduction compared with CBA
Escherichia coli O157	12900	PCA	30°C/48 h	65% reduction compared with CBA
Enterococcus faecalis	775	PCA	30°C/48 h	2-12% reduction compared with CBA
Klebsiella aerogenes	9528	PCA	30°C/48 h	0-33% reduction compared with CBA
Listeria monocytogenes	11994	PCA	30°C/48 h	None compared with CBA
Listeria monocytogenes	11994	Chromogenic Listeria agar	37°C/48 h	None compared with CBA
Listeria monocytogenes	11994	Brilliance Listeria agar	37°C/48 h	None compared with CBA
Listeria monocytogenes	11994	Oxford agar	37°C/48 h	None compared with CBA
Listeria monocytogenes	11994	PALCAM agar	37°C/48 h	None compared with CBA
Pseudomonas aeruginosa	10662	PCA	30°C/48 h	0-42% reduction compared with CBA
Salmonella Nottingham	7832	PCA	30°C/48 h	None compared with CBA
Salmonella Nottingham	7832	Brilliant green agar	37°C/24 h	15% reduction compared with CBA
Salmonella Nottingham	7832	XLD agar	37°C/24 h	25% reduction compared with CBA
Salmonella Nottingham	7832	XLT4 agar	37°C/24 h	25% reduction compared with CBA
Salmonella Nottingham	7832	Brilliance Salmonella	37°C/24 h	25% reduction compared with CBA
Salmonella Typhimurium	12023	PCA	30°C/48 h	17% reduction compared with CBA
Salmonella Typhimurium	12023	Brilliant green agar	37°C/24 h	32% reduction compared with CBA
Salmonella Typhimurium	12023	XLD agar	37°C/24 h	42% reduction compared with CBA
Salmonella Typhimurium	12023	XLT4 agar	37°C/24 h	52% reduction compared with CBA
Salmonella Typhimurium	12023	Brilliance Salmonella	37°C/24 h	37% reduction compared with CBA
Staphylococcus aureus	6571	PCA	30°C/48 h	7-26% reduction compared with CBA
Staphylococcus aureus	6571	Baird Parker agar	30°C/48 h	17-36% reduction compared with CBA
Staphylococcus aureus	6571	Chapman agar	30°C/48 h	17-36% reduction compared with CBA