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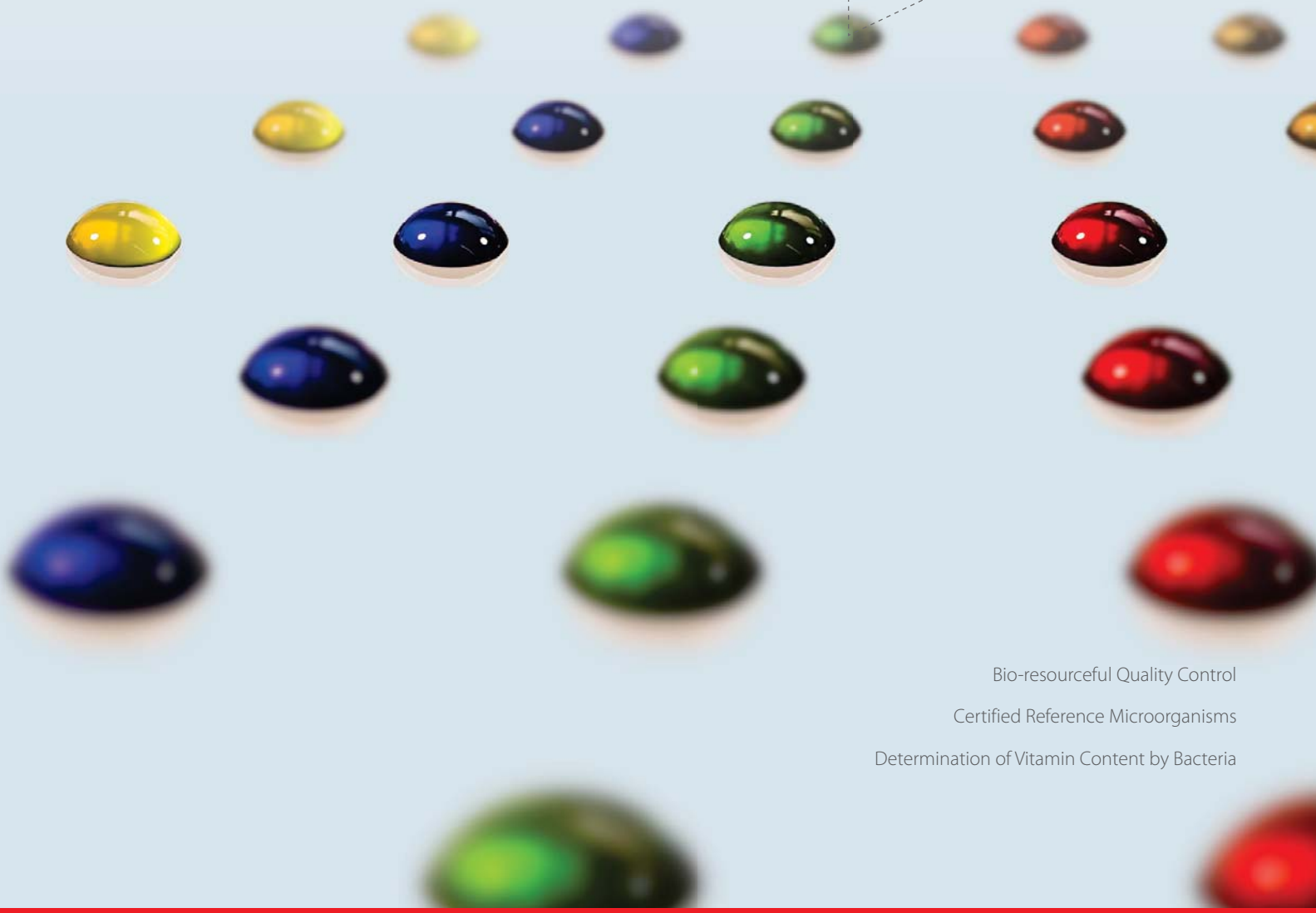
Microbiology Focus

Certified Reference Microorganisms



Bio-resourceful Quality Control

Certified Reference Microorganisms

Determination of Vitamin Content by Bacteria

SIGMA-ALDRICH®

Bio-resourceful Quality Control

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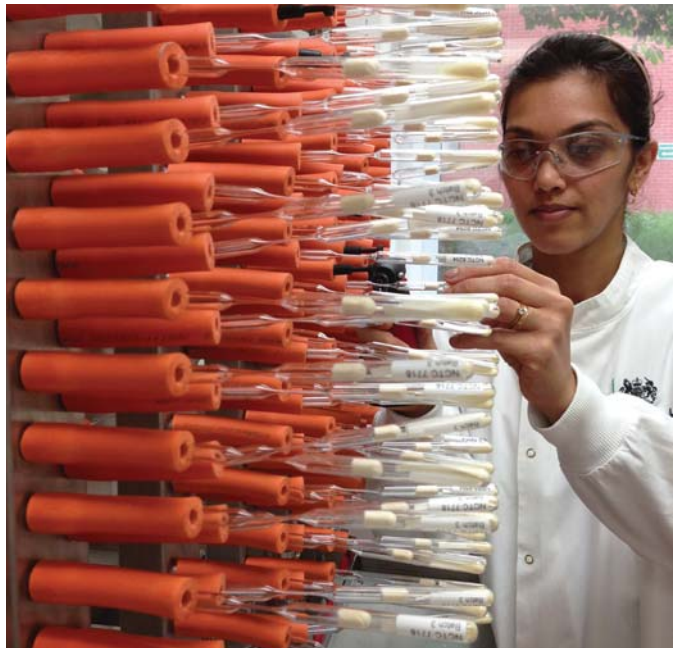
Authentic controls in microbiology – optimized for quality

It's easy to source microbial strains from colleagues for use as controls for microbiology testing. Invariably the cultures will be provided free-of-charge and can then be preserved within the testing laboratory for long periods of time. While this may appear to be a cost-saving means of procuring control strains, there are numerous documented accounts of laboratories maintaining incorrect or contaminated control strains, sometimes over many years[1]. Effective robust maintenance of control strains depletes laboratory resources because the source, authenticity, means of storage, purity and the number of subcultures (passages) that the strains have been subjected to need to be carefully controlled and recorded. Repeated subculture of microorganisms can introduce mutations, meaning that stored cultures may gradually diverge genetically from the original parent strain.

National Biological Resource Centres (BRCs) with global distribution networks have been established in many countries to provide authenticated biological reference and control strains. The very first BRC was the UK's National Collection of Type Cultures (NCTC) established in 1920 with a remit "to provide a trustworthy source of authentic bacteria for use in scientific studies", and that remit remains essentially unchanged today. Now one of four collections of microorganisms and cell cultures sustained and developed by Public Health England, NCTC was initially a general microbiology collection, similar to the US's American Type Culture Collection (ATCC), established a few years after NCTC in 1925. However, in 1947 the focus of NCTC changed in order to allow the curators to focus on bacteria of medical and veterinary interest, so the industrial and environmental strains were sent to other institutes with specialists in those particular fields. Long-established BRCs such as NCTC provide important links between the past and the present. NCTC reflects the history of clinical bacterial infections from the late 19th century to those that challenge us today.

Most BRCs are dynamic with new strains added to the collections on a very regular basis, and one of the unique characteristics of BRCs relates to their ability to preserve and maintain large numbers of authentic strains over many decades, ensuring there are no changes to physical characteristics, such as morphology and nutritional requirements, the genome and the proteome. Emerging technologies such as whole genome sequencing (WGS) and mass spectrometry, particularly matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) present challenges to BRCs. NCTC is responding by undertaking an exciting project in collaboration with the Wellcome Trust Sanger Institute (WTSI) to provide whole genome sequences, using long-read technology, for 3,000 bacteria of clinical importance.[2] Those genomes will provide valuable reference data for scientists studying microbial evolution, for supporting reference and specialist testing procedures, epidemiology projects that are increasingly using genomic technologies, and also for improving our understanding of the emergence of virulence factors and antimicrobial resistance.

Figure 1: Preparation of lyophilized culture tubes at Public Health England



As an example, the genome of NCTC 1, the first strain to be deposited in NCTC, was assembled by WTSI scientists. This strain, *Shigella flexneri*, was isolated in 1915 from a British soldier who became ill in the trenches in World War 1. *S.flexneri* infection causes bacterial dysentery, a life-threatening disease that continues to kill hundreds of thousands of children under five years old every year in developing nations and is becoming increasingly hard to treat. The genome of NCTC 1 indicates resistance to penicillin and erythromycin even though the organism was isolated before the discovery and widespread application of antibiotics to treat infectious diseases.[3]

The cultures provided by BRCs are reference cultures, many of which are control strains stipulated for use in international standards for testing food, water, environmental and clinical samples and pharmaceutical products. It is usually possible to source control strains from more than one BRC and the online World Data Centre for Microorganisms (WDCM)[4], set up by the World Federation for Culture Collections (WFCC), serves as a useful information source for laboratories who want to identify the different BRCs that can provide a particular control strain. It should be noted that microorganisms from different BRCs have different accession numbers (the identification number allocated when a strain is accepted for inclusion in a particular BRC) even though they are all exactly the same strain. For example, NCTC 9001, a strain of *Escherichia coli* used frequently as a control strain, can also be sourced from several other collections in Europe, Asia and the US. NCTC 9001 is equivalent to ATCC 11775, CCM 5172, CIP 54.8, DSM 30083, JCM 1649 and WDCM 00090.

Testing laboratories strive to provide results that are accurate and meaningful to the third parties who submit their samples for testing. Invariably laboratory managers will invest time and pay close attention to detail when procuring instruments, kits and reagents and making decisions about the quality standards that they need to apply to the operation of their tests and the results they report. However, the one factor that is repeatedly overlooked is careful sourcing of biological resources such as the quality control strains. Perhaps this is because there is insufficient awareness that the implications of this oversight can be far-reaching, expensive and cause reputational damage.

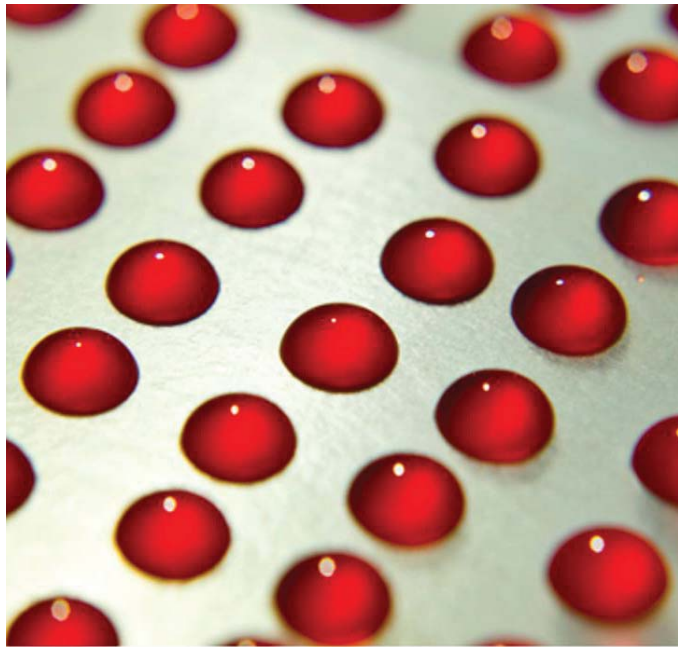
In food, water and environmental microbiology, laboratory results are an important part of a wider process that helps to confirm that samples are of an acceptable microbiological quality, are safe and comply with relevant legislation or guidelines. Quality control is an essential element of a laboratory's quality assurance system and characterized authenticated reference materials are necessary for effective quality control. Incorrect quality control materials may indicate that test results are acceptable when, in fact, there is a problem with the samples being tested. Alternatively, control results may indicate that a test is not performing correctly, instigating unnecessary investigations and repeat testing.

Ready-to-use microbiological controls minimize the need for maintaining control strains in the test laboratory and guarantee that an authenticated control culture is used for every quality control test. Such control materials must be fit-for-purpose, bearing in mind that for food, water and environmental samples the ability to accurately enumerate bacteria, yeasts and molds and reliably detect relatively low numbers of pathogenic organisms is essential. It is also important that controls can be applied to the wide range of different food and water matrices that are often tested in a single laboratory.

Figure 2: Ready to use CRM LENTICULE® disc

The application of a unique preservation technology involving controlled-drying of authenticated cultures of internationally accepted microbiology control strains has resulted in the production of single-use discs containing microorganisms, designed for use in food, water and environmental testing laboratories. These quality control materials, LENTICULE® discs and Vitroids™, are available from Sigma-Aldrich, and are manufactured under reproducible conditions compliant with ISO Guide 34:2009 (general requirements for the competence of reference material producers). The discs contain pure cultures of bacteria, yeasts or molds in a solid, water-soluble matrix. Comprehensive certificates of analysis specify the mean number of colony forming units (CFU) per disc, details about the method used to determine the product data and the number of subcultures from the original strains, provided under license by NCTC and ATCC.

Figure 3: Certified Reference Microorganism discs



Single-use controls manufactured directly from cultures provided by the BRCs mean that laboratories can be confident about the authenticity of their strains and the suitability of their quality control materials, factors that are of increasing importance as laboratories become more automated and new technologies emerge and are rapidly adopted in routine microbiology settings.

References

1. Cross, L.J.; Russell, J.E.; Desai, M. *Examining the genetic variation of reference microbial cultures used within food and environmental laboratories using fluorescent amplified fragment length polymorphism analysis.* FEMS Microbiol Lett. 2011, 1-7.
2. https://www.phe-culturecollections.org.uk/collections/nctc-3000-project.aspx
3. Baker, K.S.; Mather, A.E.; McGregor, H.; Coupland, P.; Langridge, G.C.; Day, M.; Deheer-Graham, A.; Parkhill, J.; Russell, J.E.; Thomson, N.R. *The extant World War 1 dysentery bacillus NCTC1: a genomic analysis. The Lancet*. **2014**; Volume 384, Issue 9955, Pages 1691 – 1697.
4. http://www.wdcm.org/

Certified Reference Microorganisms

By Jvo Siegrist, Product Manager Microbiology
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Public Health England (PHE) and Sigma-Aldrich are now working together in the field of Certified Reference Microorganisms.

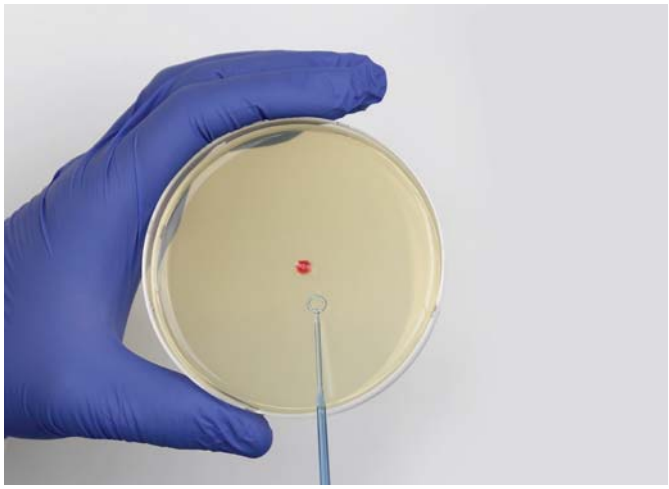
Each organization offers important contributions to this collaboration. PHE provides a great deal of knowledge through their cell culture collection organizations - NCTC and NCPF, and are recognized experts in the field of reference strains and proficiency testing programs, as well as being manufacturers of Certified Reference Microorganisms (CRMs) with their LENTICULE® discs. Sigma-Aldrich brings years of experience with regard to production according to ISO guide 34 and the certification of CRMs according to ISO/IEC 17025. In addition, Sigma-Aldrich's innovative CRM - Vitroids™- are an example of totally new microorganism standards with a precision and stability not known in the history of microbiology.

Vitroids™ and LENTICULE® discs

- Defined CFU and low standard deviation (ISO/IEC 17025)
- Economic, convenient and easy to use
- Long shelf life

Vitroids™ and LENTICULE® discs contain viable microorganisms in a certified quantity (generally accredited according to ISO/IEC 17025), produced under reproducible conditions compliant with ISO Guide 34:2009 using authenticated strains from NCTC, NCPF and ATCC®. Consisting of pure cultures of bacteria or fungi in a solid, water soluble matrix, they are stable for at least one year and are in a viable state with a shelf life of 1-3 years. The within batch variation for every product is very low (in the best case, 4% standard deviation at 100 CFU). Each batch is provided with a comprehensive certificate of analysis that specifies the mean number of colony forming units (CFU), an expanded uncertainty about the mean, details about the method used to determine the product data and the number of passages (subcultures) from the original strain.

Figure 1: A disc is streaked out on a plate



Applications

- QC to assure the quality of test results (water, food, beverage,
- environmental, etc.)
- Performance testing of media acc. to ISO 11133
- Validation of new methods
- Start material for proficiency testing or ring trials
- Method development
- Staff training
- Starter cultures

Stability

Certified Reference Microorganisms in this unique format are very stable and in most cases, will remain so for many years at -20 °C. The number of CFUs does not change, and the organisms need no recovery time and have no lag phase. A short period of time at ambient temperature, such as during shipment, is not an issue for product stability.

Save Time and Costs

Using Vitroids™ and LENTICULE® discs saves a lot of time because it removes the need for preparing stock cultures. The organisms need no recovery time and no pre-enrichment step is necessary. In addition the product concentrations are designed in a range where no or only minimal dilutions are needed. The discs readily dissolve in liquid media and on agar plates, resulting in easy handling and a very economical solution.

What is New Compared to Existing Reference Strain Products?

Utilization of new technology has allowed us to make major improvements in the field of Microbiological Reference Materials. The main areas of development are stability, temperature tolerance, adjusting the narrowly defined CFU range, rehydration time and better within batch reproducibility. In addition, each disc is certified according to ISO Guide 34 and ISO/IEC 17025.

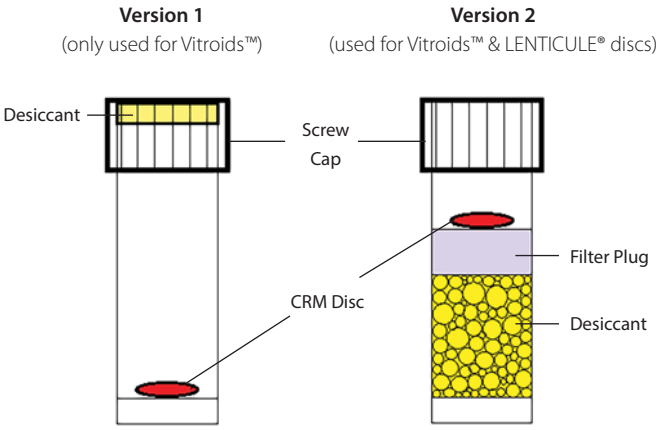
Preparation

Most solid and liquid media or a rehydration buffer can be used. The disc can be rehydrated in as little as 100 µL, or in larger volumes, e.g. 100 mL medium. It is also possible to add the disc to a cooled molten medium used for pour plate techniques. The rehydration process takes approximately 10 minutes. On solid media, the disc forms a droplet that can be spread with a sterile loop. In liquid media, the disc dissolves very quickly.

Packaging

The discs are packed individually in vials. The vials have a special screw-cap with seal and contain a desiccant at the bottom or in the cap. The vials are packed in a mylar bag with a zip.

Figure 2: Packaging of the CRMs



Strains

LENTICULE® discs are prepared from a freeze-dried, traceable culture obtained from the National Collection of Type Cultures (NCTC) or National Collection of Pathogenic Fungi (NCPF) and are manufactured by Sigma-Aldrich under license and control from Public Health England.

Vitroids™ are prepared from a freeze-dried, traceable culture obtained from the American Type Culture Collection (ATCC®) and are produced according to a Sigma-Aldrich patented technology.

Table 1: Certified Reference Microorganisms portfolio.

Species	Origin	Strain No.	Approx. Mean CFU / Mean CFU Range	Cat. No.	CRM	ATCC®	NCTC/NCPF	WDCM
Acinetobacter baumanni	ATCC®	19606™	80	**RQC22003**	✓	19606™	12156	—
Acinetobacter baumanni	ATCC®	19606™	200	**RQC22005**	✓	19606™	12156	—
Aspergillus brasiliensis (formerly *Aspergillus niger*)	NCPF®	2275	30-120	**RMF02275L**	—	16404™	2275	00053
Aspergillus brasiliensis (formerly *Aspergillus niger*)	ATCC®	16404™	80	**RQC15003**	✓	16404™	2275	00053
Bacillus cereus	NCTC®	7464	30-120	**CRM07464L**	✓	—	7464	—
Bacillus cereus	NCTC®	7464	500 - 5 × 10^4	**CRM07464M**	✓	—	7464	—
Bacillus subtilis	ATCC®	6633™	80	**RQC16003**	✓	6633™	10400	00003
Bacillus subtilis	ATCC®	6633™	10,000	**RQC02258**	✓	6633™	10400	00003
Campylobacter jejuni	NCTC®	11322	10^2	**RM11322Q**	—	29428™	11322	00156
Candida albicans	ATCC®	10231™	80	**RQC14003**	✓	10231™	3179	00054
Candida albicans	ATCC®	10231™	100	**RQC14004**	✓	10231™	3179	00054
Candida albicans	ATCC®	10231™	1,000	**RQC14007**	✓	10231™	3179	00054
Candida albicans	ATCC®	10231™	10,000	**RQC14008**	✓	10231™	3179	00054
Candida albicans	NCPF®	3255	30-120	**RMF03255L**	—	2091™	3255	00055
Candida albicans	NCPF®	3255	2 × 10^4	**RMF03255H**	—	2091™	3255	00055
Citrobacter freundii	ATCC®	8090™	200	**RQC02105**	✓	8090™	9750	—
Citrobacter freundii	ATCC®	8090™	10,000	**RQC02108**	✓	8090™	9750	—
Citrobacter freundii	NCTC®	9750	30-120	**RM09750L**	—	8090™	9750	—
Clostridium bifermentans	NCTC®	506	30-120	**CRM00506L**	✓	—	506	00079
Clostridium perfringens	NCTC®	13170	30-120	**CRM13170L**	✓	—	13170	00201
Clostridium perfringens	NCTC®	13170	500 - 5 × 10^4	**CRM13170M**	✓	—	13170	00201
Clostridium sporogenes	ATCC®	19404™	80	**RQC19003**	✓	19404™	532	00008
Cronobacter sakazakii	NCTC®	11467	30-120	**CRM11467L**	✓	29544™	11467	00214
Enterobacter aerogenes	NCTC®	10006	30-120	**CRM10006L**	✓	13048™	10006	—
Enterobacter aerogenes	NCTC®	10006	500 - 5 x 10^4	**CRM10006M**	✓	13048™	10006	—
Enterobacter aerogenes	ATCC®	13048™	50	**RQC01652**	✓	13048™	10006	00175
Enterobacter aerogenes	ATCC®	13048™	100	**RQC01654**	✓	13048™	10006	00175
Enterobacter aerogenes	ATCC®	13048™	200	**RQC01655**	✓	13048™	10006	00175

*Look for the ATCC Licensed Derivative® Emblem for products derived from ATCC® cultures.

Species	Origin	Strain No.	Approx. Mean CFU / Mean CFU Range	Cat. No.	CRM	ATCC®	NCTC/NCPF	WDCM
Enterobacter aerogenes	ATCC®	13048™	1,000	RQC01657	✓	13048™	10006	00175
Enterobacter aerogenes	ATCC®	13048™	10,000	RQC01658	✓	13048™	10006	00175
Enterobacter cloacae	ATCC®	35030™	50	RQC21102	✓	35030™	11854	—
Enterobacter cloacae	ATCC®	35030™	200	RQC21105	✓	35030™	11854	—
Enterococcus faecalis	NCTC®	775	30-120	CRM00775L	✓	19433™	775	00009
Enterococcus faecalis	NCTC®	775	500 - 5 × 10^4	CRM00775M	✓	19433™	775	00009
Enterococcus faecalis	NCTC®	775	>10^5	CRM00775H	✓	19433™	775	00009
Enterococcus faecalis	ATCC®	19433™	50	RQC01772	✓	19433™	775	00009
Enterococcus faecalis	ATCC®	19433™	100	RQC01774	✓	19433™	775	00009
Enterococcus faecalis	ATCC®	19433™	200	RQC01775	✓	19433™	775	00009
Enterococcus faecalis	ATCC®	19433™	500	RQC01776	✓	19433™	775	00009
Enterococcus faecalis	ATCC®	19433™	1,000	RQC01777	✓	19433™	775	00009
Enterococcus faecium	ATCC®	19434™	80	RQC20003	✓	19434™	7171	00010
Enterococcus faecium	ATCC®	19434™	200	RQC20005	✓	19434™	7171	00010
Escherichia coli	NCTC®	13216	30-120	CRM13216L	✓	—	13216	—
Escherichia coli	NCTC®	9001	30-120	CRM09001L	✓	11775™	9001	00090
Escherichia coli	NCTC®	9001	500 - 5 × 10^4	CRM09001M	✓	11775™	9001	00090
Escherichia coli	NCTC®	9001	>10^5	CRM09001H	✓	11775™	9001	00090
Escherichia coli	ATCC®	11775™	50	RQC01702	✓	11775™	9001	00090
Escherichia coli	ATCC®	11775™	200	RQC01705	✓	11775™	9001	00090
Escherichia coli	ATCC®	11775™	1,000	RQC01707	✓	11775™	9001	00090
Escherichia coli	ATCC®	11775™	10,000	RQC01708	✓	11775™	9001	00090
Escherichia coli	ATCC®	25922™	100	RQC02704	✓	25922™	12241	00013
Escherichia coli	ATCC®	25922™	10,000	RQC02708	✓	25922™	12241	00013
Escherichia coli	ATCC®	8739™	80	RQC11003	✓	8739™	12923	00012
Escherichia coli O157 (NT)	NCTC®	12900	30-120	CRM12900L	✓	700728™	12900	00014
Heterotrophic Organisms	—	—	100	RQC02504	✓	—	—	—
Klebsiella oxytoca	NCTC®	8167	30-120	CRM08167L	✓	—	8167	—
Klebsiella pneumoniae	ATCC®	13883™	30	RQC02601	✓	13883™	9633	00097
Klebsiella pneumoniae	ATCC®	13883™	1,000	RQC02607	✓	13883™	9633	00097
Klebsiella pneumoniae	ATCC®	33495™	200	RQC03105	✓	33495™	—	—
Klebsiella pneumoniae	ATCC®	33495™	1,000	RQC03107	✓	33495™	—	—
Legionella bozemanii	NCTC®	11368	500 - 5 × 10^4	CRM11368M	✓	33217™	11368	—
Legionella micdadei	NCTC®	11371	500 - 5 × 10^4	CRM11371M	✓	33218™	11371	—
Legionella pneumophila	NCTC®	12821	30-120	CRM12821L	✓	—	12821	—
Legionella pneumophila	NCTC®	12821	500 - 5 × 10^4	CRM12821M	✓	—	12821	—
Listeria innocua	NCTC®	11288	30-120	CRM11288L	✓	33090™	11288	00017
Listeria monocytogenes	ATCC®	19115™	30	RQC01901	✓	19115™	—	—
Listeria monocytogenes	NCTC®	11994	30-120	CRM11994L	✓	—	11994	00019
Listeria monocytogenes	NCTC®	11994	500 - 5 × 10^4	CRM11994M	✓	—	11994	00019
Negative Control LENTICULE® discs, no growth	—	—	0	RMBLANK0	—	—	—	—
Negative Control (Vitroids™, no growth)	—	—	0	RQC0001	✓	—	—	—
Proteus hauseri	ATCC®	13315™	1,000	RQC03207	✓	13315™	4175	—
Proteus mirabilis	ATCC®	25933™	1,000	RQC03407	✓	25933™	—	—
Pseudomonas aeruginosa	ATCC®	10145™	200	RQC01855	✓	10145™	10332	00024
Pseudomonas aeruginosa	NCTC®	10662	30-120	CRM10662L	✓	27853™	10662	00114

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Species	Origin	Strain No.	Approx. Mean CFU / Mean CFU Range	Cat. No.	CRM	ATCC*	NCTC/NCPF	WDCM
Pseudomonas aeruginosa	NCTC®	10662	500 - 5 × 10^4	**CRM10662M**	✓	27853™	10662	00114
Pseudomonas aeruginosa	ATCC®	27853™	50	**RQC03302**	✓	27853™	10662	00114
Pseudomonas aeruginosa	ATCC®	27853™	100	**RQC03304**	✓	27853™	10662	00114
Pseudomonas aeruginosa	ATCC®	27853™	1,000	**RQC03307**	✓	27853™	10662	00114
Pseudomonas aeruginosa	ATCC®	9027™	30	**RQC02201**	✓	9027™	12924	00026
Pseudomonas aeruginosa	ATCC®	9027™	50	**RQC12002**	✓	9027™	12924	00026
Pseudomonas aeruginosa	ATCC®	9027™	80	**RQC12003**	✓	9027™	12924	00026
Pseudomonas aeruginosa	ATCC®	9027™	100	**RQC02204**	✓	9027™	12924	00026
Pseudomonas aeruginosa	ATCC®	9027™	200	**RQC12005**	✓	9027™	12924	00026
Pseudomonas aeruginosa	ATCC®	9027™	1,000	**RQC12007**	✓	9027™	12924	—
Raoultella planticola (formerly *Klebsiella aerogenes*)	NCTC®	9528	30-120	**CRM09528L**	✓	—	9528	—
Raoultella planticola (formerly *Klebsiella aerogenes*)	NCTC®	9528	500 - 5 × 10^4	**CRM09528M**	✓	—	9528	—
Saccharomyces cerevisiae	NCPF®	3191	30-120	**RMF03191L**	—	13119™	3191	—
Saccharomyces cerevisiae	NCPF®	3191	500 - 5 × 10^4	**RMF03191M**	—	13119™	3191	—
Salmonella enterica subsp. enterica serovar Abaetetuba	ATCC®	35640™	500	**RQC03006**	✓	35640™	—	—
Salmonella enterica subsp. enterica serovar Abaetetuba	ATCC®	35640™	1000	**RQC03007**	✓	35640™	—	—
Salmonella enterica subsp. Enterica serovar Typhimurium	ATCC®	14028™	50	**RQC17002**	✓	14028™	12023	00031
Salmonella enterica subsp. Enterica serovar Typhimurium	ATCC®	14028™	80	**RQC17003**	✓	14028™	12023	00031
Salmonella Enteritidis	NCTC®	6676	30-120	**CRM06676L**	✓	—	6676	—
Salmonella Nottingham	NCTC®	7832	30-120	**CRM07832L**	✓	—	7832	—
Salmonella Typhimurium	NCTC®	12023	30-120	**CRM12023L**	✓	14028™	12023	00031
Staphylococcus aureus	NCTC®	6571	30-120	**CRM06571L**	✓	9144™	6571	00035
Staphylococcus aureus subsp. Aureus	ATCC®	6538™	50	**RQC13002**	✓	6538™	10788	00032
Staphylococcus aureus subsp. Aureus	ATCC®	6538™	80	**RQC13003**	✓	6538™	10788	00032
Staphylococcus aureus subsp. Aureus	ATCC®	6538™	100	**RQC13004**	✓	6538™	10788	00032
Staphylococcus aureus subsp. Aureus	ATCC®	6538™	200	**RQC13005**	✓	6538™	10788	00032
Staphylococcus aureus subsp. Aureus	ATCC®	6538™	1,000	**RQC13007**	✓	6538™	10788	00032
Staphylococcus aureus	NCTC®	6571	500 - 5 × 10^4	**CRM06571M**	✓	9144™	6571	00035
Staphylococcus epidermidis	NCTC®	11047	30-120	**CRM11047L**	✓	14990™	11047	00132
Vibrio furnissi	NCTC®	11218	30-120	**RM11218Q**	—	—	11218	00186
Vibrio parahaemolyticus	NCTC®	10903	30-120	**RM10903Q**	—	17802™	10903	00037
Yersinia enterocolitica	NCTC®	11176	30-120	**RM11176L**	—	—	11176	—

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NCTC National Collection of Type Cultures
Operated by Public Health England

NCPF National Collection of Pathogenic Fungi
Operated by Public Health England

For more information visit
sigma-aldrich.com/mibi-crm

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Determination of Vitamin Content by Bacteria

By Jvo Siegrist, Product Manager Microbiology
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Microbiologists can help analytical chemists when things become complex.

Functional food is currently an important topic for human and animal health, and is also a growing market. Some people eat fresh BIO (organic) food, while others just add daily vitamin pills to their diet. A lot of food labels state that their product contains certain vitamins, dietary fiber, minerals, trace elements or polyunsaturated fatty acids.

Determining vitamins in a complex mixture like juice is not easy using classical analytical methods. In some cases, such analysis would be very costly and time consuming; however, microbiologists have an easy solution. With the help of some specific bacteria such as *Lactobacillus delbrueckii, Lactobacillus leichmanii, Lactobacillus plantarum* or *Streptococcus faecium*, the problem can be easily solved, as they are excellent indicators of the level of certain vitamins. For example, if the vitamin is not present, some species will not grow anymore, and if there is a low concentration, the growth is limited by the vitamin. Several tests known today can be used to detect vitamins such as B12, niacin, and folic acid.

Table 1: Typical indicator organisms for vitamin determination

Vitamin	Typical Indicator Organism
Folic acid	*Streptococcus faecium* (ATCC 8043)
Niacin or Niacinamide	*Lactobacillus plantarum* (ATCC 8014)
Vitamin B_{12}	*Lactobacillus leichmannii* (ATCC 7830)

Figure 2: Vitamins and vitamin-packed food

Principle of an analysis:

Sample preparation:

In cases where the vitamin is present only in a free form, the examination material (e.g. powders or levigated tablets) can be simply extracted with water. Should the sample contain a bonded vitamin, decomposition with buffer solution or enzymatic hydrolysis is necessary.

The decomposition with buffer solution works quite simply by adding and homogenizing 1 g of sample into 50 mL buffer containing, for example, disodium hydrogen phosphate, citric acid or sodium metabisulfite. Afterwards the sample is autoclaved for 10 minutes at 121 °C. After cooling, the pH is adjusted and the solution is filtered to get a solution without particles.

It is also possible to use an enzymatic hydrolysis by homogenizing 1 g sample in 80 mL of acetate buffer. Papain, amylase and a few drops of chloroform or toluene are added to the homogeneous suspension. The two enzymes can also be replaced by a corresponding diastase. Then the samples are incubated for about 24 hours at 37 °C, and then heated for 30 minutes at 100 °C. When the samples have cooled down, the pH is adjusted with sodium hydroxide solution and filled up to 100 mL with acetate buffer. The suspension is filtered or centrifuged to separate particles. It is recommended that a preliminary test is performed, if the content of the vitamin is completely unknown. For this preliminary test, a concentrated extract is prepared and examined in different dilutions; a dilution factor of 10 is recommended.

Preparation culture of test organism

Bacteria, which depend on the specific vitamin for growth, are used as test organisms. The organisms are inoculated in a specific broth and incubated for 20 hours at 37 °C. Then the culture is centrifuged and washed with physiological saline and adjusted to a microbial count of 10^8 bacteria/mL.

Calibration

The calibration is done by a vitamin standard which is diluted to a level of 0-50 pg/mL.

Test

For the assay, 5 ml sterile standard or sample solution must be mixed with 5 mL Vitamin Assay broth. To each tube, excluding the sterile controls, one drop of the freshly prepared culture is added and incubated 24 hours at 37 °C.

The calibration standards and samples are measured photometrically at 546 nm and compared against the control. A linear calibration curve is recorded with the optical density (OD) values against the vitamin concentration. The vitamin concentration of the samples can be calculated based on the calibration curve.

Table 2: Media for determination of vitamins

Cat. No.	Description
B3801	B_{12} Assay Medium
82897	Vitamin B_{12} Assay Medium*
N7404	Niacin Assay Medium
F5422	Folic Acid Assay Medium

* Not available in USA

References:

1. AACC, Approved methods of the American Association of Cereal Chemists. 8th edition. American (1994).
2. U.S. Pharmacopeia 21st rev., p 1183, (1985).

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