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Microbiology Focus

QPCRScan – A PCR Kit Using Rehybridization Probe System Technology

SIGMA-ALDRICH®

QPCRScan for Qualitative Detection of Food Pathogens like *Salmonella, Listeria* and *Campylobacter* by Real-Time PCR Method

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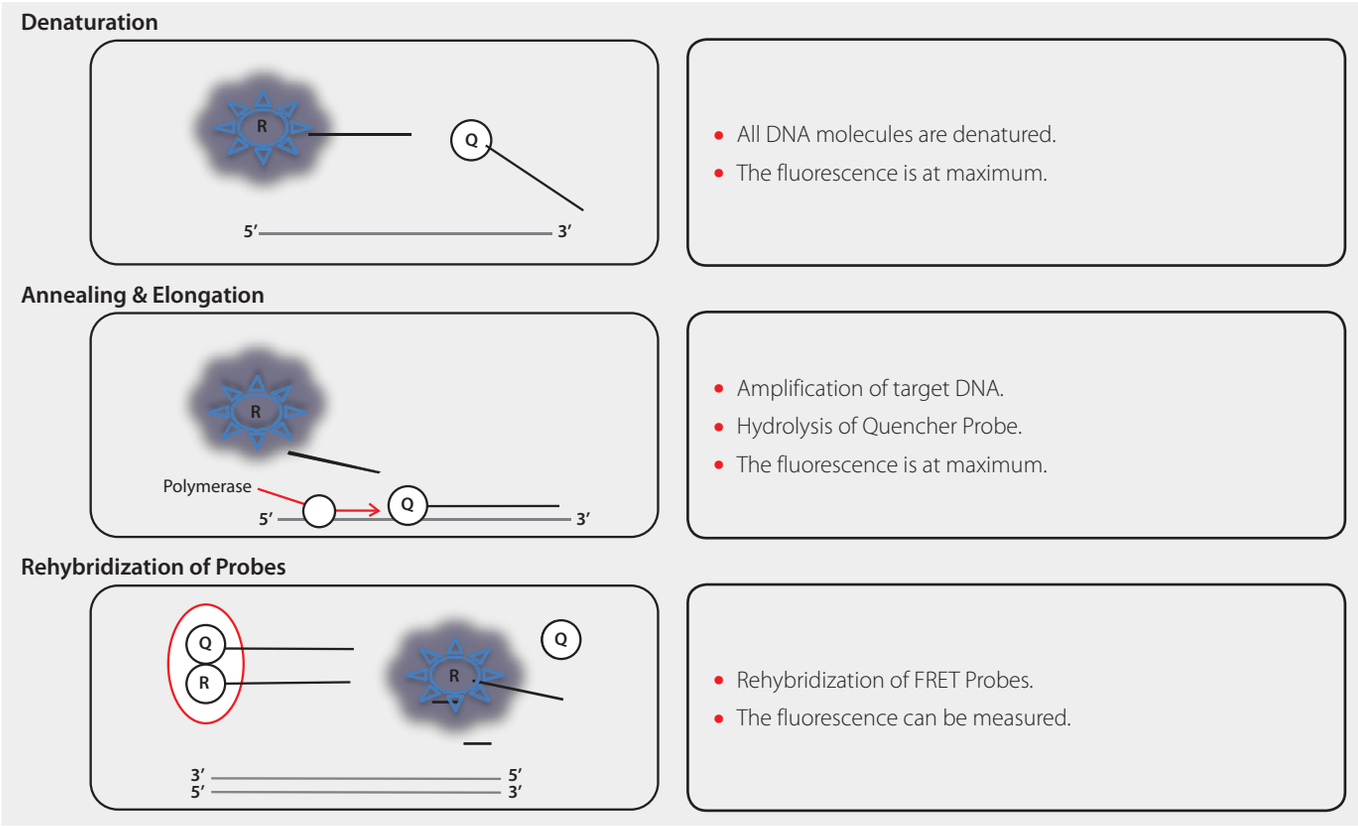
The QPCRScan portfolio is used for the qualitative detection of several food pathogens by Real-Time PCR method. The kits are based on specific primer sets and fluorescence-labeled probes. Flexibility in the use of all common PCR instruments is possible.

The Polymerase Chain Reaction (PCR) is a method used to make a large number of copies of a specific DNA fragment from the genome in a relatively short time. In 1993, the American Biochemist Kary Mullis was awarded the Nobel Prize for the development of the PCR technique (Malmström et al., 2013). PCR technology counts as a major breakthrough because it solved the problem of how to produce multiple copies of any specific gene sequence using a relatively simple, economical and reliable procedure. PCR is also an important tool for the analysis of DNA in samples. The application range of PCR is broad, beginning with analysis of metabolic pathways, to the identification of plants and animals, trace research, forensic samples, fossils and archaeology, as well as the detection, identification and classification of microorganisms (Hutzler et al., 2008). The identification and phylogenetic classification of bacteria is now mainly carried out by analysis of the ribosomal RNA (rrn) operon, in most cases the 16S rDNA gene and the 16S-23S spacer region.

The PCR method used for QPCRScan is based on a duplex system (internal amplification control) with specific primers and fluorescence-labeled probes and utilizes the patented Rehybridization Probe System (RPS) technology. Using this novel probe technique, the method is characterized by the system allowing a homogenous and high-throughput nucleic acid detection that can be automated. Two probes, oligonucleotides, are used: at the 3' end of one probe is the acceptor and at the other probe, on the 5' end, a donor is linked. The probes are selected so that they are partially complementary to each other, and the Förster resonance energy transfer (FRET) effect does occur if they are hybridized during the fluorescence measurement at a temperature which is lower than the annealing temperature of primers and probes. During the amplification step, the quencher-labeled probe is hybridized with the target DNA and can be destroyed by the exonuclease activity of the Taq-Polymerase. Therefore, the appropriate donor-labeled probe is not quenched during the next measurement.

Figure 1: Principle of Rehybridization Probe System (RPS) technology: R = Rehybridization probe with fluorescence label; Q = Quencher



sigma-aldrich.com/qpcrscan

The probes specified for the pathogen of interest are labeled with the fluorophore FAM (Carboxyfluorescein; λ_{max} 494 nm excitation, λ_{max} 518 nm emission), while probes for the internal control are labeled with the fluorophore HEX (hexachloro-fluorescein, λ_{max} 535 nm excitation, λ_{max} 556 nm emission).

Any PCR instrument which can measure these two dyes can be used, which makes the system flexible.

This method offers the following advantages:

- Method is very sensitive (1–10 cfu)
- Economic format for 96-well microtiter plate
- Flexible use of any PCR instrument capable of detecting FAM and HEX

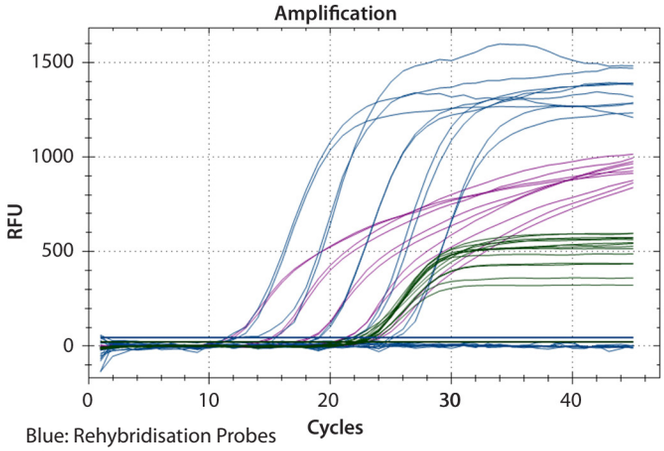
The portfolio comprises the following rapid molecular test systems:

- QPCRScan – *Salmonella* Real-Time Kit
  - for the qualitative detection of bacteria of the genus *Salmonella*, including the detection of all serovars like *S. enteritidis*, *S. typhimurium, S.typhi* and *S. paratyphi*.
  - QPCRScan – *Salmonella* kit is suitable for the detection of *Salmonella* after pre-enrichment according to EN ISO 6579: 2002+ Amd 1:2007 and for the confirmation of *Salmonella* spp. colonies.
- QPCRScan *Listeria monocytogenes* Real-Time Kit
  - for the specific detection of *L. monocytogenes*
  - The kit is suitable for the detection of *L. monocytogenes* according to DIN EN ISO 11290-1 (2005) and for the confirmation of *L. monocytogenes* colonies.
- QPCRScan *Listeria* Real-Time Kit
  - for the specific detection of bacteria of the genus *Listeria*
  - The kit is suitable for the detection of *Listeria* after pre-enrichment according to DIN EN ISO 11290-1 (2005) and for the confirmation of *Listeria* spp. colonies.
- QPCRScan *Campylobacter* Real-Time Kit
  - for the qualitative detection of *C. jejuni* and *C. coli*.
  - The QPCRScan *Campylobacter* kit is suitable for the detection of *C. jejuni* and *C. coli* according to EN ISO 10272-1 (2006) and for the confirmation of *Campylobacter* colonies.

All of the kits contain a standard PCR setup and include resuspension buffer, water (PCR grade), master mix, probes and internal amplification control. QPCRScan kits contain lyophilized reagents and should be stored until dissolving at 4 °C. Once the reagents are dissolved, they should be stored at –20 °C.

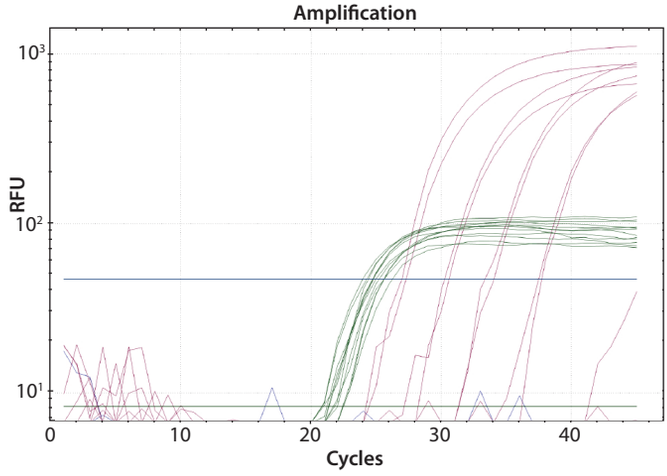
Figure 2: Comparison of TaqMan probe principle with new principle Rehybridization Probe System (RPS)

Higher level of sensitivity is seen with the RPS technology starting with *Listeria monocytogenes*. 1,000, 100, 10 cells/mL from cell culture.



Blue: Rehybridisation Probes
Lila: TaqMan
Green: Internal Control

Figure 3: Example of an amplification plot, *Salmonella* (lilac) RPS with Internal Control (green); 1:10 dilution from 10,000 to 1 copy of *Salmonella* DNA (The detection limit is between 1 and 10 DNA copies)



Ordering Information

Cat. No.	Kit	Test Kit Description
05829-96TESTS	QPCRScan *Campylobacter*	For qualitative detection of *C. coli* and *C. jejuni* by Real-Time PCR
06040-96TESTS	QPCRScan *Listeria* spp.	For qualitative detection of *Listeria* spp. by Real-Time PCR
06238-96TESTS	QPCRScan *Listeria monocytogenes*	For qualitative detection of *Listeria monocytogenes*. by Real-Time PCR
91289-96TESTS	QPCRScan *Salmonella*	For qualitative detection of *Salmonella* spp. by Real-Time PCR

References:

1. Malmström G. and Andersson B., (2013). `The Nobel Prize in Chemistry: The Development of Modern Chemistry` available from: http://www.nobelprize.org Nobelprize.org. Nobel Media AB.
2. Hutzler M., Schuster E., Stettner G., (2008) `Ein Werkzeug in der Brauereimikrobiologie. Real-time PCR in der Praxis`, Brauindustrie, 4, 52–55.

Comparison of Dehydrated Culture Media for Efficient Media Fill Testing by Evaluating Cold Filterability

By Volker Lanz, Product Manager Microbiology — *volker.lanz@merckgroup.com*

It is frustrating if you have done your preparation and are ready to begin filtering your media, only to find that it is not possible since the filter is clogged. Unfortunately, the quality of media and filters on the market varies greatly.

Introduction

Good Manufacturing Practices (GMP) require media fill testing to verify that aseptic procedures are adequate for preventing contamination during drug production. The standard medium used for fill tests under aerobic conditions is Soybean Casein Digest Medium (SCDM), also referred to as Tryptic Soy Broth (TSB). The European Pharmacopeia (EP; 5.2.8) recommends minimizing contamination risk by using materials from "non TSE-relevant animal species" or non-animal origin and for peptone, the use of vegetable protein in place of animal source protein. In alignment with these recommendations, there is a market tendency to replace animal-derived peptones with vegetable-sourced materials in order to reduce the risk of contaminating controlled production areas with prions. Furthermore, avoiding the use of animal-derived materials eliminates the need for animal origin documentation requirements.

The majority of aseptic filling processes do not permit autoclaving prior to filling; therefore, process simulations should also avoid this step. The inability to autoclave presents a contamination risk to the aseptic filling lines. Although sterile filtration is conducted in most cases, this technique is inefficient for eliminating the risk of contamination, as a number of organisms such as Mycoplasma, found in plant-derived peptones, and Alcholeplasma, found in animal-derived peptones, are small enough to pass through the 0.2 µm filters. In order to absolutely counter this risk, gamma-irradiation is an absolute necessity.

A study was conducted to compare dehydrated culture media for efficient media fill testing using cold filterability. As Vegetable Peptone Broth (VPB) has become a well-accepted animal component-free alternative to TSB, both media types are included in this study. Gamma-irradiated products that are deemed suitable for safe introduction into controlled areas were also used. During the sterile filtration step of the aseptic process simulation, a high-throughput rate of the media through the 0.2 µm filter membrane is essential and is the main focus of this study.

Methods

An important contributing factor to an effective media fill process simulation is the complete solubility of the dehydrated culture media. Only complete solubility will prevent particles from clogging the membrane. To change the filter during the process simulation is time-consuming, expensive and a contamination risk. Therefore, a good solubility and a high liquid throughput rate of the membrane filter are high priority criteria for selecting the most suitable media for process simulations.

merckmillipore.com/media-fill

Did you know …

In 1918, Nobel Prize winner Richard Zsigmondy developed the first membrane filters for commercial use in the pharma industry?

After the observation that *Pseudomonas diminuta* (now *Brevundimonas diminuta*) could pass the 0.45 µm pore filters, the standard for sterilizing changed to 0.2 µm filters.

Figure 1. Membrane syringe filter

Tables 1 and 2 contain the types of dehydrated culture media and filtration materials used in the comparison study. The dehydrated culture media was dissolved in 2 L of purified water and only used when the medium had completely dissolved.

TSB and VPB from Merck Millipore and Brands A and B can be dissolved in cold purified water. A heating step is unnecessary. The solubility of the unheated Merck Millipore TSA and VBP, Brand B TSA and VBP, and Brand B TSB was very good, dissolving completely. However, the VPB of Brand B did not dissolve. Solubility could not be improved by replacing purified water with Milli-Q®-water or by leaving the broth overnight to dissolve in purified water. Therefore, the dissolving time for this medium was set to 4 hours under agitation for this comparison study.

Brand C and Brand D were heated according to manufacturers' instructions, to ensure the broth was dissolved, and allowed to cool to room temperature. It is important to note that growth promotion tests were not performed after the defined heating time.

Three runs of every combination of media and membrane filters were then performed. Two batches per type of filter membranes were evaluated.

The filterability of culture media is often measured in liters per square meter of membrane and does not take into account the time elapsed until clogging occurs. As filtration time is a critical factor when assessing the practical usability of culture media for media fill applications, an alternative approach was selected for measuring the time required to filtrate a given volume regarded as a minimum requirement. In laboratory scale, this volume is set to 2 L on a 47 mm diameter membrane, with a maximum acceptable filtration time of 30 minutes.

For this study, it was decided to filter until completion or clogging, irrespective of the 30-minute threshold. Two liters on a 47 mm diameter membrane (with an actual filtration area of 40 mm diameter) are equivalent to about 1,592 L per square meter.

To confirm vacuum generation, the EZ-Stream™ vacuum pump was checked once by the Merck Millipore calibration service. Filtration time measurement was initiated daily with a system check referred to as the "blank reference test," which comprises three runs of time measurement with 2 L of purified water and the defined filter membrane. The reference values for water shown in **Figures 2** and **3** are the average values of all these blank reference tests.

Results

Figures 2 and 3 illustrate the average time to filtrate 2 L of TSB and VPB, respectively. The displayed results illustrate the average values from three filtration runs of each media and membrane batch combination. With some combinations of membrane filter and broth, the membrane clogged, and it was not possible to filtrate 2 L through the membrane.

Discussion

Before dehydrated culture media undergoes sterile filtration, it must first be brought into solution. In many cases, media preparation takes place close to the aseptic filling area, typically a clean room D area. To prevent contamination of this area, it is preferable to use a product that is suitable for safe transfer into clean rooms. Surprisingly, not all gamma-irradiated products on the market are triple-wrapped, and the overall packaging quality is heterogeneous (see **Table 1**).

Even more important is solubility itself. The volume of culture medium required for a single media fill run can be substantial, and dissolving powder in several hundred liters of water can be a time- and labor-consuming process. Even in the laboratory scale of this study, it was observed that granulated products dissolve much easier and faster than any powder products. The different powdered products also showed important differences, such as the color indicator of one product failing to dissolve completely.

Some manufacturers recommend heating their media to support dissolution. In addition to providing an additional process step, heating is often not an option in the facilities where the media are prepared. Users who cannot heat the media should be aware of potential issues with some brands. Furthermore, this study also demonstrates that heating does not guarantee acceptable filtration times.

This comparison study includes four types of membrane filters for sterile filtration. The filter types applied largely depend on the substances produced, and culture media should provide sufficient filtration performance with all filter types. While PVDF and PES membranes are the most commonly used on the market, there are considerable differences between the two filters with regard to how easily culture media – and even water – can be filtered.

PES membranes are the least demanding filters for both TSB and VPB. While Nitrocellulose membranes require only slightly more time, PVDF and Nylon are much more challenging. With these filters, the differences between TSB and VPB become more obvious. It should be noted that, overall, vegetable-sourced peptones are more difficult to filtrate than the animal-derived peptones in standard TSB. This is true for all tested brands and on all filter types.

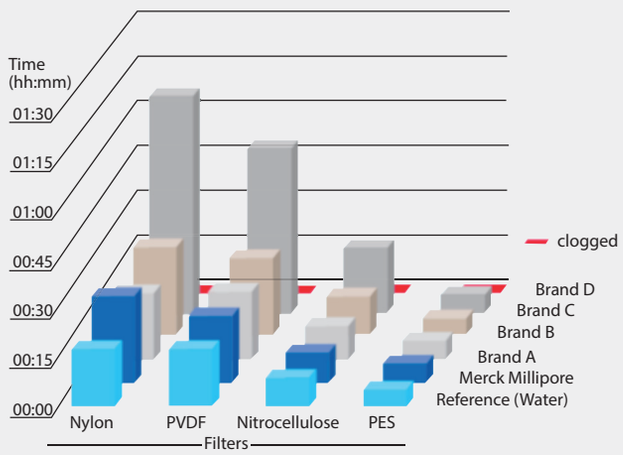
Table 1: Dehydrated Culture Media

Culture Media Type	Brand	Gamma-irradiated	Packaging	Format
Tryptic Soy Broth (Cat. No. 1.00800.0500)	Merck Millipore	Yes	Triple-wrapped	granulated
Tryptic Soy Broth	Brand A	Yes	Triple-wrapped	powder
Tryptic Soy Broth	Brand B	Yes	Triple-wrapped	powder
Tryptic Soy Broth	Brand C	Yes	Double-wrapped	powder
Tryptic Soy Broth	Brand D	No	Single-wrapped	powder
Vegetable Peptone Broth (Cat. No. 1.00550.0500)	Merck Millipore	Yes	Triple-wrapped	granulated
Vegetable Peptone Broth	Brand A	Yes	Triple-wrapped	powder
Vegetable Peptone Broth	Brand B	Yes	Triple-wrapped	powder
Vegetable Peptone Broth	Brand C	Yes	Double-wrapped	powder

Table 2: Filtration Materials

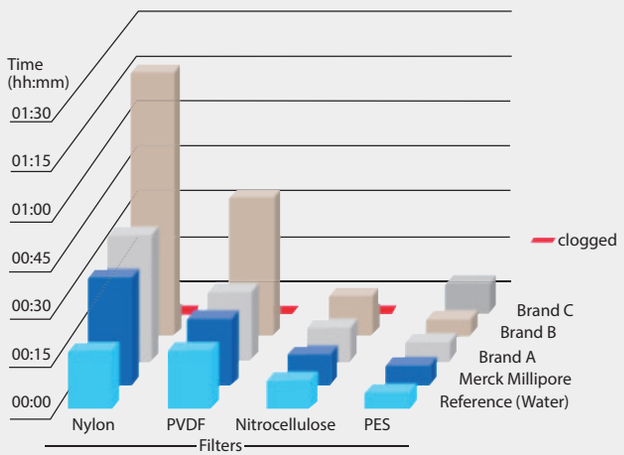
Product Name	Cat. No.	Filter Type	Pore Size	Diameter
Millipore Express PLUS Membrane Filter	**GPWP04700**	PES (Polyethersulfon)	0.2 µm	47 mm
Durapore Membrane Filter	**GVWP04700**	PVDF (Polyvinylidenfluorid)	0.22 µm	47 mm
Nylon Membrane Filter	**GNWP04700**	Nylon	0.2 µm	47 mm
MF-Millipore Membrane Filter	**GSWP04700**	Mixed Cellulose Esters	0.22 µm	47 mm
Microfil® Funnel, 250 mL	**MIHAWG250**	N/A	N/A	N/A

Figure 2: Filterability comparison of Tryptic Soy Broth



	Nylon	PVDF	Nitrocellulose	PES
Reference (Water)	00:17:38	00:17:40	00:08:07	00:05:29
Merck Millipore	00:27:06	00:20:14	00:09:23	00:06:09
Brand A	00:21:49	00:21:54	00:09:58	00:06:30
Brand B	00:28:44	00:25:52	00:10:51	00:06:26
Brand C	01:14:14	00:58:33	00:16:27	00:08:00
Brand D	clogged	clogged	clogged	clogged

Figure 3: Filterability comparison of Vegetable Peptone Broth



	Nylon	PVDF	Nitrocellulose	PES
Reference (Water)	00:17:38	00:17:40	00:08:07	00:05:29
Merck Millipore	00:33:54	00:21:17	00:10:13	00:06:09
Brand A	00:39:56	00:22:52	00:10:26	00:06:32
Brand B	01:23:32	00:44:51	00:13:27	00:06:40
Brand C	clogged	clogged	clogged	clogged

New Vitroids™ Product Range and Cross Reference Guide

By Coralie Leonards, Product Manager Applied Analytics — *coralie.leonard@sial.com*

Vitroids™ and LENTICULE® discs are Certified Reference Microorganisms (CRMs). In the case of Vitroids™, many changes have occurred in the last few months, which is why we are updating you by means of this article.

What are Vitroids™ and LENTICULE® discs?

Vitroids™ and LENTICULE® discs contain viable microorganisms with a certified colony forming unit (CFU) count. They are reference materials (RMs) and Certified Reference Materials (CRMs) compliant with ISO Guide 34:2009 and certified in an ISO 17025 accredited laboratory under reproducible conditions. They are traceable to an authenticated reference strain from NCTC®, NCPF® or CECT®.

These RMs and CRMs consist of pure cultures of bacteria or fungi in a solid water-soluble matrix; they are stable from 1 to 3 years in a viable state. The intra-batch variation is low (down to 4% standard deviation). Each product is provided with a downloadable comprehensive certificate of analysis that contains the mean number of 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 authenticated strain.

Figure 1: Microbiology Vitroids™ Lenticule disc on solid media

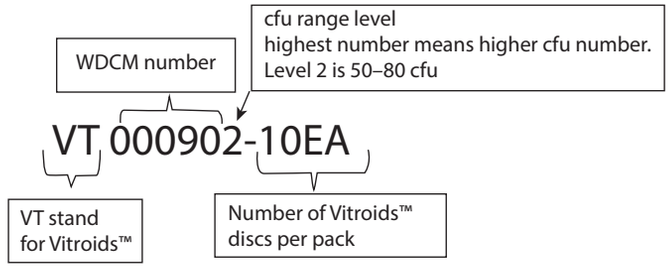
New Vitroids™ Product Range Based on WDCM Numbers

Important changes to the Vitroids range of Microorganism Certified Reference Materials are currently taking place. The new range of Vitroids is conveniently matched to WDCM numbers and has cfu ranges that more closely align with ISO 11133. To achieve this, we are utilizing the CECT Spanish Type Culture Collection.

We produced a cross reference table with the previous range, and the WDCM numbers have been used to match our old part number to the new strain used for our new Vitroids ™ range.

For added convenience, our new part numbers contain the strain WDCM equivalent number, when available.

For example:



Did you know …

Why the WDCM (World Data Centre for Microorganisms) reference strain catalog was built?

The intention was to enable broader and easier access to the reference strains listed by the ISO TC 34 SC 9 Joint Working Group 5 and by the Working Party on Culture Media of the International Committee on Food Microbiology and Hygiene (ICFMH-WPCM) in their publication *Handbook of Culture Media for Food and Water Microbiology*. It fulfills a need expressed by these bodies for a unique system of identifiers for strains recommended for use in quality assurance.

Figure 2: Website of WDCM

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Where there is no WDCM number available for a specified strain, the closest related product has been used, utilizing the CECT® strain description located on the CECT® website.

Please note the new Vitroids™ range from which the new part numbers are shown in the table below are not derived from ATCC® stains.

Strain Name	Old Cat. No.	ATCC*	CFU Median	WDCM	New Cat. No.	CECT*	CFU Range
Acinetobacter baumanni	RQC22003	19606™	80	—	**VT091112**	911	50–80
	RQC22005	19606™	200	—	**VT091114**	911	130–270
	RQC22007	19606™	1,000	—	**VT091115**	911	600–1,400
Aspergillus brasiliensis	RQC15003	16404™	80	00053	**VT000532**	2574	50–80
	RQC15008	16404™	10,000	00053	**VT000533**	2574	80–120
Bacillus subtilis	RQC16003	6633™	80	00003	**VT000032**	356	50–80
	RQC02258	6633™	10,000	00003	**VT000036**	356	3,000–7,000
Bacillus cereus	New product - no previous number available			00001	**VT000013**	193	80–120
Candida albicans	RQC14004	10231™	100	00054	**VT000543**	1394	80–120
	RQC14007	10231™	1,000	00054	**VT000545**	1394	600–1,400
	RQC14008	10231™	10,000	00054	**VT000546**	1394	3,000–7,000
Citrobacter freundii	RQC02105	8090™	200	—	**VT004014**	401	130–270
	RQC02108	8090™	10,000	—	**VT004016**	401	3,000–7,000
Clostridium sporogenes	RQC19003	19404™	80	00008	**VT000082**	485	50–80
Enterobacter aerogenes	RQC01652	13048™	50	00175	**VT001752**	684	50–80
	RQC01654	13048™	100	00175	**VT001753**	684	80–120
	RQC01655	13048™	200	00175	**VT001754**	684	130–270
	RQC01657	13048™	1,000	00175	**VT001755**	684	600–1,400
	RQC01658	13048™	10,000	00175	**VT001756**	684	3,000–7,000
	New product - no previous number available			00083	**VT000834**	194	130–270
Enterococcus faecalis	RQC01772	19433™	50	00009	**VT000092**	481	50–80
	RQC01774	19433™	100	00009	**VT000093**	481	80–120
	RQC01775	19433™	200	00009	**VT000094**	481	130–270
	RQC01776	19433™	500	00009	**VT000095**	481	600–1,400
	RQC01777	19433™	1,000	00009	**VT000096**	481	3,000–7,000
	RQC20003	19434™	80	00010	**VT000102**	410	50–80
	RQC20005	19434™	200	00010	**VT000104**	410	130–270
	RQC20007	19434™	1,000	00010	**VT000105**	410	600–1,400
Escherichia coli	RQC01702	11775™	50	00090	**VT000902**	515	50–80
	RQC01705	11775™	200	00090	**VT000904**	515	130–270
	RQC01707	11775™	1,000	00090	**VT000905**	515	600–1,400
	New product - no previous number available			00090	**VT000906**	515	3,000–7,000
	RQC01708	11775™	10,000	00090	**VT000909**	515	variable
	RQC01778	11775™	80	00090	**VT000902**	515	50–80
	RQC02704	25922™	100	00013	**VT000133**	434	80–120
	RQC02708	25922™	100,000	00013	**VT000136**	434	3,000–7,000
	RQC11003	8739™	10,000	00012	**VT000122**	516	50–80
	RQC11008	8739™	10,000	00012	**VT000127**	516	50,000–150,000
Legionella pneumophila (serogroup 1)	RQC02008	—	100,000	00205	**VT002057**	8734	50,000–150,000
Legionella pneumophila	RQC04008	—	100,000	00107	**VT001077**	7109	50,000–150,000
Fluoribacer bozemanae	RQC39008	33217™	10,000	—	**VT072766**	7276	3,000–7,000

Strain Name	Old Cat. No.	ATCC*	CFU Median	WDCM	New Cat. No.	CECT*	CFU Range
Fluoribacer bozemanae	New product - no previous number available			—	VT072767	7276	50,000–150,000
Klebsiella pneumoniae	RQC02601	13883™	30	00097	VT000971	143	15–40
	RQC02607	13883™	1,000	00097	VT000975	143	600–1,400
Proteus hauseri	RQC03207	13315™	1,000	—	VT004835	484	600–1,400
Proteus mirabilis	RQC03407	25933™	1,000	—	VT000233	4168	80–120
	RQC03407	25933™	1,000	—	VT000237	4168	50,000–150,000
Pseudomonas aeruginosa	RQC01855	10145™	200	—	VT000244	110	130–270
	RQC01857 & RQC01859	10145™	variable	00024	VT000249	110	variable
	RQC03302	27853™	50	00025	VT001142	108	50–80
	RQC03304	27853™	100	00025	VT001143	108	80–120
	RQC03307	27853™	1,000	00025	VT001145	108	600–1,400
	RQC02201	9027™	30	00026	VT000261	111	15–40
	RQC12002	9027™	50	00026	VT000262	111	50–80
	RQC12003	9027™	80	00026	VT000262	111	50–80
	RQC02204	9027™	100	00026	VT000263	111	80–120
	RQC12005	9027™	200	00026	VT000264	111	130–270
	RQC12007	9027™	1,000	00026	VT000265	111	600–1,400
	RQC12008	9027™	–	00026	VT000266	111	3,000–7,000
	New product - no previous number available			00026	VT000267	111	50,000–150,000
Salmonella enterica subsp. Enterica serovar Typhimurium	RQC17002	14028™	50	00031	VT000312	4594	50–80
	RQC17003	14028™	80	00031	VT000313	4594	80–120
Salmonella enterica subsp. Enterica serovar Abony	RQC18003	—	80	00029	VT000292	545	50–80
Salmonella enterica subsp. Enterica serovar Enteridis	New product - no previous number available			—	VT000303	—	80–120
Staphylococcus aureus susp. Aureus	RQC13002	6538™	50	00032	VT000322	239	50–80
	RQC13003	6538™	80	00032	VT000322	239	50–80
	RQC13004	6538™	100	00032	VT000323	239	80–120
	RQC13005	6538™	200	00032	VT000324	239	130–270
	RQC13007	6538™	1,000	00032	VT000325	239	600–1,400
	RQC13008	6538™	—	00032	VT000326	239	3,000–7,000

* ATCC and catalog marks are trade marks of American Type Culture Collection, Manassas, Va., U.S.A

Simple Tools for a More Efficient Microbiological Testing Workflow in Food Safety Labs

By Diana Spitznagel, Global Product Manager Microbiology Release Testing — *diana.spitznagel@merckgroup.com*

Many workflow steps can be repetitive and tedious in the food safety testing workflow. The advancement of automation, which includes small and simple instruments like gravimetric dilutors and sample homogenizers, eases repetitive workflow steps greatly and reduces hands-on time.

Food testing laboratories have become more and more automated for various tasks, including the pathogen testing workflow. Instruments have the potential to change a food testing lab from outdated manual processes to an automated instrumentation process and, thereby, improve overall method performance. This can have a positive impact on the numbers for sample throughput and lab efficiency, reducing overall labor costs, which is an important factor in food testing laboratories.

Ultimately, the less time lab employees have to spend on manual lab processes, the more they are able to work on other, more important tasks. Increased employee satisfaction is another positive outcome since the burden of cumbersome and repetitive activities is removed, which could also help alleviate certain health risks such as shoulder pain. Another advantage of bringing automated solutions into testing labs is that method variability can be greatly reduced since the human error aspect has been eliminated.

A fully automated food testing laboratory is not yet a reality, but there are several workflow steps which can be easily automated and have been for many years with the use of simple bench-top instruments, which can be operated by moderately skilled staff. Two of these types of instruments include gravimetric dilutors and homogenizers and are used at the front-end of the testing workflow.

These small, bench-top instruments in the food testing sample preparation workflow simplify the sample handling enormously and speed up the overall workflow time with less hands-on time for the technicians. A gravimetric dilutor system is used to automatically dispense the required amount of diluent, which is standard enrichment media such as buffered peptone water, into the food sample located in the enrichment bag. This can be done in a sterile manner and with a very high level of precision. The required media is attached through a tubing system to the dispensing unit, which is operated through peristaltic pumps. The dilution system is less prone to dilution errors, and dispensing of the media is commenced by simply pressing two buttons. Weighing and dispensing happens automatically. This has a positive ergonomic impact on handling movements since repetitive motions with heavy media bottles are greatly reduced. Another advantage of these automated systems is the option to include digital features for easier traceability of each sample tested in the lab.

Did you know …

Microbiologists are seen as traditionalists?

Since microbiologists continue to work with plates and use methods developed by Robert Koch in the late 1800s, many people view them as traditionalists. They predict that in the future, classical methods will no longer be used. However, microbiologists and bacteria laugh at these experts.

Figure 1: Microbiologist 1981 (Source: CDC)

Gravimetric sample preparation is an accurate way of measuring the solid sample and the required diluent. The exact amount of substance dispensed using an automated dosing unit is recorded and used to accurately calculate the amount of liquid to weigh into the container. Automated liquid dispensing compensates for any inaccurate weighing by delivering the exact amount of diluent required to achieve the specified concentration. This guarantees accurate sample and solution preparation.

DiluCult™ and DiluCult 2 dilutor instruments are accurate gravimetric dilutors which are designed to facilitate work in a microbiological safety cabinet due to their low height. They are easy to clean and have a removable drip tray for better handling. The two versions differ in the maximum weight they can, which is limited to 3,000 g for DiluCult and 5,000 g for DiluCult 2. Thus, the DiluCult 2 can be used for large pooling samples. The media is dispensed through a peristaltic pump system and is accurately weighed to the level of concentration the user chooses on the front settings. After the media is dispensed, the sample can be further processed by a homogenizer; or, in the case of liquid or powdery food samples, the enrichment bag can be immediately placed into the incubator for further analysis.

Figure 2: Gravimetric Dilutor standard (left) and for pooling samples (right)

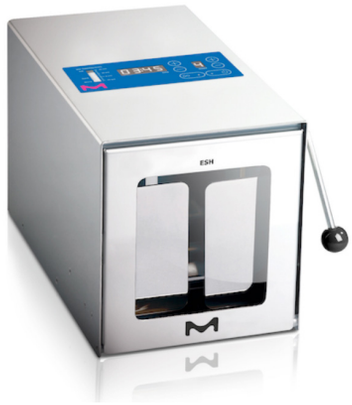
After diluting the sample with the appropriate media, the subsequent step is handled by a sample homogenizer. It blends the food sample in various volumes, depending on the size of the instrument, with repetitive massaging pedals in the sterile enrichment bag. The homogenizers process solid samples quickly and efficiently without compromising the sample or any potential bacteria present in the sample. Homogenizers have almost entirely replaced previously used lab blenders in food testing labs.

The bench-top Enrichment Sample Homogenizer (ESH) instrument is used for this preparation of solid sample. The instrument is used to mix the tested food sample with specific enrichment media for a chosen amount of time. Through the mechanical homogenization of the food sample, better access is given to potentially hidden bacteria. Depending on which sample is tested, the intensity of the paddling motion can be adjusted. These instruments are very robust and easy to use.

ESH Instrument Components:

- Multi-function digital display/control panel
- Variable speed
- Variable time
- Close-tight window door
- Adjustable blending power
- Side-by-side paddle stop
- Silent, brushless motor
- Security drip tray
- Removable autoclavable paddles, making it very easy to clean

Figure 3: Sample Homogenizer for solid food samples



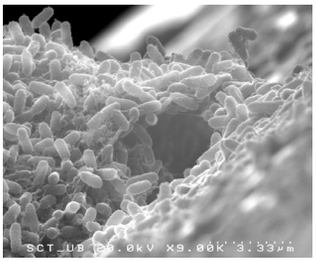
Order information

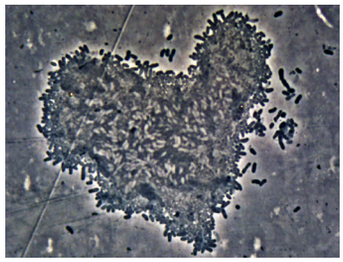
Cat. No.	Instrument	Description
5427650001	ESH	Enrichment Sample Homogenizer for solid food samples
5427530001	DiluCult™	Gravimetric Dilutor
5427600001	DiluCult™ 2	Gravimetric Dilutor for pooling samples

SIGMA-ALDRICH®

Win a Tablet! Microbiology Photo Competition

This photography competition is sponsored by Merck to encourage microbiologists to show something about their work and science. The best photographic entries will win nice prizes such as a tablet, Swiss army knife, USB stick and laser pointer. The images will appear in a future issue of Microbiology Focus, and the best one will be featured on the cover.





Rules of the Competition and Conditions of Entry

1. The competition is open to all applicants worldwide.
2. Entries should illustrate microorganisms (living or dead) or microbiologists in action at work.
3. Picture size should be at least 400 dpi and 90 × 120 mm (max. 5 MB). The file format must be in jpg, tiff or pdf.
4. The entries will be judged on:

   - clarity of presentation
   - composition
   - illumination and contrast
   - congruency of subject matter and title of photograph
   - scientific interest and relevance
   - originality

5. Winning entries will be retained by Merck, who will have sole rights of publication, reproduction and display.
6. Closing date is January 31, 2017.
7. Entries submitted after the closing date will not be considered. Entries received incomplete, illegible, mutilated, altered or not complying exactly with the instructions and theme may be disqualified.
8. Decisions of the judges in all matters affecting the competition will be final and legally binding.

The competition will be judged by:

Dr. Lars Fieseler
Zurich University of Applied Sciences - ZHAW
Supervisor, Department of Microbiology

Prof. Mohammad Manafi
Medical University of Vienna
Head of Department for Food Hygiene

Jvo Siegrist
Sigma-Aldrich
Product Manager, Sigma-Aldrich Microbiology

Method of Entry

There is no entry fee, but an entry form must be completed for each entry (maximum of three entries per submitter).

For more information on this competition, visit
sigma-aldrich/mibi-competition

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