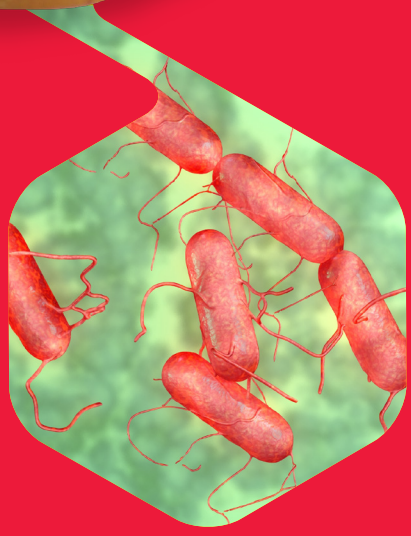
Volume 9.1, 2017

Microbiology focus

Detection of Sublethally Damaged *Salmonella*



Content

Detection Of Damaged *Salmonella* Cells

By Jvo Siegrist, Product Manager S-A Microbiology

Ivo.siegrist@sial.com

Resuscitation and improved growth of *Salmonella* cells by the addition of the hydroxamate siderophore ferrioxamine E.

Salmonella are Gram-negative, rod-shaped bacteria consisting of the two species *Salmonella enterica* and *Salmonella bongori*. *Salmonella enterica* is further divided into six subspecies - *Salmonella enterica*, *Salmonella salamae*, *Salmonella arizonae*, *Salmonella diarizonae*, *Salmonella houtenae* and *Salmonella indica*. Salmonella is one of the most significant foodborne pathogens for both humans and animals.

Sometimes *Salmonella* appears in unexplainable places, even among food or raw materials which were tested according to ISO standards. Often these are food and raw materials which were dried, heat treated, set under high osmotic pressure or came into contact with inhibiting chemicals. Examinations have shown that one reason this happens is that some *Salmonella* cells are in the state of "**viable but nonculturable**" (VNC) bacteria. That means they do not grow in normal standard media within the usual incubation time. In the case of Salmonella, we talk about injured cells which are not immediately culturable and the standard media are often not sufficient for the recovery. According to the latest VNC definition, VNC cells are regarded as viable and potentially replicative, but the methods required for resuscitation are beyond our current knowledge.

VNC cells are sensitive or sublethally damaged, which can mean the loss of some ribosomes, damaged enzymes, damaged cell membranes and other problems causing malfunctions in cells. The determination of such cells is done by direct epifluorescent filter technique (DEFT) by staining the double stranded DNA by DAPI or staining by acridin orange, which intercalates to RNA and results in red-orange fluorescence while the intercalation to DNA shows a green fluorescence. These stains are used in combination with an unspecific enzyme activity detection assay, for example the hydrolysis of CTC (5-Cyano-2,3-ditolyl tetrazolium chloride; red fluorescence) or reduction of INT (2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyl tetrazolium chloride; red coloration) to demonstrate metabolic activity.[1-2]

For *Salmonella*, it has recently been shown that supplementing the pre-enrichment and enrichment broths with an iron complex, called ferrioxamine E, significantly improves the recovery of *Salmonella* from artificially or naturally contaminated foods.[3-4] A concentration of 75 ng/mL **ferrioxamine E** (**Cat. No. 38266**) or **Peptone Water, phosphate-buffered with Ferrioxamine E (Cat. No. 67331)** improves the recovery rate and supports growth. Ferrioxamine E provides the essential micro-nutrient iron (III) to the organisms. This leads to a reduced lag-phase in the medium and reactivates damaged bacteria. The motility of *Salmonella* is also improved, which helps to improve the identification by semisolid selective motility media like MRSV, DIASSALM or SMS. Ferrioxamine E is recommended when isolating small quantities of cells from dried powders like tea, spices, dried fruits, etc., as a modified pre-enrichment medium in the ISO 6579:2002 workflow. It is also used to improve the pre-enrichment of *Salmonella* by shortening the incubation time even in highly competitive flora (semi-selective enhancement of growth).[5]

Did you know ...

that the viable but non-culturable state was discovered and defined in 1982?

Xu and coworkers introduced the term "viable but nonculturable bacterial cells" to distinguish particular cells that could not form colonies on solid media but retained metabolic activity and the ability to elongate after the administration of nutrients. Since then many bacterial species have been found to exist in such a state.

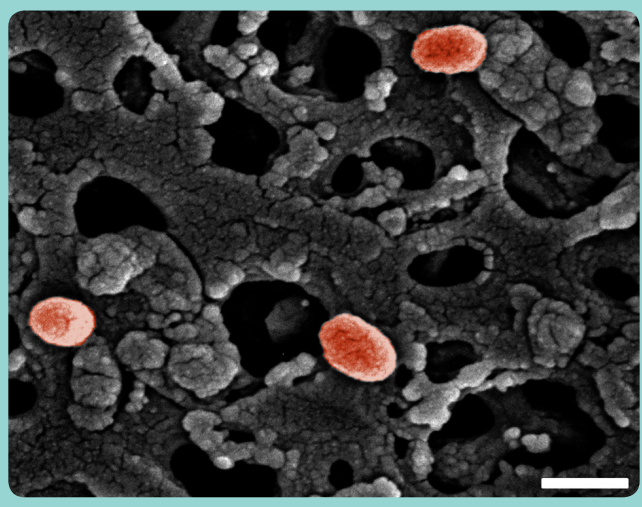


Figure 1. *Aeromonas hydrophila* showing coccoid cells instead of rods, lost its culturability (Source: Prof. José Ramos Vivas, IDIVAL Research Institute)

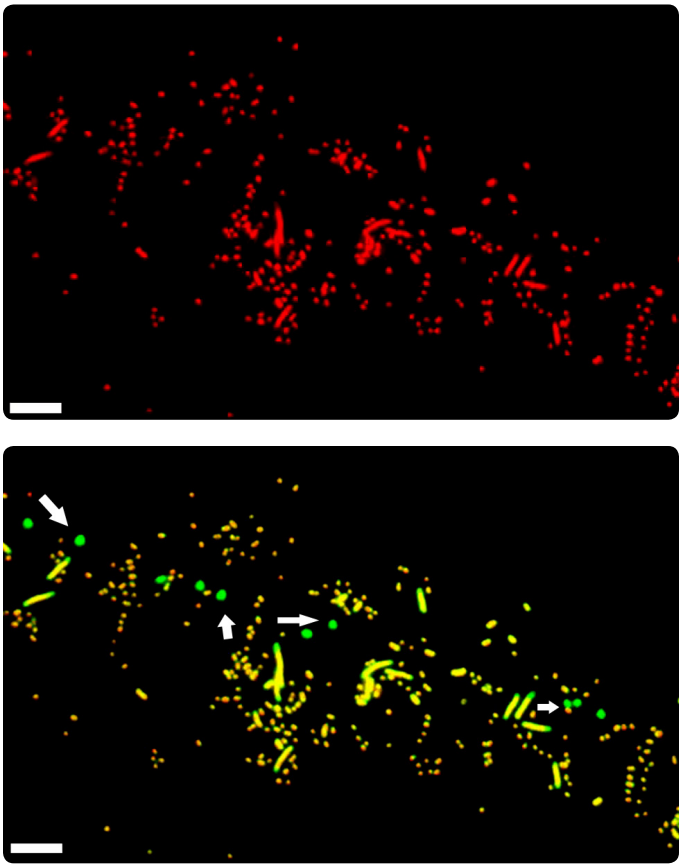


Figure 2. Example of a live/dead cell staining of nonculturable bacteria (genus Acinetobacter). The dead bacteria (upper panel) appear red (propidium iodid). The lower panel is a merged fluorescence image: in green (SYTO 9) are the live bacteria with coccoid instead of rod shaped forms and the dead bacteria are yellow. The arrows indicate some VBNC bacteria. (Source: Prof. José Ramos Vivas, IDIVAL Research Institute)

References:

1. Roszak, D. B., and Colwell, R. R. Survival Strategies of Bacteria in the Natural Environment, Microbiological Reviews, 1987, 365-379.
2. Oliver, J. D.The Viable but Nonculturable State in Bacteria, The Journal of Microbiology, 2005, 93 -100.
3. Barcina, I., Lebaron, P., Vives-Rego, J. Survival of Allochthonous Bacteria in Aquatic Systems: A Biological Approach, FEMS Microbiol. Ecol., 1997, 23:1–9.
4. Cho, J. C., Kim, S. J. Viable, but Non-culturable, State of a Green Fluorescence Protein-tagged Environmental Isolate of *Salmonella typhi* in Groundwater and Pond Water, FEMS Microbiol. Letters, 1999,170:257–264.
5. Reissbrodt, R. et al., Ferrioxamine E-supplemented Pre-enrichment and Enrichment Media Improve Various Isolation Methods for Salmonella., Int. J. Food Microbiol., 1996, 81-91.

Ordering Information

Product	Cat No.
Ferrioxamine E	**38266**
Peptone Water, phosphate-buffered with Ferrioxamine E	**67331**

Table 1. Ferrioxamine E products

Great Reliability, Superior Wine - Ez-Fluo™ for Rapid Spoilage Detection in Beverages

By Juan Martin Oteiza, Laboratorio de Microbiologia de Alimentos, Argentina

juano@ciati.com.ar

It only takes a single microorganism to cause spoilage in beverage production, which can potentially lead to product recall and brand damage. EZ-Fluo™ assay can shorten the detection time for contaminants and increase safety.

Many beverage manufacturing processes are susceptible to spoilage organisms like yeast or bacteria contamination. Contamination can alter the scent, flavor or turbidity of a beverage, resulting in customer dissatisfaction and, in some cases, in product recall. For these microorganisms, traditional monitoring methods require up to 10 days to obtain microbiological results to allow the release of the product. A rapid microbiology system that can detect potential contamination 3 times faster than traditional monitoring methods would result in significant cost savings and preserve company reputation. The EZ-Fluo™ System uses fluorescence-based technology and is a convenient and sensitive platform for the quantitative detection of contaminants in filterable samples. This rapid microbiological method is based on a universal enzymatic fluorescent staining of viable and culturable microorganisms. The fluorescent staining procedure is non-destructive, allowing microorganism identification following a positive result. The EZ-Fluo™ system offers a fast and reliable alternative for the rapid detection of spoilage microorganisms in wines. This evaluation study, performed by the accreditated lab Centro de Investigación y Asistencia Técnica a la industria (CIATI AC) in Argentina, shows that the system enables a faster response and corrective action when used during the wine manufacturing process. It improves process control, product yield and a faster release of final product to market.

Figure 1. EZ™-Fluo test equipment

Principle of Detection

The principle of the fluorescence detection is based on an enzymatic reaction. The fluorogenic substrate used is a non–fluorescent viability marker which is cleaved by non-specific ubiquitous intracellular enzymes resulting in a fluorescent product. Natural amplification of fluorescence by accumulation inside cells is an indicator of microbial metabolism. The dye is diluted in a staining buffer allowing cell membrane permeability and thus dye introduction into cells.

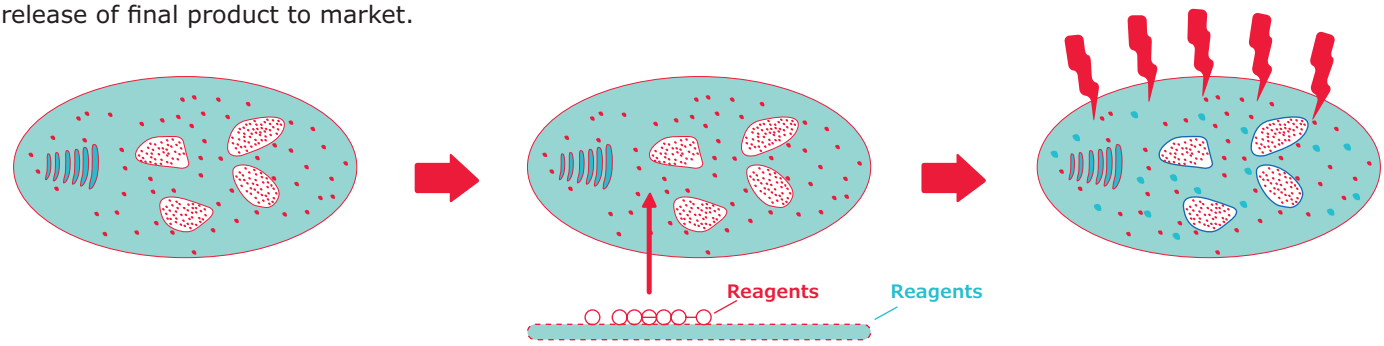


Figure 2. EZ™-Fluo Test Principle

Note: Fluorescence detection is a non-destructive method that enables the microorganisms to continue to grow after they have been stained in order to identify them using standard ID technology.

Protocol for Rapid Detection

The procedure used was a standard protocol to detect spoilage microorganisms in samples of interest with fluorescence detection:

- A filtration unit is installed onto the filtration system
- The appropriate volume of sample is poured into the filtration unit
- After filtration, the membrane is disconnected from the device and aseptically transferred onto a media cassette
- The incubation is performed according to the specifications
- After the incubation, the membrane is stained with the fluorogenic reagent for 30 min at 32.5 °C (± 2.5)
- The fluorescent micro-colonies are counted using the fluorescence reader
- After detection, the stained membrane can be re-incubated on fresh media for traditional plate count and identification if required.

Definition of a Rapid Incubation Time

An appropriate incubation time is defined as the minimal time which allows a percentage of recovery above 70% compared to the traditional method. The calculation is based on both formulas:

- The fluorescence recovery is the fluorescent dot count compared to the traditional method count.

$$\text{Fluorescence recovery (\%)} = \frac{\text{Average of fluorescence counts}}{\text{Average of traditional method count}} \times 100$$

- The viability recovery is the colony count on stained membranes after re-incubation compared to the traditional method count

$$\text{Viability recovery (\%)} = \frac{\text{Average of Colony-Forming Unit counts after re-incubation}}{\text{Average of traditional method counts}} \times 100$$

An optimal incubation time should allow a sufficient fluorescent signal intensity and fluorescence and viability recoveries above 70%.

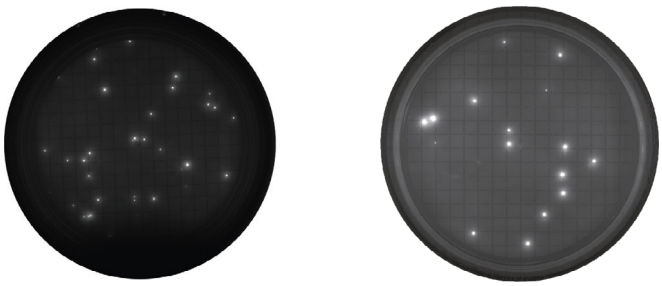


Figure 3. The picture on the right illustrates a sufficient fluorescent signal intensity translating to an appropriate incubation time. The picture on the left shows that an accurate count is not possible if the intensity of fluorescence is too low due to an insufficient incubation time.

CIATI AC Evaluation Results

A series of tests on red wine were carried out to determine the performance of the rapid count system compared to traditional microbiological analysis, as established by the OIV and wine industry. Results in the tables are average counts of the performed tests.

1. *Brettanomyces spp.* counts

Counts of the test yeast were performed using both the traditional method (filtration through a 0.45 µm membrane, and incubation on *Brettanomyces* agar at 25 °C (±2) for 10 days under aerobic conditions) and the EZ-Fluo™ rapid system. The results were read at several times during incubation.

a. Inoculated samples

Three (n = 3) 500 mL samples of red wine were inoculated with different concentrations (N_0) of a *Brettanomyces spp.* strain previously isolated and characterized in CIATI AC microbiology laboratory.

Results

Sample Details	Days of incubation (25 °C)	Traditional microbiological test	EZ-Fluo™ System (CFU/10 mL)
Sample 1, estimated N_0 = 3 CFU/10 mL	3	<1	<1
	5	<1	3
	7	2	2
	10	3	4
Sample 2, estimated N_0 = 30 CFU/10 mL	3	<1	6
	5	<1	28
	7	13	33
	10	29	30
Sample 3, estimated N_0 = 30 X 10^2 CFU/ 10 mL	3	<1	52
	5	<1	295
	7	252	283
	10	304	307

b. Naturally contaminated samples

Five (n = 5) samples of red wine naturally contaminated with *Brettanomyces spp* were analyzed. Counts were performed using both the traditional method (filtration through a 0.45 µm membrane, and incubation on Brettanomyces agar at 25 °C (± 2) for 10 days) and the EZ-Fluo™ rapid system (with incubation at 25°C (± 2) for 5 days).

Results

Samples	Days of incubation (25 °C)	Traditional microbiological test	EZ-Fluo™ System (CFU/10 mL)
1	49	45	0
2	570	601	0
3	1	2	0
4	2	1	0
5	55	37	0

2. Lactic acid bacteria (LAB) counts

a. Inoculated samples

Three (n = 3) 500 mL samples of red wine were inoculated with different concentrations (N_0) of an *Oenococcus oeni* strain previously isolated and characterized at the CIATI AC. Counts were performed using both the traditional method (filtration through a 0.45 µm membrane, and incubation on MRS agar + tomato juice at 30°C (± 2) for 10 days under aerobic conditions) and the EZ-Fluo™ rapid system. The results were read at several times during incubation.

Results

Sample Details	Days of incubation (25 °C)	Traditional microbiological test	EZ-Fluo™ System (CFU/10 mL)
Sample 1, estimated N_0 = 3 CFU/10 mL	3	<1	2
	5	1	3
	7	2	2
	10	4	5
Sample 2, estimated N_0 = 30 CFU/10 mL	3	<1	14
	5	3	21
	7	23	30
	10	36	31
Sample 3, estimated N_0 = 30 X 10^2 CFU/ 10 mL	3	<1	42
	5	17	310
	7	254	303
	10	311	320

b. Naturally contaminated samples

Five (n = 5) samples of red wine naturally contaminated with lactic acid bacteria were analyzed. Counts were performed using both the traditional method (filtration through a 0.45 µm membrane, and incubation on MRS agar + tomato juice at 30 °C (± 2) for 10 days) and the EZ-Fluo™ rapid system (with incubation at 25 °C (± 2) for 5 days).

Results

Samples	Days of incubation (25°C)	Traditional microbiological test	EZ-Fluo™ System (CFU/10mL)
1	144	152	9
2	3	2	0
3	623	489	21
4	105	97	6
5	18	22	0

3. Acetic acid bacteria (AAB) counts

a. Inoculated samples

Three (n = 3) 500 mL samples of red wine were inoculated with different concentrations (N_0) of an *Acetobacter spp*. strain previously isolated and counts were performed using both the traditional method (filtration through a 0.45 µm membrane, and incubation on Carr agar at 25 °C (± 2) for 4 days under aerobic conditions) and the EZ-Fluo™ rapid system. The results were read at several times during incubation and characterized at the CIATI AC.

Results

Sample Details	Days of incubation (25°C)	Traditional microbiological test	EZ-Fluo™ System (CFU/10mL)
Sample 1, estimated N_0 = 3 CFU/10 mL	1	<1	<1
	2	<1	3
	3	2	4
	4	5	2
Sample 2, estimated N_0 = 30 CFU/10 mL	1	<1	<1
	2	<1	17
	3	11	25
	4	27	22
Sample 3, estimated N_0 = 30 X 10^2 CFU/ 10 mL	1	<1	<1
	2	<1	252
	3	199	277
	4	246	280

b. Naturally contaminated samples

Five (n = 5) samples of red wine naturally contaminated with acetic acid bacteria were analyzed. Counts were performed using both the traditional method (filtration through a 0.45 µm membrane, and incubation on Carr agar at 25 °C (± 2) for 4 days) and the EZ-Fluo™ rapid system (with incubation at 25°C (± 2) for 2 days).

Results

Samples	Days of incubation (25 °C)	Traditional microbiological test	EZ-Fluo™ System (CFU/10 mL)
1	6	9	0
2	70	101	9
3	26	489	3
4	12	97	1
5	137	22	11

4. Yeast Counts

a. Inoculated samples

Three (n = 3) 500 mL samples of red wine were inoculated with different concentrations (N_0) of a *Saccharomyces cerevisiae* strain previously isolated and characterized at the CIATI AC. Counts were performed using both the traditional method (filtration through a 0.45 µm membrane, and incubation on YEPD agar at 25 °C (± 2) for 4 days under aerobic conditions) and the EZ-Fluo™ rapid system. The results were read at several times during incubation.

Results

Sample Details	Days of incubation (25 °C)	Traditional microbiological test	EZ-Fluo™ System (CFU/10 mL)
Sample 1, estimated N_0 = 3 CFU/10 mL	1	<1	<1
	2	<1	2
	3	2	3
	4	3	2
Sample 2, estimated N_0 = 30 CFU/10 mL	1	<1	<1
	2	<1	32
	3	18	35
	4	33	29
Sample 3, estimated N_0 = 30 X 10^2 CFU/ 10 mL	1	<1	<1
	2	<1	289
	3	125	307
	4	296	301

b. Naturally contaminated samples

Five (n = 5) samples of red wine naturally contaminated with yeasts were analyzed. Counts were performed using both the traditional method (filtration through a 0.45 µm membrane, and incubation on YEPD agar at 25 °C (± 2) for 4 days) and the EZ-Fluo™ rapid system (with incubation at 25 °C (± 2) for 2 days).

Results

Samples	Days of incubation (25 °C)	Traditional microbiological test	EZ-Fluo™ System (CFU/10 mL)
1	28	24	7
2	1.97 x 103	2.33 x 103	46
3	115	175	15
4	42	36	9
5	5	3	0

Main Outcomes of the CIATI AC Evaluation Study

- According to the results obtained, the EZ-Fluo™ rapid system performed very well in obtaining counts of wine spoilage microorganisms, since it reduced the incubation time by at least 50% in every case analyzed, in comparison to that required for traditional methods (OIV).
- The EZ-Fluo™ rapid system enabled the detection and quantification of *Brettanomyces spp.* and lactic acid bacteria (LAB) in as little as 5 days of incubation, and of acetic acid bacteria (AAB) and yeasts in as little as 2 days of incubation.
- The EZ-Fluo™ system is a useful tool for the rapid (and non-destructive) detection and quantification of wine spoilage microorganisms.

Conclusion

Using the fluorescence-based technology as a microbiology quality control tool dramatically reduces the time needed to detect yeast and bacterial contamination in wine. This study demonstrated that this technology could easily replace the compendial microbiological method with a 2 to 4 times faster time to result, and a full compatibility with the standard culture media traditionally used for the detection of spoilage organisms in beverages. Moreover, as the method is non-destructive, each fluorescent micro-colony detected will continue to grow to yield visible colonies allowing the identification of the contaminants using available identification methods. With the EZ-Fluo™ system, beverage manufacturers can improve their quality control by detecting contamination earlier, and implementing corrective actions more quickly. This early identification creates savings of raw materials and manufacturing capacities. It can also help identify the root cause of a process failure, providing a clearer understanding and confidence in the process and increased quality control. Most importantly, the product release can be accelerated and storage time decreased, resulting in financial savings.

For more information, visit
merckmillipore.com/EZ-Fluo

Were You Aware That

By Regina Petrasch,
Head of Application Development Team

regina.petrasch@merckgroup.com

By Andreas Bubert,
Senior Global Product Manager Culture Media

andreas.bubert@merckgroup.com

EN ISO 11290-1:2014 Allows A Maximum Of 72 Hours Cold Room Step Before Transfer To Fraser Broth And That The Readybag® Half-Fraser Composition Is Acc. To ISO 11290?

Outbreaks of Listeria infections are mainly associated with contaminated foods such as seafood, milk products, meat and vegetables in which these bacteria can multiply even at low temperatures.[1] According to EN ISO 11290-1:2014 for detection of *Listeria monocytogenes* in food and animal feed, the primary enrichment (half-Fraser broth) incubation time is 24 h ± 2 h and can be refrigerated before transfer to Fraser broth for a maximum of 72 h. The cold room step allows flexibility of lab work activities.

Readybag® Half FRASER (Demi FRASER) broth with supplements acc. ISO 11290, irradiated, is a granulated culture medium, but provided in prefilled, gamma irradiated pouches of 12.5 g or 62.0 g for testing of 25 gr and 125 gr samples, respectively. For the Readybag® pouches the need for upfront media preparation is eliminated and no supplement handling is necessary. The food testing routine is simplified because only sterile water is added before use, thus avoiding the time-consuming autoclaving step.

To evaluate a maximum of 72 h cold room storage and the influence of gamma-irradiation, we have commissioned a study by the Institute of Veterinary Food Science – Department of Veterinary Medicine, Justus Liebig University of Giessen, Germany. The study included growth promotion tests and food trials. GranuCult™ Half FRASER (Demi FRASER) broth acc. ISO 11290 was used as a reference of a non-gamma-irradiated dehydrated culture media.

Method

Test strain	
Listeria monocytogenes WDCM 00020	Official culture collection test strain
Listeria monocytogenes WDCM 00021	Official culture collection test strain; included in performance testing culture media according to ISO/DIS 11290-1:2014
Listeria monocytogenes	Food isolate No. 1
Listeria monocytogenes	Food isolate No. 2

Table 1. Test strains

Food Trials Listeria monocytogenes

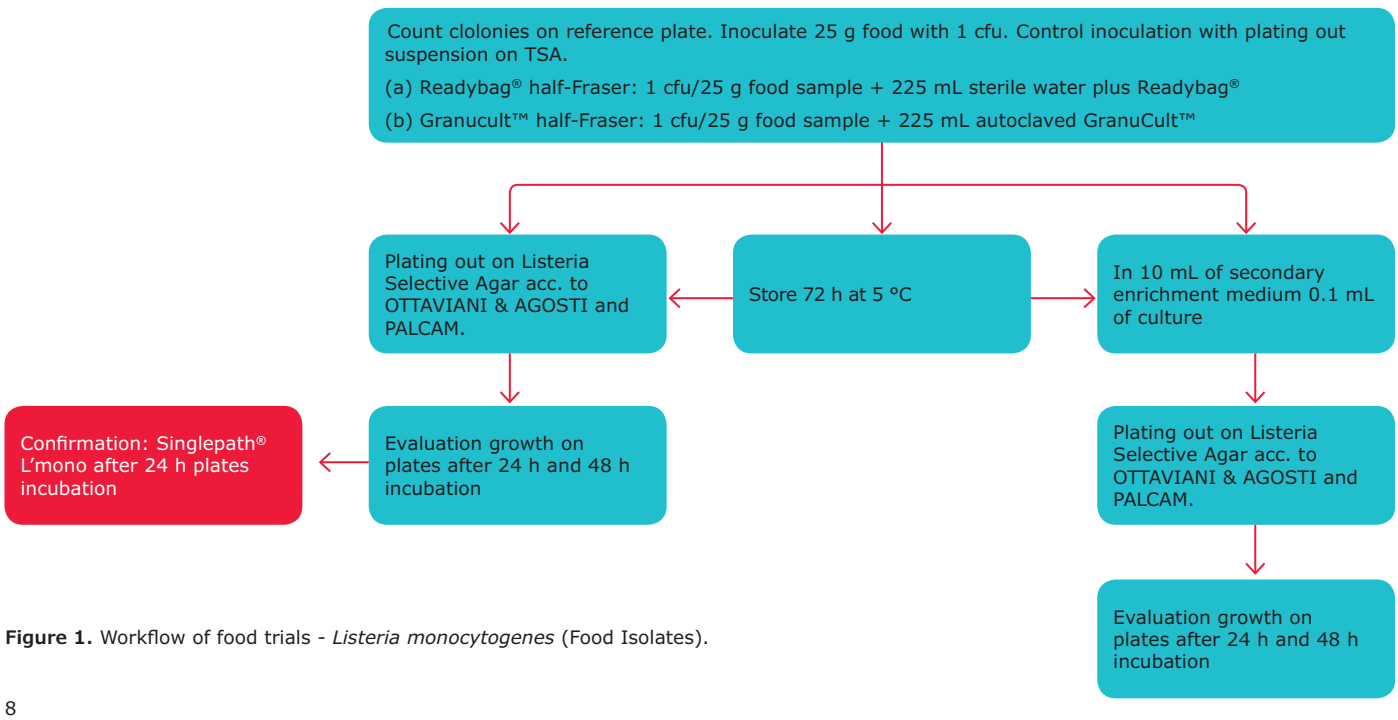


Figure 1. Workflow of food trials - *Listeria monocytogenes* (Food Isolates).

For the growth promotion test, the shortest (22 h) and longest (26 h) incubation times were selected to evaluate the final concentration of colony forming units (cfu) per sample. In addition, bacterial counts (cfu/ml) were calculated after cold storage. Growth promotion data were collected for all four test strains and repeated 10 times (inoculation level was 10 cfu).

The initial inoculation level (cfu per sample) was determined by plating out on Tryptic Soy Agar (TSA).

Readybag® and GranuCult™ half-Fraser media were inoculated with the same suspension to ensure comparability and the tests were performed in parallel.

Cooked prawns and cream cheese were selected as food matrices to evaluate growth promotion with matrix. The cold room storage step of 72 h was also included in the food test procedure.

For comparison, inoculation and detection of *Listeria monocytogenes* in one food matrix was performed in parallel with Readybag® and GranuCult™ culture media using the same bacterial suspension. A total of 20 food samples were inoculated (10 samples per week).

Singlepath® L 'mono, a rapid immunochromatographic assay specific for *Listeria monocytogenes*, was used for confirmation after the first enrichment. This step was done once per sample and plating medium after 24 h agar plate incubation. Test performance and interpretation were carried out as described in the package insert[2] of Singlepath® L 'mono.

All food samples were not naturally contaminated.

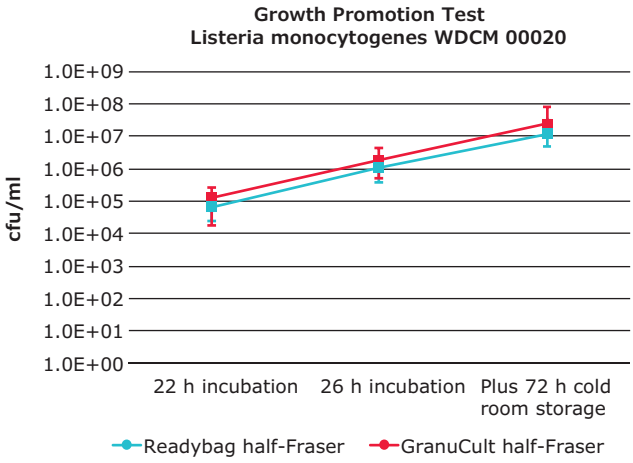


Figure 2. Colony forming units after 22, 26 h incubation and 72 h cold room storage of *Listeria monocytogenes* WDCM 00020.

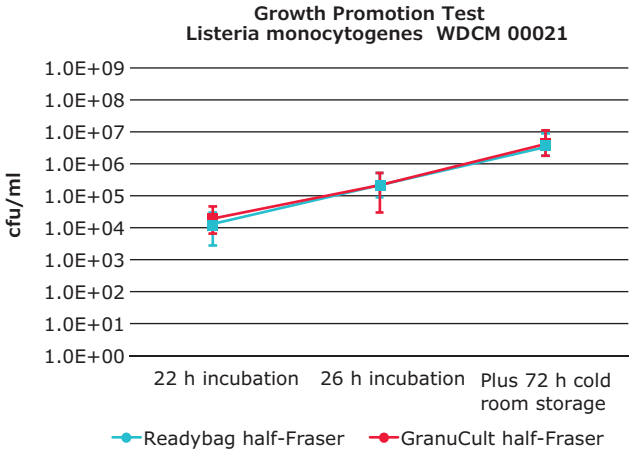


Figure 3. Colony forming units after 22, 26 h incubation plus 72 h cold room storage of *Listeria monocytogenes* WDCM 00021.

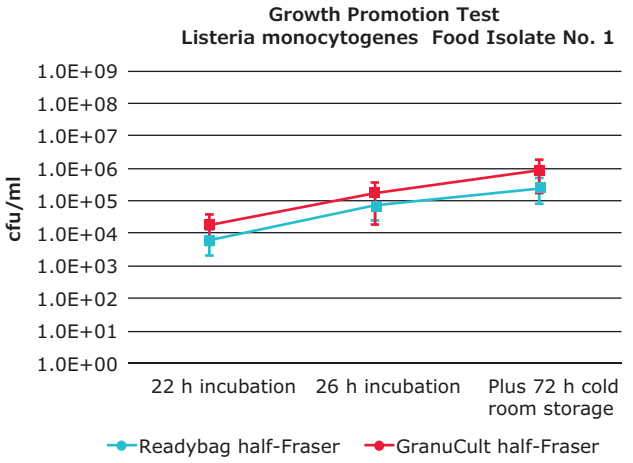


Figure 4. Colony forming units after 22, 26 h incubation plus 72 h cold room storage of *Listeria monocytogenes*. Food Isolate No. 1.

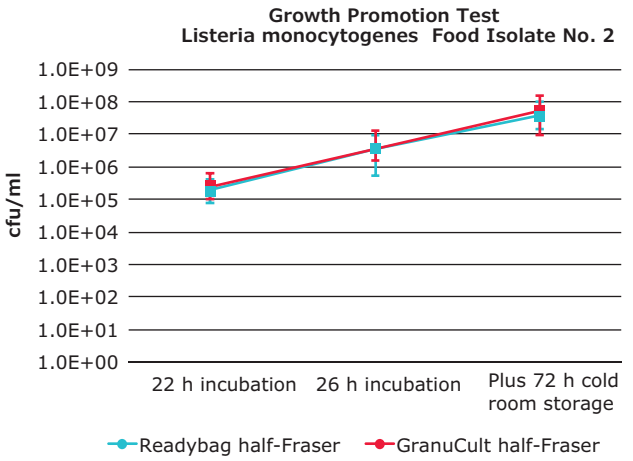


Figure 5. Colony forming units after 22, 26 h incubation plus 72 h cold room storage of *Listeria monocytogenes*. Food Isolate No. 2.

Results

The results of the growth promotion tests are shown in Figures 3 to 6. Growth curves are based on the median values of 10 results per test strain and sampling time point. The 25th percentile (lower quartile) and 75th percentile (upper quartile) are marked.

Enrichment of the target bacteria was efficient with all samples tested. The slope of the curves between 22 h and 26 h incubation is similar, which indicates that the rate of growth is equal. The 25th and 75th percentile of both growth curves overlay in most cases.

The growth promotion test confirmed that half-Fraser can be refrigerated before transfer to the second enrichment as a reduction of growth was not detected. In all samples an increase of bacterial growth was measurable.

The results of the food sample testing (n=20) are summarized in Tables 2 and 3. The Tables show the positive and negative results of culture media evaluation after 24 h plate incubation (ALOA and PALCAM) and the sensitivity of Singlepath® L 'mono which correctly indicated the presence of Listeria monocytogenes in all positive samples.

The detection rate of positive and negative samples is in line with those obtained from the secondary enrichment and the cold room storage.

Food Matrix: Cooked Prawns	Positive	Negative	Total Sample	Sensitivity Singlepath® L 'mono
Readybag® half-Fraser	10	10	20	100%
GranuCult™ half-Fraser	12	8	20	100%

Table 2. Evaluation of half-Fraser and Singlepath® L 'mono. Food Matrix is cooked prawns with *Listeria monocytogenes.* Food Isolate No. 1.

Food Matrix: Cooked Prawns	Positive	Negative	Total Sample	Sensitivity Singlepath® L 'mono
Readybag® half-Fraser	11	9	20	100%
GranuCult™ half-Fraser	10	10	20	100%

Table 3. Evaluation of half-Fraser and Singlepath® L 'mono. Food Matrix is cream cheese with *Listeria monocytogenes.* Food Isolate No 2.

The food samples were inoculated with 1 colony forming unit and approximately 50% of test samples were positive. Comparison of the frequency of positive samples using the Fisher exact test gives a P-value for both of 0.75. Therefore the observed difference in number of positive samples is not statistically significant.

Interpretation

The currently revised (ISO EN ISO/DIS 11290-1:2014) version now also includes the opportunity for cold storage of the primary enrichment for up to 3 days before further processing. This external evaluation study investigated the potential impact of gamma irradiation on growth promotion by using four different *Listeria monocytogenes* strains including two food isolates and a cold room storage step of 72 h.

After 22 h and even after 26 h of incubation, both products show a similar rate of growth (Figure 2-5). The slope of growth promotion curves is equal. The study revealed no detrimental effects on the growth promotion for *Listeria monocytogenes*. However, the cfu/mL slightly increased as Listeria are known to grow at low temperatures (psychotropic).

The food trials using cooked prawns and cream cheese at a low level spiking of 1 cfu/25 g also showed comparable results considering the additional storage period of 3 days according to EN ISO/DIS 11290-1:2014. This also included a confirmation step with Singlepath® L 'mono.

Product Name	Cat. No.
Readybag® Half FRASER (Demi FRASER) broth with supplements acc. ISO 11290, 12,5 g, irradiated	**1024490060**
FRASER broth (base) acc. ISO 11290 GranuCult™	**1103980500**
FRASER Listeria Selective Supplement	**1000930010**
FRASER Listeria Ammonium iron(III) Supplement	**1000920010**
Singlepath® L 'mono	**1041480001**
GranuCult™ Tryptic Soy Agar acc. EP, USP, JP, ISO and FDA-BAM	**1054580500**
GranuCult™ BHI (Brain Heart Infusion) broth acc. ISO 6888	**1104930500**
GranuCult™ Sodium chloride peptone broth (buffered) acc. EP, USP, JP	**1105820500**
Listeria Selective Agar acc to OTTAVIANI and AGOSTI acc ISO 11290 ReadyPlate™ CHROM	**1461860020**
PALCAM Listeria-Selective Agar (Base)	**1117550500**
PALCAM Listeria Selective-Supplement acc. to van Netten et al.	**1121220010**

Table 4. Culture media and supplements used for growth promotion test and food trial from Merck KGaA, Darmstadt, Germany and Merck Life Science GmbH, Eppelheim, Germany.

Ordering Information

Product	Pack size	Other pack sizes available	Cat. No.
ReadyTube™ 10 Fraser ISO 11290	20 x 10 ml	100 x 10 ml	**1.46208.0020**
GranuCult™ Half FRASER (Demi FRASER) Broth (Base) with Antibiotics acc. ISO 11290	500 g		**1.00025.0500**
Readybag® Half FRASER (Demi FRASER) Broth with supplements acc. ISO 11290, 62 g, irradiated	35 bags		**1.01865.0001**
ReadyTube™ 2000 Half Fraser ISO 11290	2000 ml bag	6 x 225 ml	**1.46646.0001**
Chromocult® Listeria Agar (Base) acc. OTTAVIANI and AGOSTI acc. ISO 11290	500 g		**1.00427.0500**
Chromocult® Listeria Agar Enrichment Supplement	10 x1 vial		**1.00439.0010**
Chromocult® Listeria Agar Selective Supplement	10 x 1 vial		**1.00432.0010**
Oxford-Listeria-Selective Agar (Base)	500 g		**1.07004.0500**
Oxford-Listeria-Selective Supplement	10 x 1 vial		**1.07006.0010**
Oxford Listeria Selective Agar	20 pcs		**1.46328.0020**
PALCAM Listeria-Selective Agar (Base) acc. to VAN NETTEN et al.	500 g		**1.11755.0500**
PALCAM Listeria Selective-Supplement acc to van Netten et al	10 x 1 vial		**1.12122.0010**
PALCAM Listeria Selective Agar	20 pcs		**1.46329.0020**
L-PALCAM-Listeria Selective Enrichment Broth (Base) acc to van Netten et al.	500 g		**1.10823.0500**
ReadyTube™ 9 BPW ISO 6579, 6887, 21528	20 x 9 ml	100 x 9 ml, 6 x 225 ml, 6 x 1000 ml, 1 x 2000 ml	**1.46142.0020**

Table 5. Further culture media and supplements available from Merck KGaA, Darmstadt, Germany and Merck Life Science GmbH, Eppelheim, Germany for testing of Listeria from food and animal feeding stuffs

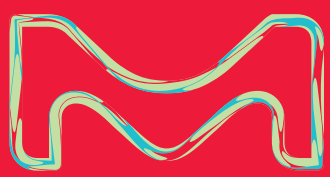
References:

1. Schuchat A., Swaminathan B., Broome C., Epidemiology of human listeriosis, Clin. Microbiol. Rev. 4 (1991) 169-183
2. Singlepath® L `mono (Cat. No. 1.04148) – introduction for use - GLISA-Rapid Test (Gold Labelled ImmunoSorbent Assay) for the qualitative detection and confirmation of Listeria monocytogenes in food and environmental samples
3. ISO International Standardisation Organisation: Microbiology of food and animal feeding stuffs -- Horizontal method for the detection and enumeration of Listeria monocytogenes - Part 1: Detection method - Amendment 1: Modification of the isolation media and the haemolysis test, and inclusion of precision data. EN ISO 11290-1:1996 + Amd 1:2004.
4. ISO International Standardisation Organisation: Microbiology of the food chain -- Horizontal method for the detection and enumeration of Listeria monocytogenes and other Listeria spp. - Part 1: Detection method. EN ISO/DIS 11290:2014.

For more information, visit
merckmillipore.com/readybag

Merck KGaA
Frankfurter Strasse 250
64293 Darmstadt
Germany
Tel.: +49 (0) 6151 72-0
Fax: +49 (0) 6151 72-2000

Merck.com



Lit. No. SJJ Ver. 1.0
sh1212
01/2017