ARTICLE

EVALUATION OF THE SOLERIS METHOD FOR STERILITY TESTING OF UHT MILK

The resazurin test is routinely used to verify UHT milk sterility in many industrial laboratories. After pre-incubation of the milk for 2 to 5 days at 30 °C, this test reveals the bacterial reductase activity. The official method (European directive 92/46) is a total plate count following sample incubation for 15 days at 30 °C, which therefore cannot be routinely used. The Soleris method, which detects microbial growth after a pre-incubation of UHT milk for 2 to 3 days at 30 °C, could allow quicker release of negative batches. In this study, we evaluated the Soleris method in parallel with the resazurin test and the official method.

Neogen, founded in the USA in 1982, has grown internationally, with its European headquarters located in Scotland. Neogen develops and markets products dedicated to food and animal safety. The company's Food Safety Division markets dehydrated culture media, and diagnostic test kits to detect foodborne bacteria, natural toxins, genetic modifications, food allergens, drug residues, plant diseases and sanitation concerns. These kits focus on topical concerns about the quality and safety of food and agricultural products, from the quality of seed that goes into the ground, right through the chain to the safety of fully processed food products.

Soleris is a rapid system for the accurate detection and enumeration of a variety of microorganisms across a spectrum of sample types – including food and beverages. Its operating principle is as follows:
A vial with a specific microbial growth medium for respective microorganisms or products is inoculated and placed in an incubator connected to an automated reader system. Optical sensors continuously monitor the microbial growth in the vial. The growth curve obtained can be visualised and edited. A maximum of 512 samples can be tested simultaneously, using four instruments connected to a single computer.

For UHT testing in dairy products the "NF UHT medium" vial containing a trypticase soy agar is used. This medium is inoculated with UHT milk that has undergone pre-incubation at 30 °C, and then placed into the instrument set at 30°C. The vial's agar plug contains a coloured indicator that changes from green to yellow when CO_2 is released during microorganism growth. CO_2 release is regularly measured and then interpreted by the Soleris software that allows growth to be directly expressed as a curve, and gives a positive result as soon as the detection threshold (DT) is reached. The method aims to detect contamination by one cell (1 CFU) in a milk bottle.

PROCEDURE

This study was performed by the Microbiology Laboratory of Actalia Cecalait in Poligny, France, from October 2014 to April 2015 and was conducted in 2 steps:
- Establishing the method's limit of detection
- Inclusivity study

Tests were performed on semi-skimmed UHT milk, in 1 litre bottles. Milk pre-incubation was conducted in a water bath set at 30 +/- 1 °C.

ARTICLE

1. Methods

- Soleris method: after pre-incubation of the milk for 2 and 3 days at 30 °C, 5 mL are sampled in sterile fashion, and inoculated into a vial of "NF UHT medium". The vial is placed in the Soleris instrument, set at 30 +/- 1 °C, for a maximum of 48 hours.

- Resazurin test: after pre-incubation of the milk for 5 days at 30 °C, 2.5 mL of milk are added to 0.5 mL of resazurin at 0.005%. Readings are performed after 4 hrs 30 mins at 30 °C. In the event of a positive result, the blue or blue-purple colouration turns pink to white.

- Official method: 0.1 mL of milk pre-incubated for 15 days at 30 °C is inoculated into 2 PCA [Plate Count Agar] plates incubated for 3 days at 30 +/- 1 °C. A result below 10 CFU per 0.1 mL (< 100 CFU/mL) is considered negative. Within the context of this study, tests were also performed after 3 and 5 days of milk pre-incubation.

2. Determination of the limit of detection (LOD)

5 strains commonly found in UHT milk with different growth periods were tested. The 2014 protocol ISO/ FDIS 16140 was followed for this study:
- 5 negative samples
- 20 samples with contamination of 0.5 CFU/litre
- 10 samples with contamination of approximately 5 CFU/litre

When it was possible, the limit of detection ($LOD_{50\%}$) was calculated: it is the lower number of cultivable microorganisms detectable in the sample with a probability of 50 %.

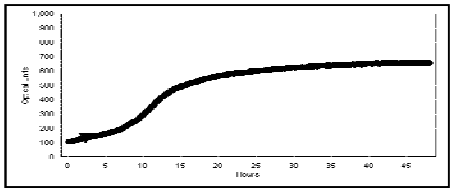
3. Inclusivity

The 2014 protocol ISO/ FDIS 16140 was followed for this study:
A panel of 32 representative microorganism strains that can contaminate UHT milk (including the 5 strains tested as part of the LOD step), from which the majority of strains specific to UHT milk or raw milk, were inoculated into milk at an approximate level of 10 CFU/mL.
The official method was only used in the event of negative results for the other methods, or as additional verification.

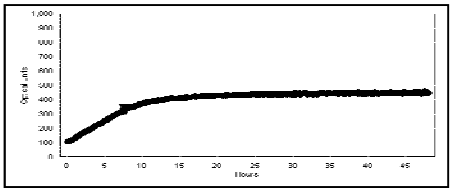
RESULTS

1. Determination of the limit of detection

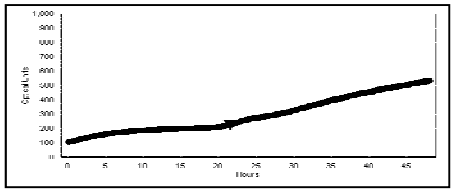
STRAIN		***Candida parapsilosis***			***Pseudomonas***			***Cellulosimicrobium cellulans***		
CFU/litre D0		**0**	**0.27**	**2.7**	**0**	**0.39**	**3.9**	**0**	**0.79**	**7.9**
Number of bottles (1 L)		5	20	5	5	20	5	5	20	5
Number of positive results	SOLERIS D+2	0	3	4	0	8	5	0	11	5
	D+3	0	3	4	0	8	5	0	11	5
	RESA D+5	0	3	4	0	8	5	0	11	5
	PCA D+15	0	3	4	0	8	5	0	11	5
SOLERIS $LOD_{50\%}$		**1.2** [0.5 – 2.6]			**0.5** [0.2 – 1.0]			**0.7** [0.3 – 1.3]		



C. parapsilosis: D+2 DT=7.7–17.2 hrs



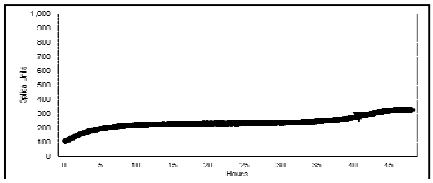
Pseudomonas: D+2 DT = 7.8 hrs



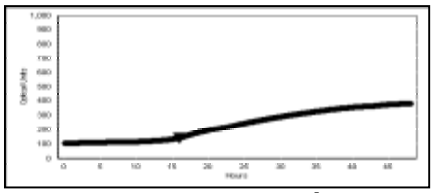
C. cellulans: D+2 DT =18.7 to 29.7 hrs

For these 3 strains, the detection threshold for the Soleris method is around 1 CFU/L of UHT milk, with $LOD_{50\%}$ values ranging from 0.7 to 1.2 CFU/L, starting at 2 days of milk pre-incubation. These results are perfectly in line with the resazurin test and the official method.

STRAIN		*Mycobacterium vaccae*					Spores of *Bacillus sporothermodurans*		
CFU/litre D0		0	1.1	9.2	92	920	0	1.3	13
Number of bottles (1 L)		5	20	5	3	3	5	20	5
Number of positive results	SOLERIS D+2	0	0	0	0	3	0	0	0
	D+3	0	0	0	3	3	0	0	0
	RESA D+5	0	0	0	0	0	0	0	0
	PCA D+15	0	9	5	3	3	0	0	0
SOLERIS $LOD_{50\%}$ D+2		**280** [80 – 990]					-		
D+3		**27** [7 – 98]					-		



M. vaccae : D+2 DT= 40.0 – 44.9 hrs



B. sporothermodurans : 4 10^5 CFU/ vial, DT = 16 hrs

- For *Mycobacterium vaccae*, the $LOD_{50\%}$ of the Soleris method is approximately 300 CFU/litre after a 2 days pre-incubation of UHT milk and 30 CFU/litre after a 3 days pre-incubation (also detectable in PCA). Resazurin gives negative results after 5 days incubation of the milk, even at high levels of around 1000 CFU/litre. At very low levels, after 5 days incubation of the milk, the colonies are detectable (about 20 to 50 colonies for 0.1 mL).

- *B. sporothermodurans* was not detected by any method at low levels. The same strain was tested using the MPN [Most Probable Number] technique (3 bottles/ dilution), with and without thermisation, at levels ranging from 1 to 5000 CFU/L, giving negative results in the Soleris method and resazurin tests, while the highest levels were detectable in PCA. The Soleris method was positive when a massive inoculation was performed (4 10^5 CFU/ vial), demonstrating that the method is capable of detecting this bacteria. These results show that *B. sporothermodurans* survives, but slowly develops in milk.

Inclusivity Testing

	STRAINS	LEVEL/ L	SOLERIS D+2	SOLERIS D+3	RESA D+5	PCA D+3	PCA D+5	PCA D+14
BACILLI GRAM - OXYDASE +	*Stenotrophomonas maltophilia*	23	+	+	+			
	Stenotrophomonas maltophilia	40	+	+	+			
	Aeromonas hydrophila	4.6/34	-/+	+/+	+/+	/+	/+	/+
	Pseudomonas	7.8	+	+	+	+	+	+
	Pseudomonas putida	7.4	+	+	+			
	Pseudomonas aeruginosa	11	+	+	+			
BACILLI GRAM - OXYDASE -	*Enterobacter cloacae*	5.6	+	+	+			
	Escherichia coli	11	+	+	+			
	Acinetobacter rudis: CIP 110305T	4.1/11	-/+	-/+	-/+	-/+	-/+	-/+
COCCI	*Staphylococcus aureus*	9.6	+	+	+			
	Staphylococcus capitis	32	-	-	-	-	-	-
	Streptococcus faecalis	15	+	+	+			
	Lactococcus lactis	6.1	+	+	+			
BACILLI GRAM +	*Lactobacillus paracasei*	6.1	+	+	+			
	***Cellulosimicrobium cellulans*: CIP 81.28**	16	+	+	+	+	+	+
	Microbacterium lacticum: CIP 101097	16/15	-/-	-/-	+/+	+/+	+	+
	Microbacterium liquefaciens: CIP 102402T	10/7	+/+	+/+	+/+	+/+	+	+
	Mycobacterium mucogenicum	10-100	-	-	-	-	+	+
	***Mycobacterium vaccae*: CIP 105934T**	9.2	-	-	-	-	+	+
BACILLI GRAM + SPORES	*Paenibacillus lactis:* CIP 108827T	5.5/78	-/-	-/-	-/+	+/-	+/+	+/+
	Bacillus cereus	4.6	+	+	+			
	Bacillus licheniformis	5.5/4.9	-/+	-/+	-/-	-/-	-/+	+/+
	Bacillus sporothermodurans	6.5	-	-	-	-	-	-
	Bacillus sporothermodurans	11	-	-	-	-	-	-
	***Bacillus sporothermodurans* (spores)**	26	-	-	-	-	-	-
	Bacillus subtilis (spores): ATCC 6633	10	+	+	+	+	+	+
	Bacillus stearothermophilus (spores): C953	10	-	-	-	-	-	-
	Clostridium perfringens (spores)	10	-	-	-	-	-	-
YEAST	Yeast	8.0/0.2	-/ -	+/ +	+/ +	/ +	/ +	/ +
	Candida parapsilosis	5.5	+	+	+	+	+	+
MOULD	*Geotrichum*	15	-	+	-	+	+	+
	Penicillium candidum	20	-	-	-	-	-	-

In bold: strains tested for LOD / Variance between Soleris method and resazurin test / Soleris method positive at D+3 and negative at D+2

Some negative strains were tested twice to confirm the results.
Out of 32 strains tested for inclusivity (of which 5 strains were tested for LOD) at levels of approximately 10 CFU/litre, the Soleris method allowed the detection of 21 strains, of which 3 strains (*Aeromonas hydrophila*, *Acinetobacter rudis* and *B. licheniformis*) indicated a negative result in the first trial, and a positive result in the second trial.

The Soleris results are aligned with the resazurin test, except for:
- 1 strain of mould (*Geotrichum*) and 1 bacterial strain (*B. licheniformis*), detected with the Soleris method and not with the resazurin method
- 2 bacterial strains (*Microbacterium lacticum* and *Paenibacillus lactis*), detected with the resazurin method and not with the Soleris method.

Detection with the Soleris method occurred after a 2 days incubation of milk at 30 °C, except for 3 strains: 1 yeast and 1 mould, detected in 3 days, and 1 bacterium (*Aeromonas hydrophila*), detected in 2 days for 1 test out of 2.

CONCLUSIONS

Conclusion and results of the study

The study for the limit of detection of 5 strains gave the following results:
- For 3 strains (*Candida*, *Pseudomonas* and *Cellulisomicrobium*) the Soleris method, after a 2 days pre-incubation at 30°C, detected approximately 1 CFU/litre of semi-skimmed UHT milk, in perfect concordance with the resazurin test and the official method.
- The *Mycobacterium vaccae* strain was detected in the Soleris method, starting from about 100 CFU/litre, while the resazurin test made no detection at 1000 CFU/litre.
- The strain of *B. sporothermodurans* in sporulated or vegetative form was only detected at very high levels, regardless of the method used, undoubtedly because of the slow growth of this bacterium in milk.

The inclusivity study reveals that 21 out of 32 strains were detected using the Soleris method after a 3 days pre-incubation period of UHT milk at 30 °C, and 19 strains after a 2 days pre-incubation period. Four discrepancies were observed versus the resazurin test: two in favour of the Soleris method and two in favour of the resazurin test.

Conclusion using the Soleris method

The Soleris method is an easy method to implement: 5 mL of UHT milk are inoculated into a ready-to-use broth. The optical reading and interpretation of the results are performed automatically (without visual assessment), as opposed to the resazurin test. The results obtained are plotted, and the growth curve allows additional information to be obtained for the interpretation of results.

It enables results equivalent to the resazurin test to be obtained after 2 or 3 days of UHT milk pre-incubation, versus 5 days for the resazurin test.

The Soleris method can be used for all types of UHT milk: white, flavoured or supplemented (not tested in this study) while the resazurin test cannot be used on coloured flavoured milks and certain types of supplemented milks.

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