EVALUATION OF PROMICOL SYSTEM
FOR STERILITY TESTING OF UHT MILKS

Actually, the resazurin test is used in many industrial laboratories to test in routine the sterility of UHT milks After a pre-incubation 2 to 3 days at 30°C, this test underlines the activity of the bacterial reductases but it cannot be used on a large range of products. The official method (European decree 92/46), which consists in the enumeration of the total flora after an incubation of the samples 15 days at 30°C, cannot be used in routine. The Promicol system, which permits to underline the microbial ATP after incubation 2 to 3 days at 30°C, appears then as an interesting alternative. In this study, we have tested in parallel the resazurin test and the Promicol system, using also the enumeration of the total flora as reference method.

Promicol BV is situated in the Netherlands and manufacture an extensive range of microbial tests kits on ATP bioluminescence. Specific tests are available for finished products as wines, beverages, fruit juices and dairy UHT and ESL products.

The Promicol system includes a specific microbial ATP (adenosine triphosphate) detection kit for UHT and ESL dairy products. The Promilite III instrument, the flagship in Promicol's family of luminometers, is a very compact analyser controlled by PC and operating with Proscreen software under Windows XP. It is able to test up 400 samples per hour.

The method is used to detect microbial APT in UHT and ESL dairy products, including flavoured milk, lactose reduced milks, desserts, baby milks, creams and non-dairy creamers. After incubation 2 or 3 days at 30°C, just after their production, milk products are analysed by the Promicol method for the evaluation of the growth of bacteria, by extraction and detection of their ATP. The aim of this method is to be able to detect one bacterium in a bottle of milk.

PROCEDURE

3 types of UHT milks were studied:
- white UHT milk
- supplemented UHT milk (minerals, vitamins)
- flavoured UHT milk

This evaluation was carried out in 2 steps:
- Examination of the negative milks and determination of the control RLU (Relative Light Unit) of each type of milk.
- Examination of artificial contaminated milks.

1.   Examination of the negative samples

The tests were performed on milk bottles sent to CECALAIT just after their packaging (to reduce the incubation time at room temperature).

a) **After 3 days of incubation at 30 +/-2°C**: 10 samples of each type of milk were analysed according to 3 methods:

- ATP detection by the Promicol system with 2 determinations on each sample. The control RLU was evaluated by calculation of the mean of the values obtained on ten samples of each type of milk.
- Resazurin test with 2 determinations on each sample.

- Microorganisms enumeration at 30°C (according to ISO 4833 standard) with 2 plates inoculated by 0.1 ml of milk.

b) **After 15 +/- 2 days of incubation at 30°C:**

The enumeration of microorganisms at 30°C was performed again, to follow the European directive 92/46: the criterion is < 10 CFU for 0.1 ml of milk corresponding to < 100 CFU/ml.

2. Examination of the artificially contaminated milks

One batch of each type of UHT milk was contaminated with a natural positive milk according to the resazurin test. 10 negative samples of each type were contaminated at different levels, lower and higher than 1 bacterium / litre, in order to obtain negative samples and to evaluate the limit of detection.

The samples were then analysed:

a) After 3 days of incubation at 30 +/-2°C :
according to 3 methods

- ATP detection by the Promicol system with 2 analyses by sample. The control RLU obtained by the negative samples analysis was used to calculate the limit values for results:

• If the RLU of the samples **less than 2 x** the control RLU, the sample is then considered as sterile.

• If the RLU of the sample is **more than 2 x but less than 3 x** the control RLU, the sample is then considered as suspect and should be tested again after an additional incubation.

• If the RLU of the sample is **more than 3 x** the control RLU, the sample is then considered as non-sterile.

- Resazurin test with 2 analyses by sample.

- Microorganisms enumeration at 30°C on dilutions from –1 to –4.

b) After15 +/- 2 days of incubation at 30 +/- 2°C:

The enumeration of microorganisms at 30°C was performed again only on the negative samples after 3 days of incubation with 2 plates inoculated by 0.1 ml of :milk.

RESULTS

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1. Examination of the negative samples

1.1. Comparison of the methods for the control of milk sterility

- For white milks, we obtained negative results, except for goat milk, which can not be tested with the Promicol system.
- For negative supplemented milks, we obtained positive or doubtful results on 4 samples using the resazurin test. Indeed, according to the level and the supplement type, the redox potential can be modified.
- As the flavoured milks were coloured, the resazurin test could not be used.
- For all the samples we obtained negative results with the ATP method and the enumeration of the total flora after pre-incubation of milks 3 or 15 days at 30°C (< 100 CFU/ml).

1.2. ATP method

For all the types of negative milks, we obtained very closed values for the mean and the standard deviation on the RLU control. Moreover, repeatabilities are concordant for the different tested milks.

Table 1:   ATP results in RLU on negative samples analysed in duplicate after 3 days of incubation at 30°C

Samples	White milks	Supplemented milks	Flavoured milks chocolate	All samples
n	10	10	10	**30**
Mean (Control RLU)	27	26	30	**27**
SD	6,0	5,2	5,7	**5,8**
Negative limit	53	52	59	**55**
Positive limit	80	78	89	**82**
Sr	4,4	4,0	3,7	**4,1**
Sr %	17%	16%	13%	**15%**
r	12	11	10	**11**

n : number of samples

SD: standard-deviation

Sr: standard-deviation of repeatability

Sr%: standard-deviation of repeatability in % of the mean value

r : repeatability

2.   Examination of the contaminated milks

2.1.   Analyses of the positive milks

Total flora of positive milks was enumerated according to ISO 4833 standard, in order to obtain an evaluation of the contamination. These milks positive to the resazurin test were naturally contaminated by UHT spoilage bacteria, presenting different characteristics.

Table 2:   Enumeration and characteristics of the bacterial contamination of the positive milks

Positive milks	2	19	20
CFU / ml	6,8E+06	1,4E+06	4,6E+03
Colonies	medium	very small	yellow small
Characteristics	Rods Gram - Oxidase -	Cocci Gram + Catalase +	Rods Gram + Catalase +

2.2   Contaminations and examination of the samples after contamination

- Comparison between the ATP test, the enumeration of the total flora and the resazurin test

For all the samples (positive or negative), we obtained after pre-incubation 3 days at 30°C concordant results between the ATP method and the enumeration of the total flora. For all the negative samples, the total flora was < 10 CFU/ml after pre-incubation 15 days at 30°C.

It was not possible to test flavoured chocolate milks with the resazurin test. For the other supplemented milks and white milks, the resazurin test gave concordant results with the ATP method and the enumeration of the total flora.

- ATP results

Concerning the limit of detection of the Promicol system, it is possible to detect, after 3 days of incubation at 30°C, 1 CFU in 1 litre of milk (present before incubation).

We can observe also, that the values of positive samples were not the same according to the positive milk used for the contamination. The lower values were between 90 and 200 RLU and the higher between 1000 and 1500 RLU.

Concerning repeatability of the positive samples, Sr is between 2 and 7 % (in percentage of the mean value).

Table 3:   Rate of bacteria present in milks before incubation, ATP results and positive milks used for the contamination

Samples	White milks		Supplemented milks		Flavoured chocolate milks	
	CFU/litre	ATP	CFU/litre	ATP	CFU/litre	ATP
1	1	-	0,1	-	0,08	-
2	1,6	-	0,3	+	0,16	+*
3	2	+	0,4	-	0,24	-
4	4	-	1,1	+	0,6	+
5	6	+	1,4	+	0,8	+
6	10	+	2,9	+	1,6	+
7	16	+	4,3	+	2,4	+
8	20	+	7,1	+	4	+
9	100	+	11	+	6,4	+
10	200	+	14	+	7,9	+
Positive milk	2		19		20	

ATP - : non-contaminated milk
ATP + : contaminated milk
ATP +* : accidental contamination (not from added milk)

Table 4: ATP results in RLU (relative light units) on the positive samples analysed in duplicate after 3 days of incubation at 30°C

	White milks	Supplemented milks	Flavoured chocolate milks
n	6	7	6
RLU	110 à 250	1000 à 1500	90 à 200
Sr	11	30	10
Sr%	6%	2%	7%
r	31	83	26

n : number of samples
Sr : standard deviation of repeatability
Sr% : standard deviation of repeatability in % of the mean value
r : repeatability

CONCLUSIONS

Conclusion of the study results

With the Promicol system, after pre-incubation 3 days at 30°C, we obtained concordant results with the enumeration of total flora (after pre-incubation of 3 to 15 days at 30°C) for all the positive and negative results.

The Promicol system can be used for all types of UHT cow milks including white milks, supplemented or flavoured milks, but it cannot be used for goat milks.

The Promicol system can detect 1 bacterium in 1 litre of UHT milk after incubation 3 days at 30°C. It is possible to reduce this pre-incubation to 2 days, according to the type of milk or contaminating bacterium. Before using the Promicol system in routine, each laboratory must test each type of products to evaluate their control RLU value and to confirm the pre-incubation time and temperature.

The standard-deviation of repeatability is about 14% for the negative samples and between 2 and 7% for the positive samples, lower for example than the standard deviation obtained by the method of enumeration of total flora (about 23% CFU/ml).

During this study, we used different batches of the Promicol test kit, and no significant variations were observed on the values of negative samples.

Conclusion on the Promicol system use

The Promicol system can be used for all the types of UHT milks: white, flavoured or supplemented, contrary to the resazurin test which cannot be used on coloured flavoured milks and on certain types of supplemented milks.

The Promicol system can measure and classify results of 96 different samples in only about 20 minutes (from inoculation to reading), against 1 to 4 hours for the resazurin test and 3 days for the enumeration of total flora.

The Promicol system is easy to use and a half-day's training is sufficient for a technician to use the system. The results are obtained by optical measures (without visual appreciation as with the resazurin test) and are not dependent of the good aseptic laboratory skills as those of the traditional plate count method.

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