NOTE ON THE ACTIVITY OF THE "TBX MEDIUM" WORK GROUP OF THE AFNOR COMMISSION V08B "FOOD MICROBIOLOGY"

PART 1: REPORT

Tryptone-bile-glucuronide (TBX) and peptone-tergitol-glucuronide (PTX) agars are two chromogenous media which allow the enumeration of *E. coli* by their positive character for the β-D-glucuronidase, present in about 95% of *E. coli* found in food products. *E. coli* 0157:H7 for example, is deprived of this character.
Since 1993, the medium PTX is described in a routine standard at a national level (NF V 08-053). However, following the non-commercialisation of tergitol 7, a selective agent in this medium, it was replaced, at the end of 2002, by the medium TBX, which is standardised at an international level since 2001 (ISO 16649 parts 1 and 2).
TBX had been selected following the ISO SC9 meeting in 1997, where comparisons had been presented on various matrices, with various methods and chromogenous media.
However, taking into mind these results, TBX did not seem to possess much higher performances than the other chromogenous media.
With the use of this new medium, some laboratories met difficulties, particularly for the analysis of dairy products. These observations are described in this first article.

1) PROBLEMS MET IN DIFFERENT LABORATORIES

1.1 Comparison between PTX and TBX media

At the beginning of 2003, following the set up of the new standardised medium, TBX, in place of PTX, 2 laboratories, analysing dairy products, alerted us on discordant results between these agars.

- Results obtained in naturally contaminated samples:

The laboratory L1 compared results obtained from the analysis of 25 raw milk samples on TBX and PTX of a same supplier (E). Globally, this laboratory observed a strong inhibition with the medium TBX in raw milk (-0.7 log on average). However, the colonies were not identified.

The laboratory L2 compared enumerations obtained with 5 suppliers of ready-to-use TBX in relation to a medium PTX on a majority of raw milk cheese samples. Globally, few differences were observed (on average +/- 0.1 log according to the supplier). However, on a batch from a supplier of TBX (B), colonies were uncountable because they were only weakly, or not, visible, whereas important enumerations were obtained on the medium PTX and on 2 other TBX media. It should be noted that this batch was close to the use-by-date.

- Results obtained on the samples from CECALAIT's proficiency tests:

Proficiency test *E. coli* in milk in February 2003 (5 samples):

The laboratory L1 tested various suppliers of the TBX and PTX media. The 2 PTX media gave results located within the target of conformity (+/- 0.3 log in relation to the reference), whereas 3 TBX media, on average, largely underestimated the populations (-0.4 to –0.8 log).

The laboratory L2 compared 4 different suppliers of TBX and one of PTX: only one TBX (supplier C) was out of range (-0.4 log on average).

CECALAIT's laboratory, by using dehydrated TBX and PTX media from the same supplier (A), obtained identical results, within the target of conformity. With the TBX from this supplier, our laboratory has always obtained good results in the proficiency tests.

Proficiency test *E. coli* in cheese in December 2002 (5 samples):

The laboratory L1 once again compared TBX and PTX media from the same supplier (E): PTX was within the target of conformity, whereas TBX underestimated on average 0.9 log in relation to the reference

1.2 Other problems met in CECALAIT's proficiency tests

Thereafter, other laboratories related to us their difficulties of enumeration using TBX in the proficiency tests. For example, the phosphate plug diluent used with TBX can have an inhibiting effect. This effect is not observed with the Ringer diluent or on other matrices.
These problems are more often met with the ready-to-use than with the dehydrated media. They can concern all the media proposed by the different suppliers, although they appear punctually according to the batches used.

2) TOTAL INTERPRETATION OF CECALAIT'S PROFICIENCY TESTS

Note: Proficiency test allows to evaluate the performances of a laboratory, but not the performance of a method or a supplier. However, this vision can alert us on the general tendencies.

From June 2003, when the use of TBX in the laboratories increased, we noted that, globally, a more substantial dispersion of the results of *E. coli* proficiency tests, and more significantly in cheese than in milk. Generally, the results were more closely grouped with the medium PTX.

So, we tried to interpret in more detail the *E. coli* proficiency tests in cheese. We observed, for the medium TBX, a more important percentage of results off target, generally underestimations, than for the other media.

By locating the suppliers on the target, it was not possible to show a constant supplier effect with time. In the same way, the underestimation of the ready-to-use media compared to the dehydrated formula was not clearly demonstrated. However, recently the results have tended to improve.

Following these reports, the AFNOR commission V 08B decided to create a work group, which must gather and study these observations and try to provide solutions. Organised by AFNOR and animated by P. ROLLIER of CECALAIT, this work group is composed of about 10 suppliers or users. The group has met 3 times between 2004 and 2005. The activities and conclusions of this work group will be presented in the second part of this article in the next Lettre de CECALAIT.