

Vos interlocuteurs :

Valentine DIGONNET
valentine.digonnet@afaq.afnor.org
ligne directe : +33 (0)1 41 62 85 29
télécopie : + 33 (0)1 49 17 90 19

Joël DUCHATELLE
Joel.duchatelle@afaq.afnor.org
ligne directe : +33 (0)1 41 62 83 29
télécopie : + 33 (0)1 49 17 91 91

nos références : RV/JDU/102/MICROBIO/FAB/Biotrace

objet : marque AFNOR VALIDATION

**Monsieur MICHEL
BIOTRACE
ZI du Val de Seine
20 Av Marcelin Berthelot
92396 VILLENEUVE LA GARENNE**

Bagneux, March 29th, 2007

Dear Sir,

AFNOR VALIDATION certificate of the following alternative method:

TEST TECRA ULTIMA SALMONELLA	Certificate # TEC 24/2 – 04/03
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will expire on April 2nd, 2007, before that complete results of the renewal study may be examined by AFAQ AFNOR Certification.

Following advice of AFNOR Validation Technical Committee, I declare that you can continue to refer to this certificate till October 12th, 2007.

Yours Sincerely



**Deputy General Manager
Jacques BESLIN**



**Alternative methods for agribusiness
Analytical performances certified**

**VALIDATION CERTIFICATE FOR ALTERNATIVE ANALYTICAL METHOD
ACCORDING TO NF V 03-100 STANDARD**

Certificate No.: TEC – 24/2 – 04/03

Validation date :	02.04.2003
End of validity :	02.04.2007

The Company **BIOTRACE INTERNATIONAL PLC**
(Head office and The Science Park
Distribution) Bridgend CF31 3NA Wales, UK

Production **BIOTRACE INTERNATIONAL PLC**
site 13 Rodborough Road
 FRENCHS FOREST NSW 2086
 AUSTRALIA

is hereby authorized to refer to this **AFNOR Validation certificate** for the following alternative **qualitative** analysis method:

TECRA ULTIMA™ Salmonella method

Protocol reference : **MS2427 10/02**

METHOD PRINCIPLE

TECRA ULTIMA™ Salmonella is an Enzyme linked immunosorbent assay (ELISA) performed in a sandwich configuration, using purified polyclonal antibodies prepared with flagellar and other antigens. After a pre-enrichment in non-selective media and an enrichment in selective media the ELISA test is performed.

A green coloration will occur if Salmonella was present in the original sample (the interpretation can be visual or with a microplate reader).

In the context of AFNOR Validation, all samples identified as positive by the TECRA ULTIMA™ Salmonella method must be confirmed according to classical tests described in methods standardized by CEN, ISO or AFNOR (including a purification step), starting from the enrichment broth.

SCOPE : All food and animals feeding stuffs

RESTRICTIONS OF USE : None

REFERENCE METHOD

NF EN ISO 6579- Microbiology of food and animals feeding stuffs - Horizontal method for the detection of *Salmonella* spp.

**Deputy General Manager
Jacques BESLIN**

AFAQ AFNOR Certification

Head office : 11, avenue Francis de Pressensé – 93571 Saint-Denis la Plaine Cedex - France
Office: 116, avenue Aristide Briand – BP 40 – 92224 Bagneux Cedex 6 – France
Tél +33 (0)1 46 11 37 00 – Fax +33 (0)1 46 11 39 40
certification@afaq.afnor.org - www.afnor.fr

PRACTICABILITY

- **Negative** results are obtained in 2 days using TECRA ULTIMA™ Salmonella method compared with 5 to 7 days using the reference method.
- **Positive** results are obtained in 5 to 6 days using the TECRA ULTIMA™ Salmonella method (including the confirmation step) compared with 5 to 7 days using the reference method.

SPECIFICITY

2003 study

Some complementary studies have been conducted from the previous 1996 results :

- 6 *Salmonella* strains were detected out of 6 strains tested.
- 6 strains not belonging to the genus *Salmonella* gave negative results.

1996 bibliographic study for AOAC

(This study was taken into account for the validation of TECRA Visual Immuno Assay method)

- 288 *Salmonella* strains were detected out of 300 strains tested with 10^7 cells/ml threshold. All strains were detected with 10^8 cells/ml threshold.
- The study of 100 strains not belonging to the genus *Salmonella* detected the presence of cross-reactions with strains of *Citrobacter freundii* and *Enterobacter cloacae*, which gave a positive response with both reading systems.

INTRINSIC DETECTION LIMIT

(This study was performed in 1996 during the validation of the TECRA Visual Immuno Assay method)

- The number of *Salmonella* required to obtain a positive result with the TECRA ULTIMA™ Salmonella method is 10^6 cells/ml.
- This result was obtained on pure strains of *Salmonella* (*Newport*, *typhimurium*, *pullorum*, *enteritidis* and *Dublin*)

DETECTION LIMIT

Assays were performed in 2003 using 4 different types of food (raw milk, raw minced beef, whole egg, and smoked salmon), each artificially contaminated with 4 strains of *Salmonella* (*enteritidis*, *typhimurium*, *virchow* and *infantis*) at 5 different contamination levels (0, 1 to 10 CFU/25g, 2 to 20 CFU/25g, 5 to 50 CFU/25g, 10 to 100 CFU/25g).

All samples were analysed **twice** by the **two methods**.

The following results were obtained for all matrices:

Level of contamination in CFU/25 g	Alternative method % of samples detected	Reference method % of samples detected
0	0	0
1 to 10	100	100
2 to 20	100	100
5 to 50	100	100
10 to 100	100	100

Conclusion

The detection limit is between 1 and 10 cells/25g.

ACCURACY

Comparison of performance between the alternative method and the reference method

Assays were performed in 2003, using 262 samples, of which 131 were uncontaminated, 106 were artificially contaminated and 25 were naturally contaminated.

All samples were analysed in single by the two methods.

Results were as follows :

- False negatives with TECRA method: 2
- Supplementary positive samples with TECRA method: 2
- Same result with both methods: 258

Conclusion

Agreement between the TECRA ULTIMA™ Salmonella method and the reference method is 98.47%.

PRECISION

Precision data were determined during an assay performed in 2003, involving 11 independent laboratories. Analyses were performed using Pasteurised Whole Milk artificially contaminated with a *Salmonella enteritidis* strain at 4 different levels : 0, 1-10 cells/25ml, 5-50 cells/25ml, and 10-100 cells/25ml.

Laboratories tested 2 samples for each level of contamination.

Results per contamination level were as follows :

Level	Total number of samples	Number of sample analyzed (*)	Number of negative results	Number of positive results	Number of unprocessed samples
0	22	18	18	0	0
1 - 10	22	18	0	18	0
5 - 50	22	18	0	18	0
10 - 100	22	18	0	18	0

(*) 2 laboratories did not realize the analysis, due to damaged samples.

Conclusion

Agreement is 100 % compared with expected results. The method is reliable.

Please send any queries concerning the performance of the validated method to AFAQ AFNOR Certification.

On request, AFAQ AFNOR Certification will send you a summary document (in French) on the preliminary and collaborative studies.