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# EU Interlaboratory comparison study VII (2003) on bacteriological detection of *Salmonella* spp.

H. Korver, N.J.D. Nagelkerke, A.W. van de Giessen and K.A. Mooijman

Contact:
H. Korver
Microbiological Laboratory for Health Protection
Hans.Korver@rivm.nl

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RIVM, P.O. Box 1, 3720 BA Bilthoven, telephone: 31 - 30 - 274 91 11; telefax: 31 - 30 - 274 29 71
European Commission, Legislation Vétérinaire et Zootechnique, Rue de la Loi 86, B-1049 Bruxelles, Belgique, telephone 32-2-2959 928; telefax: 32-2-2953 144

# Abstract

**EU Interlaboratory comparison study VII (2003) on bacteriological detection of *Salmonella* spp.**

In 2003 a seventh interlaboratory comparison study on bacteriological detection of *Salmonella* spp. was organised by the Community Reference Laboratory for *Salmonella* (CRL-*Salmonella*, Bilthoven, the Netherlands). National Reference Laboratories for *Salmonella* (NRLs-*Salmonella*) of the EU Member States (16), the NRL of Norway and of three EU Candidate Countries participated in the study. Reference materials in combination with or without the presence of chicken faeces, as well as naturally contaminated faecal samples (containing *Salmonella* Muenchen) were tested. The reference materials existed of gelatin capsules containing *Salmonella* Typhimurium (STM), *Salmonella* Enteritidis (SE) or *Salmonella* Panama (SPan) at different contamination levels. In addition to the performance testing of the laboratories a comparison was made between the media described in ISO 6579: 2002 and the alternative media Modified Semi-solid Rappaport Vassiliadis (MSRV) and Brilliant Green Agar (BGA) resulting in six medium combinations. Significantly more positive isolations were obtained from capsules containing a high level of SE than, in declining order, with a low level of SE or with a high level of STM and a low level of STM. The overall results of all capsules as well as the results of the naturally contaminated samples revealed better (although not statistically significant) results for MSRV (with BGA and XLD as plating-out media) in comparison with the ISO 6579: 2002 method. Seven laboratories scored systematically below the average results of all laboratories for the artificially contaminated samples for all six medium combinations and nine laboratories for the naturally contaminated samples.

Keywords: CRL-*Salmonella, Salmonella*, interlaboratory comparison, reference materials, detection methods.

# Rapport in het kort

**EU Ringonderzoek VII (2003) over bacteriologische detectie van *Salmonella* spp.**

In 2003 werd door het Communautair Referentie Laboratorium voor *Salmonella* (CRL-*Salmonella*, Bilthoven, the Netherlands) het zevende bacteriologische ringonderzoek georganiseerd. Nationale Referentie Laboratoria voor *Salmonella* (NRL's-*Salmonella*) van de EU lidstaten (16), van NRL Noorwegen en van drie EU kandidaat lidstaten namen deel aan deze studie. Referentiematerialen in combinatie met of zonder de aanwezigheid van kippenfeces, zowel als natuurlijk besmette feces (bevattende *Salmonella* Muenchen) werden getest. De referentiematerialen bestonden uit gelatine capsules met verschillende besmettingsniveaus van *Salmonella* Typhimurium (STM), *Salmonella* Enteritidis (SE) of *Salmonella* Panama (SPan). Bovendien werd naast de uitvoering van de testen door de laboratoria een vergelijking gemaakt tussen de media zoals beschreven in ISO 6579: 2002 en de alternatieve media Modified Semi-solid Rappaport Vassiliadis (MSRV) en Briljant Groen Agar (BGA), resulterend in zes medium combinaties. Significant meer positieve isolaties werden gevonden met capsules welke een hoog gehalte aan SE bevatten en in afnemende volgorde, met een laag gehalte aan SE en een hoog gehalte aan STM en vervolgens met een laag gehalte aan STM. De totale resultaten van zowel alle capsules als van de natuurlijk besmette monsters lieten betere (alhoewel niet statistisch significant) resultaten zien voor MSRV (met BGA en XLD als uitplaat-medium) in vergelijking met de ISO 6579: 2002 methode. Zeven laboratoria scoorden systematisch onder de gemiddelde resultaten behaald door alle laboratoria met de kunstmatig besmette monsters voor alle zes medium combinaties en negen laboratoria met de natuurlijk besmette monsters.

Trefwoorden: CRL-*Salmonella, Salmonella*, ringonderzoek, referentiematerialen, detectie-methoden.

# Contents

**Summary** **7**

**List of abbreviations** **8**

**1. Introduction** **9**

**2. Participants** **11**

**3. Materials and Methods** **13**

*3.1 Reference materials* *13*

*3.2 Faecal samples* *14*
3.2.1 General 14
3.2.2 MPN of *Salmonella* in naturally contaminated faeces 14
3.2.3 Total bacterial count in faeces 15

*3.3 Optimisation dissolving procedure of capsules in BPW* *15*

*3.4 Design of the interlaboratory comparison study* *16*
3.4.1 Samples 16
3.4.2 Methods 17
3.4.3 Temperature recording during shipment 17

*3.5 Accreditation/certification* *18*

*3.6 Statistical analysis of the data* *18*

**4. Results** **19**

*4.1 Reference materials* *19*

*4.2 Faecal samples* *20*

*4.3 Optimisation dissolving procedure of capsules in BPW* *21*

*4.4 Technical data collaborative study for artificially and naturally contaminated samples* *22*
4.4.1 Pre-warming temperature of BPW 22
4.4.2 Incubation time and temperature for dissolving the capsules 22
4.4.3 Incubation time and temperature of pre-enrichment 22
4.4.4 Incubation time and temperature of selective enrichment media 23
4.4.5 Composition of MKTTn 25

*4.5 Control samples* *26*

*4.6 Results faeces samples artificially contaminated with Salmonella spp.* *28*
4.6.1 Results per type of capsule and per laboratory 28
4.6.2 Results per medium combination tested by all laboratories 32
4.6.3 Results of other medium combinations 33

*4.7 Comparison between laboratories* *34*

*4.8 Results faeces samples naturally contaminated with Salmonella spp.* *36*

*4.9 PCR* *39*

*4.10 Transport of samples* *40*

**5. Discussion** **43**

**6. Conclusions** **47**

**References** **49**

**Annex 1. Calculation of $T_2$** **51**

**Annex 2. Results by laboratory, sample and medium combination** **52**

**Annex 3. Information on the media used** **57**

**Annex 4. Results of PCR detection** **73**

**Annex 5. Temperature recording** **77**

**Annex 6. Protocol** **83**

**Annex 7. Standard Operation Procedure** **87**

**Annex 8. Test report** **97**

# Summary

The Community Reference Laboratory for *Salmonella* (CRL-*Salmonella*) organised the seventh interlaboratory comparison study on bacteriological detection of *Salmonella*. Participants were the sixteen National Reference Laboratories for *Salmonella* (NRLs-*Salmonella*) of the EU Member States, the NRL from Norway and 3 NRLs from EU Candidate Countries.

The main objective of the seventh interlaboratory comparison study was to make a comparison of the results obtained with the different levels of contamination and different serotypes of *Salmonella* in the presence or absence of competitive micro-organisms between and within the NRLs. Furthermore, the results obtained with Modified Semi-solid Rappaport Vassiliadis (MSRV) as the selective enrichment medium were compared with the results obtained with Mueller-Kauffmann Tetrathionate-novobiocin (MKTTn) and Rappaport-Vassiliadis Soya Broth (RVS) as mentioned in the ISO 6579: 2002. As selective isolation media Brilliant Green Agar (BGA, ISO 6579: 1993) and Xylose Lysine Deoxycholate agar (XLD) were used. Due to this, 6 combinations of media were created that were compared mutually, namely RVS/BGA, RVS/XLD, MKTTn/BGA, MKTTn/XLD, MSRV/BGA and MSRV/XLD. Optionally, a laboratory could also use other, own media for the detection of *Salmonella* in addition to the prescribed media.

Thirty five individually numbered capsules and 20 naturally contaminated samples had to be tested by the participants for the presence or absence of *Salmonella*. Twenty five of the capsules had to be examined in combination with 10 gram of *Salmonella* negative chicken faeces. The 25 capsules were divided over the following groups: 5 capsules with ca 10 colony forming particles (cfp) of *Salmonella* Typhimurium (STM10), 5 capsules with ca 100 cfp *S*. Typhimurium (STM100), 5 capsules with ca 100 cfp *S*. Enteritidis (SE100), 5 capsules with ca 500 cfp *S*. Enteritidis (SE500) and 5 blank capsules. The other 10 capsules, to which no faeces had to be added, were control samples, existing of 3 capsules with ca 10 cfp *S*. Typhimurium, 3 capsules with ca 100 cfp *S*. Enteritidis, 2 capsules with ca 5 cfp *S*. Panama and 2 blank capsules.

Four laboratories did not test all possible six medium combinations, which made comparison of results not possible. Seven laboratories scored systematically below the average results of all laboratories for the artificially contaminated samples for all six medium combinations. Nine laboratories scored systematically below the average results for the naturally contaminated samples. Four laboratories scored below the average results of all laboratories for the artificially as well as the naturally contaminated samples. For future studies procedures will be set up to assist these laboratories in searching the possible cause of the poor performance by sending extra reference materials and/or faeces samples.

# List of abbreviations

| | |
|---|---|
| BGA | Brilliant Green Agar |
| BBLS | Bromthymol Blue Lactose Sucrose agar |
| BPW | Buffered Peptone Water |
| cfp | colony forming particles |
| CRL | Community Reference Laboratory |
| DIASALM | Diagnostic Semi-solid *Salmonella* medium |
| dPCA | Double concentrated Plate Count Agar |
| dVRBG | Double concentrated Violet Red Bile Glucose agar |
| hcmp | Highly Contaminated Milk Powder |
| ISO | International Organization for Standardization |
| LDC | Lysine Decarboxylase |
| LIS | Diagnostic Laboratory for Infectious Diseases and Perinatal Screening |
| MK | Mueller Kauffmann |
| MKTTn | Mueller Kauffmann Tetrathionate novobiocin broth |
| MLCB | Mannitol Lysine Crystal violet Brilliant green agar |
| MPN | Most Probable Number |
| MSRV | Modified Semi-solid Rappaport Vassiliadis |
| NRL | National Reference Laboratory |
| PCR | Polymerase Chain Reaction |
| RM | Reference Material |
| RV | Rappaport Vassiliadis |
| RVS | Rappaport Vassiliadis Soya broth |
| SC | Selenite/Cystine broth |
| SE | *Salmonella* Enteritidis |
| SOP | Standard Operation Procedure |
| SPan | *Salmonella* Panama |
| STM | *Salmonella* Typhimurium |
| TBG | Tetrathionate Brilliant-Green Bile Enrichment Broth |
| TSI | Triple Sugar Iron agar |
| UA | Urea Agar |
| XLD | Xylose Lysine Deoxycholate agar |
| XLT4 | Xylose Lysine Tergitol 4 agar |

# 1. Introduction

In pursuance of the Council Directive 92/117/EEC the Community Reference Laboratory for *Salmonella* (CRL-*Salmonella*) organises bacteriological interlaboratory comparison studies with the objective that the examination of samples in the EU Member States is carried out uniformly and that comparable results should be obtained by all National Reference Laboratories (NRLs).

Earlier studies (see Table 1) have shown a significantly reduced number of positive isolations using Selenite/Cystine broth (SC) and significantly better results using Modified Semi-solid Rappaport-Vassiliadis (MSRV) compared to the use of Rappaport-Vassiliadis broth (RV) as selective enrichment medium. Since the fourth study, all laboratories used the selective enrichment medium MSRV, in addition to RV (Rappaport Vassiliadis) or RVS. In 2002 a new version of ISO 6579 was published. In this ISO the selective broths Mueller Kaufmann Tetrathionate with novobiocin (MKTTn) and Rappaport Vassiliadis Soya broth (RVS) are prescribed. Furthermore, this ISO prescribes Xylose Lysine Deoxycholate (XLD) as the plating out agar. Since study six (2002) these media are also prescribed to analyse the samples.

All media combinations were prescribed to enable comparison of results by using the new version of ISO 6579: 2002 (RVS/MKTTn and XLD) with those obtained by using earlier medium combinations. Twenty five samples of *Salmonella*-negative chicken faeces spiked with four different reference materials had to be examined, including 2 levels of *Salmonella* Typhimurium (STM10 and STM100) and 2 levels of *Salmonella* Enteritidis (SE100 and SE500). Furthermore, 20 naturally contaminated samples of chicken faeces containing *Salmonella* Muenchen were also examined by using the same six medium combinations (RVS/BGA, RVS/XLD, MKTTn/BGA, MKTTn/XLD, MSRV/BGA and MSRV/XLD).

*Table 1 History of bacteriological studies*

| Study | Year | Number of samples | Capsules | Actual number of cfp/capsule | Salmonella negative faeces added | Selective enrichment medium | Plating-out medium | Reference |
|---|---|---|---|---|---|---|---|---|
| I | 1995 | 26 | STM5 | 6 | No | RV and SC | BGA and own | N.Voogt et al., 1996 (report 284500003) |
| | | 4 | Blank | 0 | No | | | |
| II | 1996 | 15 | STM100 | 116 | 1 gram | RV, SC and own | BGA and own | N.Voogt et al., 1997 (report 284500007) |
| | | 15 | STM1000 | 930 | 1 gram | | | |
| | | 2 | SPan5 | 5 | No | | | |
| | | 1 | STM100 | 116 | No | | | |
| | | 1 | Blank | 0 | No | | | |
| III | 1998 | 14 | STM10 | 11 | 1 gram | RV and own | BGA and own | M.Raes et al., 1998 (report 284500011) |
| | | 14 | STM100 | 94 | 1 gram | | | |
| | | 7 | STM100 | 94 | 1 gram* | | | |
| | | 14 | SE100 | 95 | 1 gram | | | |
| | | 4 | STM10 | 11 | No | | | |
| | | 2 | SPan5 | 5 | No | | | |
| | | 5 | Blank | 0 | No | | | |
| IV | 1999 | 5 | STM10 | 4 | 10 gram | RV or RVS, MSRV and own | BGA and own | M. Raes et al., 2000 (report 284500014) |
| | | 5 | STM100 | 210 | 10 gram | | | |
| | | 5 | SE100 | 60 | 10 gram | | | |
| | | 5 | SE500 | 220 | 10 gram | | | |
| | | 5 | Blank | 0 | 10 gram | | | |
| | | 3 | STM10 | 5 | No | | | |
| | | 3 | SE100 | 60 | No | | | |
| | | 2 | SPan5 | 5 | No | | | |
| | | 2 | Blank | 0 | No | | | |
| V | 2000 | 5 | STM10 | 4 | 10 gram | RV or RVS, MSRV and own | BGA and XLD | M.Raes et al., 2001 (report 284500018) |
| | | 5 | STM100 | 47 | 10 gram | | | |
| | | 5 | SE100 | 63 | 10 gram | | | |
| | | 5 | SE500 | 450 | 10 gram | | | |
| | | 5 | Blank | 0 | 10 gram | | | |
| | | 3 | STM10 | 4 | No | | | |
| | | 3 | SE100 | 47 | No | | | |
| | | 2 | SPan5 | 5 | No | | | |
| | | 2 | Blank | 0 | No | | | |
| | | 20 | None | - | 25 gram** | | | |
| VI | 2002 | 5 | STM10 | 11 | 10 gram | RVS, MSRV, MKTTn and own | BGA, XLD and own | Korver et al., 2002 (report 330300001) |
| | | 5 | STM100 | 139 | 10 gram | | | |
| | | 5 | SE100 | 92 | 10 gram | | | |
| | | 5 | SE500 | 389 | 10 gram | | | |
| | | 5 | Blank | 0 | 10 gram | | | |
| | | 3 | STM10 | 11 | No | | | |
| | | 3 | SE100 | 92 | No | | | |
| | | 2 | SPan5 | 5 | No | | | |
| | | 2 | Blank | 0 | No | | | |
| | | 20 | None | - | 25 gram** | | | |
| VII | 2003 | 5 | STM10 | 12 | 10 gram | RVS, MSRV, MKTTn and own | BGA, XLD and own | This report |
| | | 5 | STM100 | 96 | 10 gram | | | |
| | | 5 | SE100 | 127 | 10 gram | | | |
| | | 5 | SE500 | 595 | 10 gram | | | |
| | | 5 | Blank | 0 | 10 gram | | | |
| | | 3 | STM10 | 12 | No | | | |
| | | 3 | SE100 | 127 | No | | | |
| | | 2 | SPan5 | 9 | No | | | |
| | | 2 | Blank | 0 | No | | | |
| | | 20 | None | - | 10 gram** | | | |

** = with antibiotics; ** = Naturally contaminated chicken faeces with Salmonella*

# 2. Participants

| Country | Institute/City |
|---|---|
| **Austria** | Institut für Medizinische Mikrobiologie und Hygiene<br>Nationale Referenzzentrale für Salmonellen, Graz |
| **Belgium** | Veterinary and Agrochemical Research Center (VAR)<br>Brussels |
| **Cyprus** | Cyprus Veterinairy Services, Laboratory for the Control of Foods of Animal Origin (LCFAO), Nicosia |
| **Denmark** | Danish Veterinary Laboratory<br>Copenhagen |
| **Finland** | National Veterinary and Food Research Institute, Kuopio Department<br>Kuopio |
| **France** | Agence Française de Sécurité Sanitaire des Aliments (AFSSA)<br>Laboratoire d'Etudes et de Recherches Avicoles et Porcines (LERAP)<br>Ploufragan |
| **Germany** | Federal Institute for Risk Assessment (BFR)<br>National Salmonella Reference Laboratory<br>Berlin |
| **Greece** | Veterinary Laboratory of Halkis<br>Halkis |
| **Hungary** | National Food Investigation Institute<br>Budapest |

| Country | Institute/City |
|---|---|
| **Ireland** | Department of Agriculture and Food<br>Central Veterinary Research Laboratory, Dublin |
| **Italy** | Istituto Zooprofilattico Sperimentale delle Venezie,<br>Centro Nazionale di Referenza per le Salmonellosi, Legnaro |
| **Luxembourg** | Laboratoire de Médecine Vétérinaire de l'Etat , Animal Zoonosis<br>Luxembourg |
| **The Netherlands** | Rijksinstituut voor Volksgezondheid en Milieu (RIVM)<br>Bilthoven |
| **Norway** | National Veterinary Institute, Section of Bacteriology<br>Oslo |
| **Portugal** | Laboratório Nacional de Investigaçã Veterinária<br>Lisboa |
| **Slovenia** | National Veterinary Institute, Veterinary Faculty<br>Ljubljana |
| **Spain** | Laboratorio de Sanidad Y Produccion Animal de Algete<br>Madrid |
| **Sweden** | National Veterinary Institute, Department of Bacteriology<br>Uppsala |
| **United Kingdom** | Veterinary Laboratories Agency , Department of Bacterial Diseases<br>New Haw, Addlestone |
| **United Kingdom** | Department of Agriculture for Northern Ireland, Veterinary Sciences Division, Bacteriology Department, Belfast |

# 3. Materials and Methods

## 3.1 Reference materials

Five batches of reference materials were prepared. For this purpose milk, artificially contaminated with a *Salmonella* strain was spray-dried (In 't Veld et al, 1996). The obtained highly contaminated milk powder (hcmp) was mixed with sterile (γ-irradiated) milk powder (Carnation, Nestlé, the Netherlands) to obtain the desired contamination level. The mixed powder was filled in gelatin capsules resulting in the final reference materials (RMs).
The target levels of the five batches of RMs were:

- 5 colony forming particles (cfp) per capsule for *Salmonella* Panama (SPan5);
- 10 and 100 colony forming particles (cfp) per capsule for *Salmonella* Typhimurium (STM10 and STM100);
- 100 and 500 colony forming particles (cfp) per capsule for *Salmonella* Enteritidis (SE100 and SE500).

Before filling the mixed powders into gelatin capsules, test batches of 60 capsules were prepared of each mixture to determine the mean number of cfp per capsule and the homogeneity of the mixture. The remaining mixed powders were stored at –20 $^{o}$C. If the test batch fulfilled the pre-set criteria for contamination level and homogeneity, the relevant mixed powders were filled into gelatin capsules and stored at -20 $^{o}$C.
The pre-set criteria were:

- mean contamination levels should lie between target level minus 30% and target level plus 50% (e.g. between 70 and 150 cfp if the target level is 100 cfp);
- for the homogeneity within one batch of capsules the maximum demand for the variation between capsules should be $T_2/(I-1) \leq 2$, where $T_2$ is a measure for the variation between capsules of one batch (see formula in Annex 1) and I is the number of capsules.

The contamination levels of the capsules were determined following the procedure as described by Schulten et al. (2000). Shortly the procedure is as follows:

- reconstitution of each capsule in 5 ml peptone saline solution in a Petri dish at (38.5 ± 1) $^{o}$C for (45 ± 5) minutes;
- repair of *Salmonella* by the addition of 5 ml molten double concentrated plate count agar (dPCA) to the reconstituted capsule solution, and after solidification incubation at (37 ± 1) $^{o}$C for (4 ± ½) hours;
- after incubation, 10 ml of molten double concentrated Violet Red Bile Glucose agar (dVRBG) was added as an overlayer and after solidification the plates were incubated for (20 ± 2) hours at (37 ± 1) $^{o}$C.

## 3.2 Faecal samples

### 3.2.1 General

Chicken faeces was obtained from poultry laying flocks. The faeces were tested for the presence or absence of *Salmonella* spp. For this purpose 10 portions of 10 g were each added to 90 ml BPW. After pre-enrichment at 37 $^{o}$C for 16-18 h, selective enrichment was carried out on MSRV. Furthermore, the cultures were plated-out on BGA and confirmed biochemically and serologically.
The suspected colonies of the positive faeces were isolated on TSI agar and sent for serotyping to the Diagnostic Laboratory for Infectious Diseases and Perinatal Screening (LIS/RIVM). All *Salmonella* cultures of the positive faeces were typed as *Salmonella* Muenchen. The faeces, bacteriologically positive for *Salmonella*, was used to prepare the naturally contaminated samples.
From another poultry laying flock, which was found negative for *Salmonella*, faeces was used to prepare the samples containing non-*Salmonella* competitive micro-organisms.
All faecal samples (*Salmonella* negative as well as *Salmonella* positive faeces) were mixed and homogenised with sterilised glycerol/peptone solution (mixing ratio 1:1). One liter of this solution consisted of 300 ml glycerol, 7 gram of peptone and 700 ml distilled water. After mixing all faeces samples with the glycerol/peptone solution, they were again analysed for the presence or absence of *Salmonella* and were stored at –(20 ± 2) $^{o}$C untill sending the samples to the National Reference Laboratories for *Salmonella*.
To test the possible influence of transport times and temperatures on the presence of *Salmonella* spp. in the positive faeces samples a limited investigation was carried out. For this purpose mixed faeces samples with glycerol/peptone solution were stored at roomtemperature and at +5 $^{o}$C for a certain period of time.

### 3.2.2 MPN of *Salmonella* in naturally contaminated faeces

To semi-quantify the number of Salmonellae in the *Salmonella* positive (mixed) faeces, a Most Probable Number (MPN) method was used. For this purpose, ten grams of faeces were each added to 90 ml of buffered peptone water (BPW) in a plastic bag and mixed by using a Stomacher 60 seconds for each sample. Next tenfold dilutions were prepared in BPW untill a concentration of 0.1 mg faeces per 100 ml BPW. This procedure was repeated ten times. The BPW jars with concentrations of 1000 mg till 0.1 mg faeces (per 100 ml BPW) were incubated and handled according to the same standard operating procedure as all other samples in this study with medium combinations MSRV/BGA, MSRV/XLD, MKTTn/BGA and MKTTn/XLD. After completion of the test the MPN was calculated using a complementary log-log link in SAS.Proc logistic (SAS Institute Inc, 2004)

### 3.2.3 Total bacterial count in faeces

For the naturally contaminated faeces with *Salmonella* as well as the negative faeces without *Salmonella* the total number of aerobic bacteria and the number of Enterobacteriaceae were investigated. The procedures of ISO 4833, respectively ISO 7402 were used for this purpose. Portions of 10 gram chicken faeces were homogenised into 90 ml pepton saline solution in a plastic bag. The content was mixed by using a stomacher (60 sec). Next tenfold dilutions were prepared in pepton saline solution. Four times one ml of each dilution was brought into 4 empty Petri-dishes (diameter 9 cm). To two of the dishes 25 ml of molten VRBG (Violet Red Bile Glucose Agar) was added to each dish. These plates were incubated at (37 ± 1) $^{o}$C for 22-26 hours after which the number of Enterobacteriaceae were counted. To the two other dishes 25 ml of molten Plate Count Agar (PCA) was added. These plates were incubated at (30 ± 1) $^{o}$C for (72 ± 3) hours for the enumeration of the total number of aerobic bacteria.

## 3.3 Optimisation dissolving procedure of capsules in BPW

For the detection of *Salmonella* spp. in the reference materials when added to faecal samples, it is of great importance that the capsules are completely dissolved. An experiment was set up to find out the best dissolving procedure by which the most number of samples were found positive. The following influencing parameters were tested: temperature of BPW, dissolving time in BPW and thawing procedure of the faeces. In each experiment jars with 90 ml BPW were labelled as follows: Blank (1 time), STM 10 (3 times), STM 100 (3 times), STM 10 control (1 time) and faeces control (1 time). Eight groups of experiments were carried out.

- The handling of the faeces in experiments numbered as ‘1’ was: thawing the faeces overnight at 5 $^{o}$C.
- The handling of the faeces in experiments numbered as ‘2’ was: thawing the faeces 4 hours at 5 $^{o}$C and subsequently 1 hour at 21-22 $^{o}$C.
- In the experiments named ‘A’ the BPW jars were, prior to the addition of the capsules, stored overnight at roomtemperature and the dissolving time of the capsules was 30 min at 37 $^{o}$C.
- In the experiments named ‘B’ the BPW jars were, prior to the addition of the capsules, stored overnight at 37 $^{o}$C and the dissolving time of the capsules was 30 min at 37 $^{o}$C.
- In the experiments named ‘C’ the BPW jars were, prior to the addition of the capsules, stored overnight at roomtemperature and the dissolving time of the capsules was 45 min at 37 $^{o}$C.
- In the experiments named ‘D’ the BPW jars were, prior to the addition of the capsules, stored overnight at 37 $^{o}$C and the dissolving time of the capsules was 45 min at 37 $^{o}$C.

All experiments were carried out with four medium combinations. These combinations were: MSRV and MKTTn as the selective enrichment medium and BGA and XLD as the plating

resulting in the four combinations MSRV/BGA, MSRV/XLD, MKTTn/BGA and MKTTn/XLD.

## 3.4 Design of the interlaboratory comparison study

### 3.4.1 Samples

Two weeks before the study the reference materials (35 individually numbered capsules) and 300 grams of negative faeces and 250 grams of positive faeces for *Salmonella* were mailed (with cooling devices) as dangerous goods to the participants. After arrival at the laboratory the capsules and faecal samples had to be stored at –20 °C until the start of the study. Details about mailing and handling of the samples and reporting of test results can be found in the Protocol (Annex 6), Standard Operation Procedure (Annex 7) and Test Report (Annex 8).
Ten control capsules had to be tested without faeces. Twenty-five capsules (numbered 1 – 25) were tested in combination with 10 grams of chicken faeces each (negative for *Salmonella*). Beside these artificially contaminated samples, also 20 samples (numbered N1 – N20) of 10 grams each of naturally contaminated faeces samples (with *Salmonella* Muenchen) were analysed. The types and the number of capsules and faeces samples to be tested are shown in Table 2.
In former interlaboratory comparison studies ten grams of faeces were each added to 225 ml BPW. However, according to ISO 6579 a dilution of the faeces in BPW of 1:10 should be respected. Therefore, in this study it was prescribed to bring 10 g of faeces (positive or negative for *Salmonella* ssp.) in 90 ml BPW.

*Table 2 Overview of the types and the number of the capsules to be tested per laboratory in the interlaboratory comparison study*

| Capsules | Control capsules (n = 10) No faeces added | Test samples (n=25) **with 10 g** *Salmonella*-negative faeces | Test samples (n=20) **with 10 g** *Salmonella*-positive faeces |
|---|---|---|---|
| *S.* Panama 5 | 2 | --- | --- |
| *S.* Enteritidis 100 | 3 | 5 | --- |
| *S.* Enteritidis 500 | --- | 5 | --- |
| *S.* Typhimurium 10 | 3 | 5 | --- |
| *S.* Typhimurium 100 | --- | 5 | --- |
| Blank | 2 | 5 | --- |
| No capsules | | --- | 20 |

### 3.4.2 Methods

During the workshop meeting at 15 and 16 May 2003 in Bilthoven (the Netherlands) it was decided that this interlaboratory comparison study would in principle have the same set-up as study IV, V and VI. Small changes were introduced in this study due to the amount of faeces which had to be added to the BPW. The following media were prescribed in this study VII (see also Standard Operation Procedure in Annex 7):

*Pre-enrichment in:*

- Buffered Peptone Water: BPW

*Selective enrichment in:*

- Rappaport-Vassiliadis medium with soya = RVS
- Mueller-Kauffmann Tetrathionate-novobiocin broth = MKTTn
- Modified semi-solid Rappaport Vassiliadis medium = MSRV

*Plating-out on:*

- Brilliant Green agar = BGA
- Xylose lysine desoxycholate agar = XLD

*Biochemical confirmation:*

- Urea, Triple Sugar Iron agar (TSI) and Lysine Decarboxylase (LDC)

Beside to the prescribed methods the NRLs were also allowed to use their own methods. This could be different medium combinations and/or investigation of the samples with a Polymerase Chain Reaction based method.

### 3.4.3 Temperature recording during shipment

To cool the content of the packages during shipment three cooling devices per package were included. For the control of exposure to abusive temperatures during shipment and storage so called micro temperature loggers were used to record the temperature during transport. These loggers are tiny sealed units in a 16 mm diameter and 6 mm deep stainless steel case. Each package contained one logger. The loggers were programmed by the CRL-*Salmonella* to measure the temperature every hour. Each NRL had to return the temperature recorder immediately after receipt to the CRL. At the CRL-Salmonella the loggers were read via the computer and all data from the start of the shipment until the arrival at the National Reference Laboratories were transferred to an Excell graphic which shows all recorded temperatures.

## 3.5 Accreditation/certification

Ten laboratories mentioned to be accredited for their quality system according to EN-ISO/IEC 17025 (labcodes 1, 2, 3, 4, 6, 9, 11, 12, 16 and 18). One laboratory mentioned to be certified according to ISO 9001. Six laboratories are planning to be accredited or certified in the near future and one laboratory reported not to be accredited or certified and mentioned no planning to do so in the near future.

## 3.6 Statistical analysis of the data

The results of the interlaboratory comparison study were statistically analysed in order to compare the results of the participating laboratories and the different types of samples and methods (selective enrichment and plating-out media).
Results were analysed using SAS (version 8.2). In order to detect differences among media, and capsules, logistic regression (using PROC GENMOD) was used. Correlation between observations were taken into account by using Generalized Estimating Equations (GEE). This was done by specifying contrasts which are shown as p-values.
The differences in performance from one particular laboratory are compared by contrasting specific laboratories to the mean of all laboratories. This is shown in figures for the artificially as well as for the naturally contaminated samples for all medium combinations and all six medium combinations separately.

# 4. Results

## 4.1 Reference materials

The level of contamination and the homogeneity of the test batches as well as of the final batches of capsules are presented in Table 3. All batches met the pre-set criteria as stated under 3.1. The enumerated minimum and maximum levels within each batch of capsules are given between brackets.

*Table 3 Level of contamination and homogeneity of SE, SPan and STM capsules*

| | Test batch (n=25) | | Final batch (n=25) | |
|---|---|---|---|---|
| | Mean cfp per capsule (min-max cfp) | Homogeneity ($T_2$ / (I-1) | Mean cfp per capsule (min-max cfp) | Homogeneity ($T_2$ / (I-1) |
| SE 100 | 117 (80-144) | 1.14 | 127 (96-156) | 1.28 |
| SE 500 | 585 (480-630) | 0.80 | 595 (400-750) | 1.21 |
| SPan 5 | 8 (5-13) | 0.53 | 9 (4-14) | 0.87 |
| STM 10 | 11 (5-17) | 0.75 | 12 (5-17) | 1.06 |
| STM 100 | 101 (80-134) | 1.11 | 96 (72-130) | 0.84 |

*cfp = colony forming particles; min = enumerated minimum cfp; max = enumerated maximum cfp*

*formula $T_2$ see Appendix 2; I is number of capsules; Demand for homogeneity T2/(I-1) ≤ 2*

## 4.2 Faecal samples

The results of the MPN method using various dilutions of positive chicken faeces and various medium combinations are shown in Table 4.

*Table 4* *Number of positive faeces samples (n = 10) with various faeces concentrations and four medium combinations*

| Medium combination | 1000 mg faeces in 100 ml BPW | 100 mg faeces in 100 ml BPW | 10 mg faeces in 100 ml BPW | 1 mg faeces in 100 ml BPW | 0,1 mg faeces in 100 ml BPW |
|---|---|---|---|---|---|
| | | | | | |
| MSRV/BGA | 9 | 10 | 10 | 3 | 1 |
| MSRV/XLD | 9 | 10 | 10 | 3 | 1 |
| | | | | | |
| MKTTn/BGA | 9 | 10 | 10 | 3 | 1 |
| MKTTn/XLD | 10 | 10 | 10 | 3 | 1 |
| | | | | | |

As shown in Table 4, the lowest dilution (1000 mg/100 ml BPW) did not always gave positive results for all ten samples, while a next dilution did. For the calculation of the actual MPN the concentration of 1000 mg faeces in a total of 100 ml BPW was not used. The calculated MPN of the positive faeces was 473 cfp per gram (95% confidence interval: 213-1054 cfp).
In Table 5 the total number of aerobic bacteria and the number of *Enterobacteriaceae* are shown of both (positive and negative) chicken faces samples.

*Table 5* *Number of aerobic bacteria and Enterobacteriaceae per gram of naturally contaminated faeces with Salmonella and faeces without Salmonella.*

| | Aerobic bacteria | *Enterobacteriaceae* |
|---|---|---|
| Naturally contaminated chicken faeces with *Salmonella* | $1.75 \times 10^9$ cfp/gram | $1.44 \times 10^4$ cfp/gram |
| Chicken faeces without *Salmonella* | $7.05 \times 10^8$ cfp/gram | $4.70 \times 10^5$ cfp/gram |

## 4.3 Optimisation dissolving procedure of capsules in BPW

The results of the experiments concerning the optimisation of the dissolving procedure of the gelatin capsules into BPW are shown in Table 6.

*Table 6 Number of positive capsules (n = 3) in relation to various treatments of capsules and/or faeces samples*

| | | Capsules + BPW 30 minutes at 37 °C | | | | | Capsules + BPW 45 minutes at 37 °C | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | MSRV | | MKTTn | | | MSRV | | MKTTn | |
| | | BGA | XLD | BGA | XLD | | BGA | XLD | BGA | XLD |
| STM 10 | 1A | 3 | 3 | 3 | 2 | 1C | 2 | 2 | 3 | 3 |
| | 2A | 0 | 0 | 1 | 0 | 2C | 3 | 3 | 3 | 1 |
| | | | | | | | | | | |
| | 1B | 3 | 2 | 3 | 2 | 1D | 3 | 3 | 3 | 3 |
| | 2B | 3 | 3 | 3 | 3 | 2D | 3 | 3 | 3 | 3 |
| | | | | | | | | | | |
| STM 100 | 1A | 3 | 3 | 3 | 2 | 1C | 3 | 3 | 3 | 3 |
| | 2A | 3 | 3 | 2 | 2 | 2C | 3 | 3 | 3 | 3 |
| | | | | | | | | | | |
| | 1B | 3 | 3 | 3 | 3 | 1D | 3 | 3 | 3 | 3 |
| | 2B | 3 | 3 | 3 | 3 | 2D | 3 | 3 | 3 | 2 |

*Group 1 : Thawing faeces overnight at 5 °C ; Group 2:Thawing faeces 4 hours at 5 °C and subsequently 1 hour at 21 °C ; Group A + C : BPW jars at 21 °C overnight prior to use ; Group B + D : BPW jars at 37 °C overnight prior to use; Group A + B: 30 min.dissolving time; Group C+D: 45 min dissolving time*

The most important difference between the left and right hand part of the table, i.e. difference in dissolving time at 37 °C, is found in group 2A. Irrespective of the medium combination almost all STM10 capsules were found to be negative when the faeces samples were thawed at +5 °C for 4 hours and subsequently for 1 hour at roomtemperature and when the capsules were dissolved in BPW for 30 minutes at 37 °C and the pre-warming temperature of the BPW was 21 °C. The outcome of these experiments resulted in a change in the Standard Operation Procedure for the dissolving of the gelatin capsules and handling of the faeces (see Annex 7), namely:

- Thawing faeces overnight at +5 °C;
- Prewarming BPW overnight at 37 °C;
- Dissolving time of capsules (before adding the faeces) for 45 min.

## 4.4 Technical data collaborative study for artificially and naturally contaminated samples

### 4.4.1 Pre-warming temperature of BPW

Before adding the capsules and/or faeces to the BPW, all jars had to be pre-warmed at (37 ± 1) $^{o}$C overnight. All laboratories except three met the criteria as set in the standard operation procedure. Laboratory 12 reported a starting incubation temperature of 35.5 $^{o}$C of the incubator and laboratory 15 a starting temperature of 33 $^{o}$C. Laboratory 19 reported an end temperature of 35.4 $^{o}$C.

### 4.4.2 Incubation time and temperature for dissolving the capsules

Before adding the chicken faeces to the pre-enrichment medium (BPW), the capsules had to be dissolved in the BPW at 37 $^{o}$C for 45 minutes. Sixteen laboratories (labcodes 1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 12, 15, 17, 18, 19 and 20) dissolved the capsules in forty-five minutes. Four laboratories used a dissolving time of more than 45 minutes, i.e. 46, 50, 50 and 80 minutes by respectively laboratories 13, 14, 16 and 21. One laboratory (labcode 15) started the dissolving with an incubator temperature of 34 $^{o}$C and another laboratory (labcode 5) at 35.8 $^{o}$C. Laboratory with labcode 19 reported a starting temperature of the incubator of 35.4 $^{o}$C and a final temperature of 35.9 $^{o}$C.

### 4.4.3 Incubation time and temperature of pre-enrichment

According to the standard operating procedure and the ISO 6579: 2002 the incubation time for the pre-enrichment is between 16 and 20 hrs. All laboratories except four laboratories (labcodes 2, 11, 12 and 16) incubated the BPW for the prescribed time (see Table 7).
The prescribed temperature for the incubation of BPW is (37 ± 1) $^{o}$C. All laboratories except four (labcode 12, 15, 19 and 20) incubated the BPW at the prescribed temperature.

*Table 7 Incubation time and temperatures of pre-enrichment medium BPW*

| Labcode | Incubation time (h:min) | Incubation temperature in °C (minimum-maximum) |
|---|---|---|
| ISO 6579: 2002 | 16 – 20 | 37 ± 1 |
| 1 | 19:48 | 36.5 – 37.1 |
| 2 | 20:30 | 37 – 37 |
| 3 | 17:45 | 36.0 – 36.2 |
| 4 | 18:00 | 36.6 – 36.6 |
| 5 | 16:50 | 36.8 - 36.8 |
| 6 | 20:00 | 37 – 37 |
| 7 | 20:00 | 37.0 - 37.0 |
| 8 | 18:00 | 37.0 – 37.0 |
| 9 | 19:23 | 37.0 – 37.8 |
| 11 | 22:20 | 37.3 – 37.7 |
| 12 | 21:30 | 35.5 – 36.8 |
| 13 | 18:00 | 36.5 – 36.5 |
| 14 | 17:45 | 36.1 – 36.8 |
| 15 | 18:00 | 33 – 37 |
| 16 | 22:00 | 37 – 37 |
| 17 | 19:25 | 37 – 37 |
| 18 | 19:55 | 36.5 - 36.6 |
| 19 | 19:40 | 35.1 – 35.7 |
| 20 | 19:35 | 35 – 37 |
| 21 | 19:15 | 36 -37 |

*Times and temperatures deviating from the prescribed ones are indicated as gray cells.*

### 4.4.4 Incubation time and temperature of selective enrichment media

*RVS*

The incubation temperature for RVS as mentioned in the standard operation procedure should be between 40.5 °C and 42.5 °C. All laboratories except for laboratory 19 incubated their RVS medium between these temperatures (see Table 8). The minimum incubation temperature for this laboratory was 40.3 °C. Sixteen NRLs incubated the RVS medium for the prescribed time of 21 to 27 hours. The minimum incubation time for NRLs 1, 11 and 21 was 20.11 h, 20.45 h and 20.45 h, respectively.

*MKTTn*

The prescribed time and temperature for incubation of MKTTn are 21-27 h at 36-38 °C, respectively. All laboratories except for NRL with labcode 1 incubated their MKTTn medium plates for the prescribed incubation time. The incubation time for laboratory 1 was 20.13 h. Three laboratories (labcode 5, 15 and 19) incubated their plates at a temperature between 35 °C and 37 °C. All other NRLs used the prescribed incubation temperature.

*Table 8 Incubation times and temperatures of selective enrichment media*

| | RVS | | MKTTn | | MSRV | |
|---|---|---|---|---|---|---|
| Labcode | Time (h:min) | Temp. (°C) | Time (h:min) | Temp. (°C) | Time (h:min) | Temp.(°C) |
| Prescribed* | 24 ± 3 | 41.5 ±1 | 24 ± 3 | 37 ± 1 | 24 ± 3 | 41.5±1 |
| 1 | 20:11-26:43 | 41.1 – 41.5 | 20:13-26:40 | 37.0 – 37.2 | 20:11-26:43 | 41.2 – 41.5 |
| 2 | 21:15-23:30 | 41 – 42 | 21:35-22:45 | 37 | 21:15-25:30 | 41 – 42 |
| 3 | 21:15-23:25 | 41.3 – 42.1 | 22:29-22:57 | 36.2 – 36.5 | 21:55-22:40 | 41.4 – 41.9 |
| 4 | 23:20-24:35 | 41.0 - 41.4 | 22:25-24:00 | 36.0 – 37.3 | 22:30-23:00 | 41.0 - 41.2 |
| 5 | 21:00-21:40 | 41.0 – 42.3 | 21:00-21:40 | 35.9 – 36.8 | 21:00-21:40 | 41.0 – 42.3 |
| 6 | 23:00-24:10 | 41.5 – 41.8 | 23:00-24:10 | 37 | 24:00 | 41.5 – 41.8 |
| 7 | 21:15-26:15 | 41.5 – 41.8 | 23:05-24:05 | 37.0 – 37.1 | 21:00-23:15 | 41.5 – 41.6 |
| 8 | 22:00-22:30 | 41.5 | 22:00-22:30 | 37.0 | 21:00-24:00 | 41.5 |
| 9 | 23:39-23:40 | 41.7 – 42.0 | 22:47-23:27 | 37.3 – 37.7 | 24:09-25:15 | 41.2 – 41.9 |
| 11 | 20:45-25:00 | 41.2 – 41.5 | 23:50-24:25 | 37.3 | 20:45-25:00 | 41.2 – 41.5 |
| 12 | -- | -- | 22:30 | 36.4 0 36.6 | 21:30-22:30 | 41.9 - 42.0 |
| 13 | 22:15-24:02 | 41.4 – 41.5 | 22:18-24:02 | 36 – 37 | 21:30-24:02 | 41.4 – 41.5 |
| 14 | 21:50-22:50 | 41.3 – 42.3 | 21:20-22:00 | 37.1 – 37.8 | 22:50-23:00 | 41.6 – 42.2 |
| 15 | 22:00 | 41 – 42 | 22:00 | 35 – 37 | 22:00 | 41 – 42 |
| 16 | 22:40-24:15 | 42 | 22:40-24:15 | 37 | -- | -- |
| 17 | 24:45-25:00 | 42.0 – 42.2 | 22:00-25:25 | 37 | 22:40-25:35 | 41.6 – 42.2 |
| 18 | 23:26-23:35 | 41.5 – 41.7 | 23:25-24:35 | 36.5 | 22:09-23:35 | 41.5 – 41.6 |
| 19 | 22:20-23:10 | 40.3 – 40.7 | 21:00-23:10 | 35.3 – 35.6 | 21:40-23:50 | 40.2 – 40.6 |
| 20 | 22:45-23:35 | 42 | 22:45-23:35 | 37 | 22:45-25:05 | 42 |
| 21 | 20:45-22:30 | 41 | 21:15-22:00 | 36.0 – 36.5 | 20:45-26:45 | 40 - 41 |

** Incubation times and temperatures according to SOP. Mminimum and maximum times and temperatures are indicated in this table (also see Annex 7). Times and temperatures deviating from the prescribed ones are indicated as gray cells.*

*MSRV*

The incubation time and temperature for MSRV according to the SOP should be between 21 – 27 h and (41.5 ± 1) °C, respectively. Three laboratories (labcodes 1, 11 and 21) used an incubation time of 20.11 h, 20.45 h and 20.45 h, respectively. All other laboratories complied with the required incubation time. All NRLs except two (labcodes 19 and 21) met the prescribed temperature of (41.5 ± 1) °C. These two laboratories incubated their plates at a temperature of 40.2 °C and 40 °C, respectively.

### 4.4.5 Composition of MKTTn

At the time of the last intercomparison study of 2002 the MKTTn medium with the composition as prescribed in ISO 6579 (2002) was not yet available as dehydrated medium. Presently, some manufacturers do prepare dehydrated MKTTn medium which is in accordance with the formula of the ISO 6579: 2002. However, the dehydrated MKTTn media of not all manufacturers are (exactly) in accordance with ISO 6579. For more details see Table 9.

*Table 9 Manufacturer and final pH of MKTTn medium*

| Manufacturer | Laboratory code | Final pH after preparation (temp.°C) |
|---|---|---|
| Oxoid according to ISO | 1 | 8.12 (25) |
| | 2 | 8.2 (21) |
| | 3 | 8.18 (25) |
| | 4 | 8.0 (25) |
| | 6 | 8.30 (23.3) |
| | 7 | 8.12 (25) |
| | 17 | 8.14 (26.5) |
| Home made according to ISO | 16 | 8.2 (25) |
| | 18 | 8.6 (20) |
| Biolife according to ISO | 13 | 8.13 (29) |
| | | |
| Becton Dickinson (own formula) | 12 | 8.2 (16) |
| Biokar (own formula) | 14 | 7.6 (23.8) |
| BioMerieux (own formula) | 8 | 8.0 (25) |
| | 15 | No information |
| | 20 | No information |
| Biorad (own formula) | 5 | No information |
| | 9 | No information |
| | 11 | No information |
| Oxoid “old formula” | 19 | 8.2 (RT) |
| | 21 | No information |

*RT = Room Temperature; pH according to ISO 6579 (2002): 8.2 ± 0.2 at 25 °C*

## 4.5 Control samples

*Control samples*

All laboratories except two (labcodes 11 and 12) tested the control samples (n = 10) with the requested six combinations of media, i.e. RVS, MKTTn and MSRV as the selective enrichment media and BGA and XLD als the isolation/plating out media. The laboratory with labcode 11 only tested the combinations RVS/BGA, MKTTn/BGA and MSRV/BGA. The laboratory with labcode 12 only tested MKTTn/XLD and MSRV/XLD.
None of the laboratories isolated *Salmonella* from the procedure control (C11: no capsule/no faeces) and one laboratory (labcode 17) isolated *Salmonella* from the faeces control (C12: no capsule/negative faeces) with medium combinations RVS/BGA and RVS/XLD.

*Blank capsules (n=2) without addition of faeces*

The blank capsules only contained sterile milk powder. For the analyses no faeces was added. All twenty participating laboratories did not isolate bacteria from these blank capsules.

Salmonella *Panama 5 capsules (n=2) without addition of faeces*

Three laboratories (labcodes 2, 9 and 16) failed to isolate *Salmonella* from one or both capsules containing S. Panama at a level of circa 5 cfp/capsule with some of the medium combinations (see Table 10).

*Table 10 Number of positive isolations per laboratory for SPan 5 (n=2) without addition of faeces*

| | Laboratory codes | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Medium combination** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **11** | **12** | **13** | **14** | **15** | **16** | **17** | **18** | **19** | **20** | **21** |
| RVS/BGA | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | -- | 2 | 2 | 2 | 1 | 2 | 2 | 2 | 2 | 2 |
| RVS/XLD | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | -- | -- | 2 | 2 | 2 | 1 | 2 | 2 | 2 | 2 | 2 |
| MKTTn/BGA | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | -- | 2 | 2 | 2 | 1 | 2 | 2 | 2 | 2 | 2 |
| MKTTn/XLD | 2 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 1 | -- | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| MSRV/BGA | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | -- | 2 | 2 | 2 | -- | 2 | 2 | 2 | 2 | 2 |
| MSRV/XLD | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | -- | 2 | 2 | 2 | 2 | -- | 2 | 2 | 2 | 2 | 2 |

*-- = not tested; Gray cells = Unexpected results*

Salmonella *Typhimurium 10 capsules (n=3) without addition of faeces*

All laboratories except the NRL with labcode 16 isolated *Salmonella* from all capsules containing *Salmonella* Typhimurium at a mean level of circa 10 cfp/capsule with all medium combinations (see Table 11). Laboratory 16 isolated *Salmonella* in one out of three capsules with RVS/XLD, MKTTn/BGA and MKTTn/XLD and in none with RVS/BGA. The combinations with MSRV were not tested by laboratory 16.

*Table 11* *Number of positive isolations per laboratory for STM 10 (n=3) without addition of faeces*

| Medium combination | Laboratory codes | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 |
| RVS/BGA | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | -- | 3 | 3 | 3 | 0 | 3 | 3 | 3 | 3 | 3 |
| RVS/XLD | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | -- | -- | 3 | 3 | 3 | 1 | 3 | 3 | 3 | 3 | 3 |
| MKTTn/BGA | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | -- | 3 | 3 | 3 | 1 | 3 | 3 | 3 | 3 | 3 |
| MKTTn/XLD | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | -- | 3 | 3 | 3 | 3 | 1 | 3 | 3 | 3 | 3 | 3 |
| MSRV/BGA | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | -- | 3 | 3 | 3 | -- | 3 | 3 | 3 | 3 | 3 |
| MSRV/XLD | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | -- | 3 | 3 | 3 | 3 | -- | 3 | 3 | 3 | 3 | 3 |

*-- = not tested; Gray cells = Unexpected results*

Salmonella *Enteritidis 100 capsules (n=3) without addition of faeces*

All laboratories except the NRL with labcode 16 isolated *Salmonella* from all capsules containing *S.* Enteritidis at a mean level of circa 100 cfp/capsule with all medium combinations (see Table 12). Laboratory 16 isolated *Salmonella* in two of three capsules with RVS/XLD, in one capsule with MKTTn/BGA and MKTTn/XLD and in none with RVS/BGA.

*Table 12* *Number of positive isolations per laboratory for SE 100 (n=3) without addition of faeces*

| Medium combination | Laboratory codes | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 |
| RVS/BGA | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | -- | 3 | 3 | 3 | 0 | 3 | 3 | 3 | 3 | 3 |
| RVS/XLD | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | -- | -- | 3 | 3 | 3 | 2 | 3 | 3 | 3 | 3 | 3 |
| MKTTn/BGA | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | -- | 3 | 3 | 3 | 1 | 3 | 3 | 3 | 3 | 3 |
| MKTTn/XLD | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | -- | 3 | 3 | 3 | 3 | 1 | 3 | 3 | 3 | 3 | 3 |
| MSRV/BGA | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | -- | 3 | 3 | 3 | -- | 3 | 3 | 3 | 3 | 3 |
| MSRV/XLD | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | -- | 3 | 3 | 3 | 3 | -- | 3 | 3 | 3 | 3 | 3 |

*-- = not tested; Gray cells = Unexpected results*

## 4.6 Results faeces samples artificially contaminated with *Salmonella* spp.

### 4.6.1 Results per type of capsule and per laboratory

*Blank capsules*

The blank capsules only contained sterile milk powder. Each NRL analysed 5 blank capsules, each in combination with 10 g *Salmonella* negative faeces. Only one NRL (labcode 6) isolated *Salmonella* from 2 of these expected blank samples, with medium combination MSRV/BGA and from 1 sample with medium combination MSRV/XLD. No other laboratory did isolate *Salmonella* from the blank capsules (as expected).

S. *Typhimurium 10 (STM10)*

In Table 13 the results are summarised of the *Salmonella*-negative faeces samples artificially contaminated with capsules containing STM10.
Five laboratories (labcode 1, 8, 13, 15 and 16) did not isolate *Salmonella* from all medium combinations. The maximum number of isolations for all medium combinations was only obtained by laboratory 19. For combinations RVS/BGA and RVS/XLD the maximum number of positives was obtained by laboratories 5, 18 and 19. For MKTTn/BGA and MKTTn/XLD the maximum number of isolations was obtained by laboratories 4, 5, 17, 19 and 21 and for MRSV/BGA and MSRV/XLD by laboratories 4, 12, 17, 18, 19 and 21.

*Table 13 Number of positive isolations per laboratory for STM 10 (n=5) with the addition of 10 g Salmonella negative chicken faeces*

| | Laboratory codes | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Medium combination** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **11** | **12** | **13** | **14** | **15** | **16** | **17** | **18** | **19** | **20** | **21** |
| RVS/BGA | 0 | 4 | 3 | 4 | 5 | 1 | 2 | 0 | 3 | 3 | -- | 0 | 3 | 0 | 0 | 4 | 5 | 5 | 0 | 3 |
| RVS/XLD | 0 | 4 | 3 | 4 | 5 | 1 | 2 | 0 | 3 | -- | -- | 0 | 1 | 0 | 0 | 4 | 5 | 5 | 0 | 4 |
| | | | | | | | | | | | | | | | | | | | | |
| MKTTn/BGA | 0 | 3 | 4 | 5 | 5 | 1 | 2 | 0 | 3 | 2 | -- | 0 | 3 | 0 | 0 | 5 | 0 | 5 | 1 | 5 |
| MKTTn/XLD | 0 | 4 | 4 | 5 | 5 | 1 | 1 | 0 | 3 | -- | 2 | 0 | 3 | 0 | 0 | 5 | 0 | 5 | 1 | 5 |
| | | | | | | | | | | | | | | | | | | | | |
| MSRV/BGA | 0 | 4 | 4 | 5 | 4 | 3 | 2 | 0 | 1 | 1 | -- | 0 | 3 | 0 | -- | 5 | 5 | 5 | 0 | 5 |
| MSRV/XLD | 0 | 4 | 4 | 5 | 4 | 2 | 2 | 0 | 1 | -- | 5 | 0 | 3 | 0 | -- | 5 | 5 | 5 | 0 | 5 |
| | | | | | | | | | | | | | | | | | | | | |
| All combinations (n=30) | **0** | **23** | **22** | **28** | **28** | **9** | **11** | **0** | **14** | **6** | **7** | **0** | **17** | **0** | **0** | **28** | **20** | **30** | **2** | **27** |

*-- = not tested*

S. *Typhimurium 100 (STM100)*

Considerably more positive isolations were found with the STM100 than with the STM10 capsules, in combination with *Salmonella*-negative faeces (see Table 14). Laboratories 4, 17 and 19 found all capsules positive for all medium combinations. Laboratories 1 and 15 were not able to isolate *Salmonella* from any medium combination.

*Table 14* *Number of positive isolations per laboratory for STM 100 (n=5) with the addition of 10 g Salmonella negative chicken faeces*

| | Laboratory codes | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Medium combination** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **11** | **12** | **13** | **14** | **15** | **16** | **17** | **18** | **19** | **20** | **21** |
| RVS/BGA | 0 | 4 | 3 | 5 | 5 | 2 | 1 | 2 | 5 | 2 | -- | 1 | 4 | 0 | 1 | 5 | 5 | 5 | 0 | 4 |
| RVS/XLD | 0 | 5 | 2 | 5 | 5 | 2 | 1 | 2 | 5 | -- | -- | 1 | 2 | 0 | 0 | 5 | 5 | 5 | 0 | 4 |
| | | | | | | | | | | | | | | | | | | | | |
| MKTTn/BGA | 0 | 5 | 3 | 5 | 5 | 3 | 2 | 1 | 5 | 4 | -- | 1 | 4 | 0 | 0 | 5 | 1 | 5 | 0 | 5 |
| MKTTn/XLD | 0 | 5 | 3 | 5 | 5 | 3 | 2 | 1 | 5 | -- | 4 | 1 | 4 | 0 | 0 | 5 | 1 | 5 | 0 | 5 |
| | | | | | | | | | | | | | | | | | | | | |
| MSRV/BGA | 0 | 5 | 3 | 5 | 4 | 5 | 1 | 1 | 4 | 2 | -- | 1 | 5 | 0 | -- | 5 | 5 | 5 | 0 | 5 |
| MSRV/XLD | 0 | 5 | 3 | 5 | 5 | 5 | 2 | 1 | 4 | -- | 5 | 1 | 5 | 0 | -- | 5 | 5 | 5 | 1 | 5 |
| | | | | | | | | | | | | | | | | | | | | |
| All combinations (n=30) | **0** | **29** | **17** | **30** | **29** | **20** | **9** | **8** | **28** | **8** | **9** | **6** | **24** | **0** | **1** | **30** | **22** | **30** | **1** | **28** |

-- = *not tested*

S. *Enteritidis 100 (SE100)*

Laboratories 13 and 15 were not able to isolate Salmonella from the SE100 capsules with any of the medium combinations (see Table 15). Only laboratories 4, 17, 18, 19 and 21 isolated the maximum number of five isolations for all medium combinations.

*Table 15* *Number of positive isolations per laboratory for SE 100 (n=5) with the addition of 10 g Salmonella negative chicken faeces*

| | Laboratory codes | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Medium combination** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **11** | **12** | **13** | **14** | **15** | **16** | **17** | **18** | **19** | **20** | **21** |
| RVS/BGA | 1 | 3 | 5 | 5 | 4 | 1 | 1 | 0 | 5 | 1 | -- | 0 | 4 | 0 | 1 | 5 | 5 | 5 | 0 | 5 |
| RVS/XLD | 1 | 3 | 5 | 5 | 5 | 1 | 1 | 0 | 5 | -- | -- | 0 | 3 | 0 | 0 | 5 | 5 | 5 | 1 | 5 |
| | | | | | | | | | | | | | | | | | | | | |
| MKTTn/BGA | 1 | 4 | 5 | 5 | 5 | 1 | 1 | 3 | 5 | 4 | -- | 0 | 4 | 0 | 0 | 5 | 5 | 5 | 2 | 5 |
| MKTTn/XLD | 1 | 4 | 5 | 5 | 5 | 1 | 1 | 3 | 5 | -- | 5 | 0 | 4 | 0 | 0 | 5 | 5 | 5 | 3 | 5 |
| | | | | | | | | | | | | | | | | | | | | |
| MSRV/BGA | 1 | 4 | 4 | 5 | 4 | 3 | 1 | 3 | 4 | 2 | -- | 0 | 4 | 0 | -- | 5 | 5 | 5 | 1 | 5 |
| MSRV/XLD | 1 | 4 | 4 | 5 | 4 | 2 | 1 | 3 | 4 | -- | 4 | 0 | 4 | 0 | -- | 5 | 5 | 5 | 2 | 5 |
| | | | | | | | | | | | | | | | | | | | | |
| All combinations (n=30) | **6** | **22** | **28** | **30** | **27** | **9** | **6** | **12** | **28** | **7** | **9** | **0** | **23** | **0** | **1** | **30** | **30** | **30** | **9** | **30** |

*-- = not tested*

*S. Enteritidis 500 (SE500)*

The maximum number of positives for capsules SE 500 and all medium combinations was only obtained by laboratories 4, 5, 14, 17, 18 and 19 (see Table 16). No *Salmonella* could be isolated from any medium combination by NRLs 1 and 15.

*Table 16* *Number of positive isolations per laboratory for SE 500 (n=5) with the addition of 10 g Salmonella negative chicken faeces*

| | Laboratory codes | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Medium combination** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **11** | **12** | **13** | **14** | **15** | **16** | **17** | **18** | **19** | **20** | **21** |
| RVS/BGA | 0 | 4 | 4 | 5 | 5 | 3 | 2 | 1 | 5 | 5 | -- | 0 | 5 | 0 | 2 | 5 | 5 | 5 | 1 | 4 |
| RVS/XLD | 0 | 4 | 4 | 5 | 5 | 3 | 2 | 1 | 5 | -- | -- | 0 | 4 | 0 | 0 | 5 | 5 | 5 | 3 | 4 |
| | | | | | | | | | | | | | | | | | | | | |
| MKTTn/BGA | 0 | 5 | 5 | 5 | 5 | 3 | 2 | 2 | 5 | 5 | -- | 1 | 5 | 0 | 1 | 5 | 5 | 5 | 4 | 5 |
| MKTTn/XLD | 0 | 5 | 5 | 5 | 5 | 3 | 1 | 4 | 5 | -- | 5 | 1 | 5 | 0 | 1 | 5 | 5 | 5 | 3 | 5 |
| | | | | | | | | | | | | | | | | | | | | |
| MSRV/BGA | 0 | 4 | 5 | 5 | 5 | 4 | 2 | 5 | 4 | 5 | -- | 0 | 5 | 0 | -- | 5 | 5 | 5 | 2 | 5 |
| MSRV/XLD | 0 | 4 | 5 | 5 | 5 | 4 | 2 | 5 | 4 | -- | 5 | 0 | 5 | 0 | -- | 5 | 5 | 5 | 2 | 5 |
| | | | | | | | | | | | | | | | | | | | | |
| All combinations (n=30) | **0** | **26** | **28** | **30** | **30** | **20** | **11** | **18** | **28** | **15** | **10** | **2** | **29** | **0** | **4** | **30** | **30** | **30** | **15** | **28** |

*-- = not tested*

The laboratory with labcode 18 found considerably less positive isolations with MKTTn than with RVS and MSRV. This laboratory used a pH of the MKTTn of 8.6 which is 0.2 above the maximum prescribed pH for MKTTn. This phenomenon was found for the STM10 and the STM100 capsules, but not for the SE100 and SE 500 capsules. Another laboratory with a

deviating pH (of 7.6) for MKTTn was laboratory 14, but here no low numbers were found with MKTTn.

In Figure 1 all positive isolations for all capsules and all medium combinations per laboratory are given.

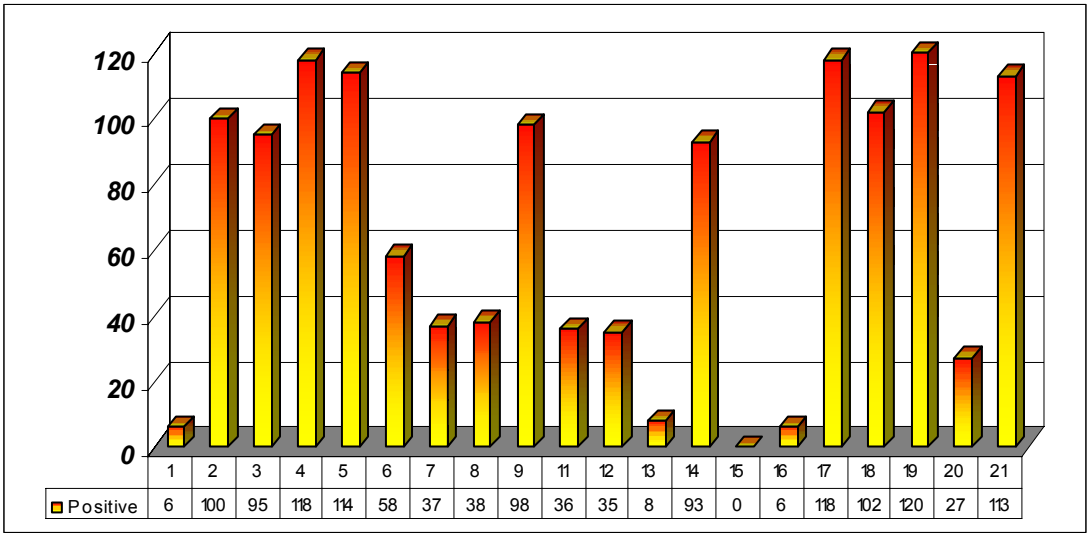


*Figure 1* *Number of positive isolations (max. 120) per laboratory (labcodes 1-9 and 11-21) for all capsules (n=20) and all medium combinations (n=6) with the addition of 10 g Salmonella negative chicken faeces*

### 4.6.2 Results per medium combination tested by all laboratories

The cumulative results per combination of selective enrichment and isolation medium for all kind of capsules with the addition of *Salmonella* negative faeces are shown in Table 17.
For the STM10 and STM100 capsules, the results obtained with MSRV are not significantly different from those obtained with MKTTn and RVS (see also Table 18).
For the SE100 and SE500 capsules significantly more positive isolations were obtained with MKTTn compared with RVS. The results of MKTTn versus MSRV are not significantly different from each other. The isolation of *Salmonella* for all capsules tested only revealed significantly more positive isolations for MSRV compared with RVS.
In case of significant differences (see Table 18) the p-value is <0.05 (indicated in gray cells) and by then the medium mentioned on the left side showed significant more psoitive results than the one mentioned on the right side in the same row.

*Table 17* *Number and percentages positive isolations for all participating laboratories (n = 20) with all capsules and all medium combinations*

| | | RVS | | MKTTn | | MSRV | |
|---|---|---|---|---|---|---|---|
| Capsules | | BGA* | XLD** | BGA* | XLD* | BGA** | XLD** |
| | | | | | | | |
| STM 10 | Positives | 45 | 41 | 44 | 44 | 47 | 50 |
| | % | 47 | 46 | 46 | 46 | 52 | 56 |
| | | | | | | | |
| STM 100 | Positives | 54 | 49 | 54 | 54 | 56 | 62 |
| | % | 57 | 54 | 57 | 57 | 62 | 69 |
| | | | | | | | |
| SE 100 | Positives | 51 | 50 | 60 | 62 | 56 | 58 |
| | % | 54 | 56 | 63 | 65 | 62 | 64 |
| | | | | | | | |
| SE 500 | Positives | 61 | 55 | 68 | 68 | 66 | 66 |
| | % | 64 | 61 | 72 | 72 | 73 | 73 |
| | | | | | | | |
| All | Positives | 211 | 195 | 226 | 228 | 225 | 236 |
| | % | 56 | 54 | 59 | 60 | 63 | 66 |
| | | | | | | | |

** = 19 participating laboratories (maximum number of positives for all capsules and all medium combinations = 380)*
*** = 18 participating laboratories (maximum number of positives for all capsules and all medium combinations = 360)*

*Table 18 Comparison of results (p-values) obtained on different media for capsules with the addition of Salmonella negative faeces*

| Media | SE 100 | SE 500 | STM 10 | STM 100 |
|---|---|---|---|---|
| MSRV vs RVS | 0.2343 | 0.1375 | 0.2896 | 0.1478 |
| MKTTn vs MSRV | 0.2101 | 0.8415 | 0.4289 | 0.2427 |
| MKTTn vs RVS | 0.0281 | 0.0116 | 0.9161 | 0.9304 |
| BGA vs XLD | 0.7328 | 0.7834 | 0.5715 | 0.7967 |

| Media | All capsules | SE capsules | STM capsules |
|---|---|---|---|
| MSRV vs RVS | 0.0232 | 0.1518 | 0.3861 |
| MKTTn vs MSRV | 0.8444 | 0.9664 | 0.4590 |
| MKTTn vs RVS | 0.1374 | 0.1472 | 0.0563 |
| BGA vs XLD | 0.0978 | 0.7105 | 0.0657 |

*In case of significant differences the p-value is <0.05 (indicated in gray cells) and by then the medium mentioned on the left side showed significant more positive results than the one mentioned on the right side in the same row*

### 4.6.3 Results of other medium combinations

Twelve laboratories also tested the capsules with their own medium combination(s). In Table 19 the results obtained with the prescribed medium combination giving the highest number of positive results (MSRV/XLD) are compared with the results of their best own medium being the own medium which give the highest number of positive results (see also Annex 2.)

*Table 19 Comparison of results between MSRV/XLD and best own medium combination*

| Labcode | Medium | STM10 | STM100 | SE100 | SE500 | All capsules |
|---|---|---|---|---|---|---|
| 1 | MSRV/XLD | 0 | 0 | 1 | 0 | 1 |
| | Own best | 0 | 0 | 1 | 0 | 1 |
| 5 | MSRV/XLD | 4 | 5 | 4 | 5 | 18 |
| | Own best | 5 | 5 | 5 | 5 | 20 |
| 7 | MSRV/XLD | 2 | 2 | 1 | 2 | 7 |
| | Own best | 2 | 2 | 1 | 2 | 7 |
| 8 | MSRV/XLD | 0 | 1 | 3 | 5 | 9 |
| | Own best | 0 | 1 | 3 | 5 | 9 |
| 9 | MSRV/XLD | 1 | 4 | 4 | 4 | 13 |
| | Own best | 3 | 5 | 5 | 5 | 18 |
| 12 | MSRV/XLD | 5 | 5 | 4 | 5 | 19 |
| | Own best | 5 | 5 | 4 | 5 | 19 |
| 13 | MSRV/XLD | 0 | 1 | 0 | 0 | 1 |
| | Own best | 0 | 1 | 0 | 1 | 2 |
| 14 | MSRV/XLD | 3 | 5 | 4 | 5 | 17 |
| | Own best | 3 | 5 | 4 | 5 | 17 |
| 15 | MSRV/XLD | 0 | 0 | 0 | 0 | 0 |
| | Own best | 0 | 0 | 0 | 0 | 0 |
| 19 | MSRV/XLD | 5 | 5 | 5 | 5 | 20 |
| | Own best | 5 | 5 | 5 | 5 | 20 |
| 20 | MSRV/XLD | 0 | 1 | 2 | 2 | 5 |
| | Own best | 1 | 0 | 3 | 4 | 8 |
| 21 | MSRV/XLD | 5 | 5 | 5 | 5 | 20 |
| | Own best | 5 | 5 | 5 | 5 | 20 |

The number of positive isolations found with MSRV/XLD is comparable to the number of positive isolations found with the lab's best own method. In four laboratories a few more positives were found with the 'best' own method when compared to MSRV/XLD (see Table 19; labcodes 5, 9, 13 and 20).

## 4.7 Comparison between laboratories

To be able to compare the positive isolations with the six medium combinations separately and all medium combinations together the differences between NRLs were calculated in relation to the average results of all laboratories (see Figure 2).
Laboratories with labcodes 4, 5, 17, 19 and 21 found significantly more positive results for all medium combinations.
The NRLs with labcodes 1, 7, 8, 13, 15 and 20 found with all medium combinations lower results when compared to the average of all laboratories.
The results of some laboratories are missing, for some medium combinations due to an incomplete set of data (laboratories 10, 11, 12 and 16).

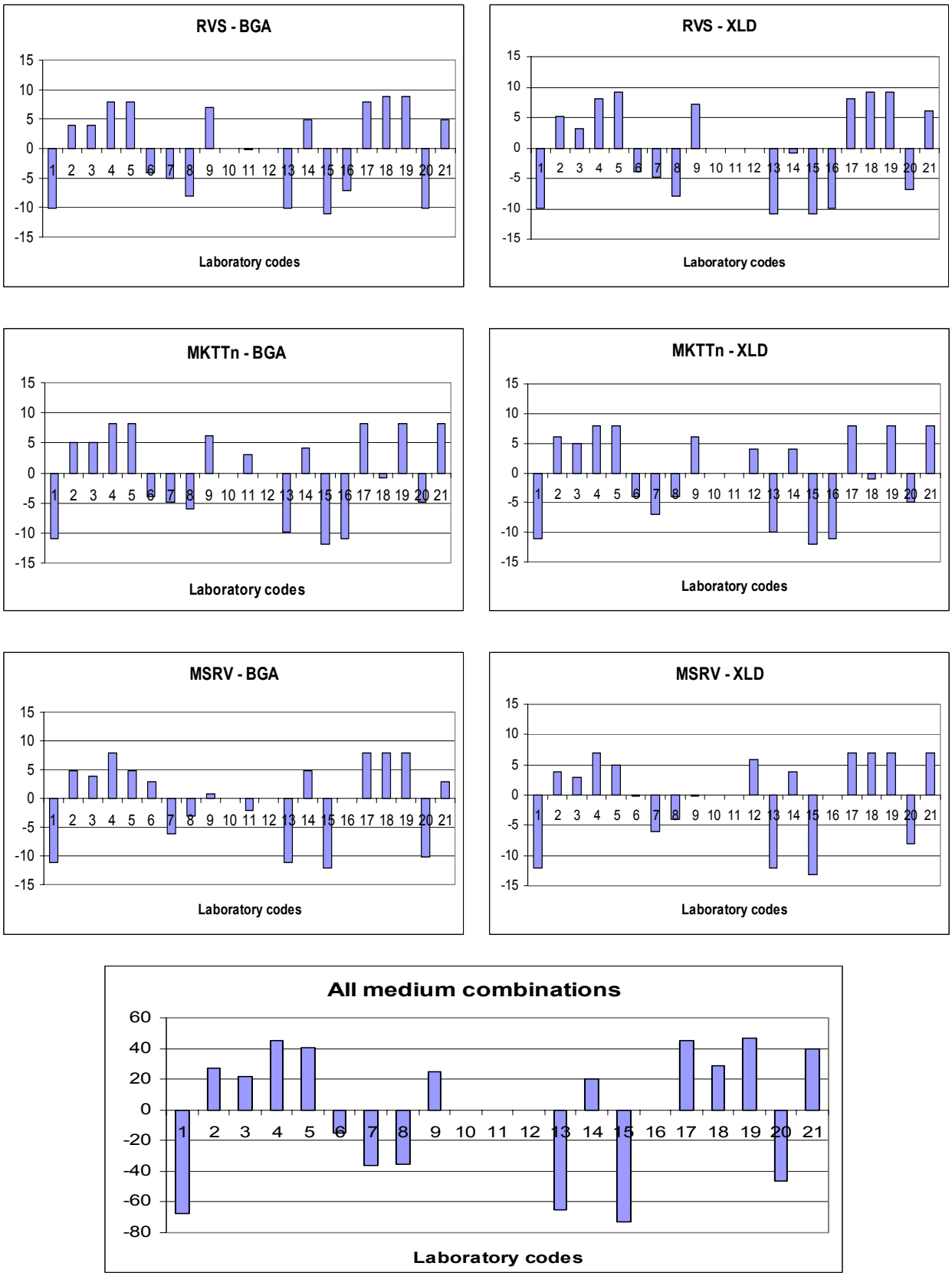


*Figure 2* *Results obtained with six medium combinations per laboratory compared to the average results of all laboratories (y-axis: arithmetical variation values) for the artificially contaminated samples*

## 4.8 Results faeces samples naturally contaminated with *Salmonella* spp.

The results in Table 20 and Figure 3 show that only one laboratory (labcode 19) was able to recover *Salmonella* from all faeces samples with the use of all medium combinations. Laboratories 5, 6 and 9 only scored the maximum of all samples with medium combinations MKTTn/BGA and MKTTn/XLD. Laboratory 14 scored the maximum with combinations MKKTn/BGA, MKTTn/XLD, MSRV/BGA and MSRV/XLD.

*Table 20* *Number of positive isolations per medium combination and per laboratory for naturally contaminated samples (n=20)*

| | Laboratory codes | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Medium combination** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **11** | **12** | **13** | **14** | **15** | **16** | **17** | **18** | **19** | **20** | **21** |
| | | | | | | | | | | | | | | | | | | | | |
| RVS/BGA | 1 | 2 | 0 | 1 | 19 | 18 | 1 | 14 | 18 | 4 | -- | 12 | 11 | 2 | 10 | 4 | 6 | 20 | 4 | 15 |
| RVS/XLD | 1 | 2 | 0 | 1 | 19 | 18 | 1 | 14 | 19 | -- | -- | 13 | 19 | 2 | 9 | 4 | 6 | 20 | 4 | 15 |
| | | | | | | | | | | | | | | | | | | | | |
| MKTTn/BGA | 8 | 2 | 15 | 7 | 20 | 20 | 4 | 0 | 20 | 14 | -- | 18 | 20 | 2 | 4 | 9 | 9 | 20 | 3 | 17 |
| MKTTn/XLD | 8 | 2 | 15 | 7 | 20 | 20 | 6 | 6 | 20 | -- | 19 | 18 | 20 | 2 | 11 | 9 | 9 | 20 | 3 | 17 |
| | | | | | | | | | | | | | | | | | | | | |
| MSRV/BGA | 3 | 0 | 7 | 2 | 17 | 19 | 3 | 17 | 14 | 9 | -- | 19 | 20 | 2 | -- | 5 | 5 | 20 | 10 | 14 |
| MSRV/XLD | 5 | 0 | 7 | 2 | 19 | 19 | 3 | 17 | 14 | -- | 15 | 19 | 20 | 2 | -- | 5 | 5 | 20 | 10 | 14 |
| | | | | | | | | | | | | | | | | | | | | |

*-- = not tested*

*Table 21* *Overall results of all participating laboratories and all medium combinations (n=6) for the naturally contaminated faeces with Salmonella (n=20)*

| | | RVS | | MKTTn | | MSRV | |
|---|---|---|---|---|---|---|---|
| Capsules | | BGA* | XLD** | BGA* | XLD* | BGA** | XLD** |
| | | | | | | | |
| None | Positives | 162 | 167 | 212 | 232 | 186 | 196 |
| | % | 43 | 46 | 56 | 61 | 52 | 54 |
| | | | | | | | |

** = 19 participating laboratories (maximum number of positives for all capsules and all medium combinations = 380)*

*** = 18 participating laboratories (maximum number of positives for all capsules and all medium combinations = 360)*

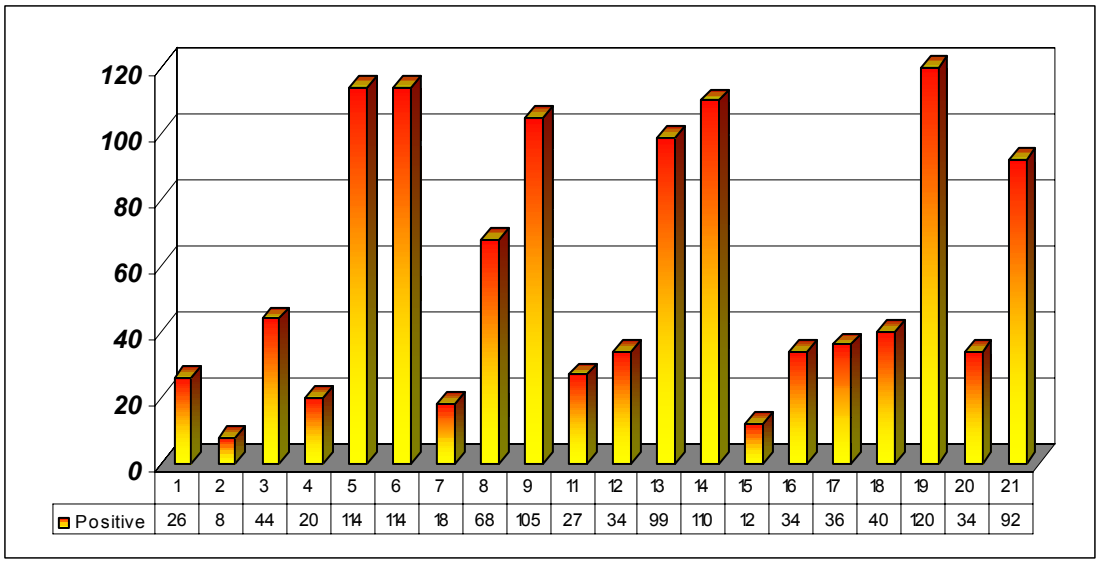


*Figure 3* *Number of positive isolations (max. 120) per laboratory (labcodes 1-9 and 11-21) for all medium combinations (n=6) when analysing 10 g Salmonella positive faeces*

The cumulative results of all laboratories for the naturally contaminated samples per medium combination are given in Table 21. The isolation of *Salmonella* from the naturally contaminated samples showed more positive isolations with MKTTn and MSRV than with RVS. These differences were significant (see Table 22). The comparison between BGA and XLD revealed that for XLD significantly more positive isolations were obtained. In case of significant differences, the medium mentioned on the left side showed significant more positive results than the one mentioned on the right in the same row.

*Table 22* *Comparison of results (p-values) obtained on different media for naturally contaminated samples*

| Media | p-values |
|---|---|
| MSRV vs RVS | 0.0454 |
| MKTTn vs MSRV | 0.2991 |
| MKTTn vs RVS | 0.0230 |
| XLD vs BGA | 0.0049 |

*In case of significant differences the p-value is <0.05 (indicated in gray cells)*

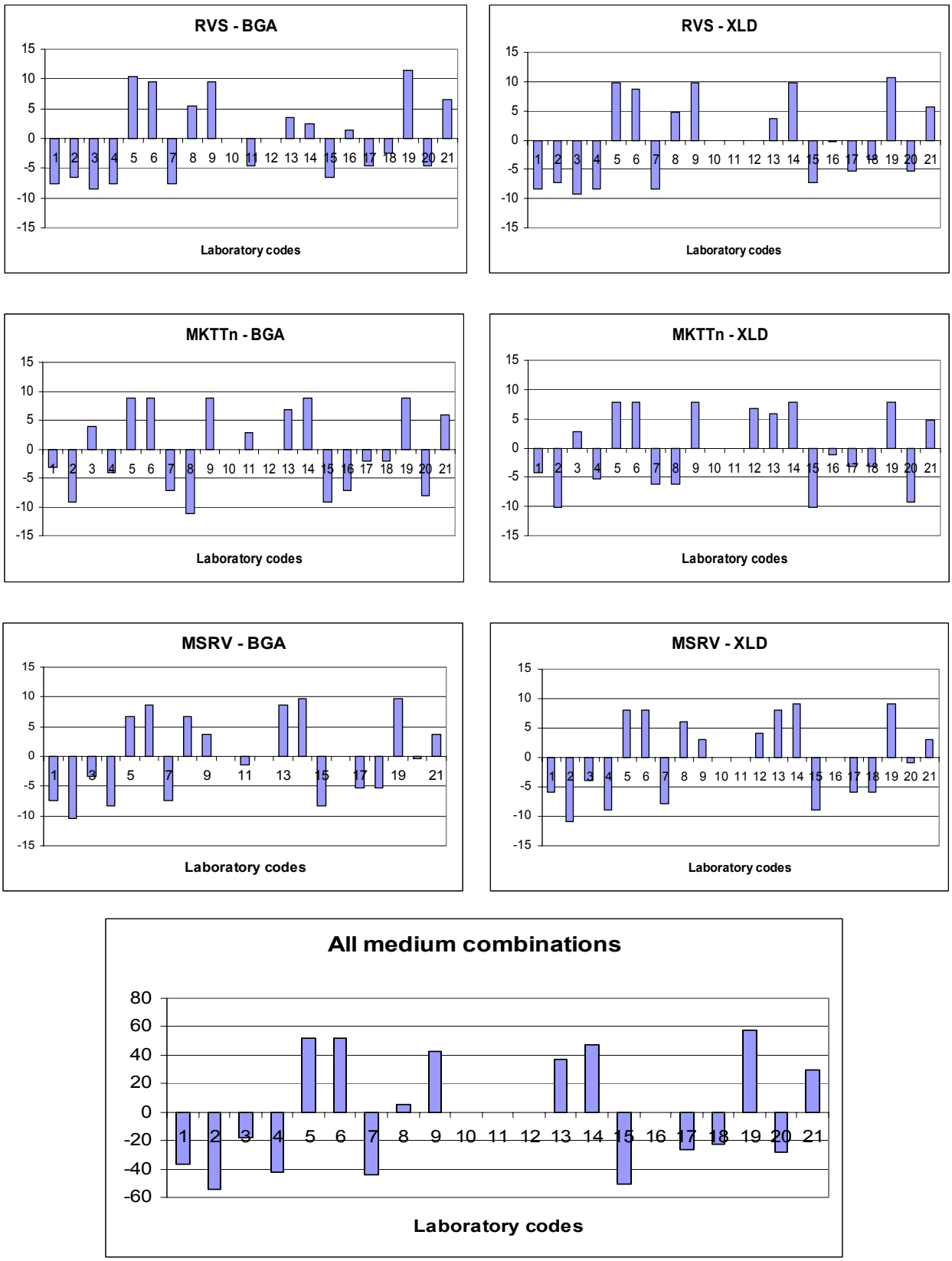


*Figure 4* *Results obtained with six medium combinations for the naturally contaminated samples per laboratory compared to the average results of all laboratories (y-axis: arithmetical variation values)*

To be able to compare the positive isolations with the six medium combinations separately and all medium combinations together the differences between NRLs were calculated in relation to the average results for all NRLs (see Figure 4).

## 4.9 PCR

Seven laboratories applied the PCR as their additional detection technique (laboratories with labcodes 2, 3, 5, 8, 18, 20 and 21).

*Table 23 Details on the Polymerase Chain Reaction method, used as own method during the interlaboratory comparison study by seven laboratories*

| Labcode | Volume of BPW (µl) | Volume of DNA sample (ml) | Volume DNA added to PCR (µl) |
|---|---|---|---|
| 2 | 2000 | 100 | 5 |
| 3 | 1000 | 80 | 5 |
| 5 | 1000 | 50 | 2 |
| 8 | 1000 | 50 | 2 |
| 18 | 10 | 200 | 5 |
| 20 – 1* | 1000 | 50 | 5 |
| 20 – 2* | 1000 | 50 | 5 |
| 20 – 3* | 500 | 650 | 5 |
| 21 | 1000 | 100 | 5 |

** Laboratory with labcode 20 used three different PCRs to detect Salmonella in chicken faeces.*

All laboratories except for laboratories with labcodes 3 and 21 used a PCR which were commercially available. Laboratory with labcode 5 only carried out PCR on the control samples C1-C12. The NRL with labcode 20 tested all samples with three different PCR tests. The PCR results of these seven laboratories are shown in the tables 4.1 - 4.6 in Annex 4. The PCR on the control samples revealed excellent results in relation to the bacteriological methods except for laboratory 21. This laboratory tested the blank control samples and the method controls as (false) positive.

For the naturally contaminated samples four laboratories (labcodes 2, 3, 8 and 21) tested all 20 samples positive in the PCR. With the bacteriological method these laboratories tested respectively two, fifteen, eighteen and seventeen samples positive. Laboratory 18 tested all samples negative in the PCR and ten samples positive with the bacteriological methods. The NRL with labcode 20 tested the samples in three different PCR tests. More positives were found with PCR-A (20) than with PCR-B (18) and also with PCR-C (10) concerning the naturally contaminated samples. With the bacteriological method this laboratory found ten positive samples.

For the artificially contaminated samples the PCR results of laboratories 2, 20 and 21 were comparable to the bacteriological methods. However, laboratory 21 tested one blank capsule (false) positive with the PCR. The results obtained by the NRLs with labcodes 3 and 18

revealed much more positive results with the bacteriological method than with their PCR methods.
Laboratory 20 found the number of positive results for the artifically contaminated samples comparable to the number of positive results as found with the PCR-A, PCR-B and PCR-C method and the bacteriological methods.

## 4.10 Transport of samples

The temperature recorders were returned immediately after receipt to CRL-*Salmonella* by eighteen NRLs. One laboratory received the package but did not return the temperature recorder in time. Print-outs of the temperature recorders can be found in Annex 5. The parcels to one NRL were sent back to CRL-*Salmonella* by customs of that particular country.
The minimum number of days for transport was 1 day and the maximum was eleven days. Four shipments were done by air (labcodes 1, 6, 8 and 16) and the rest by road transport. The average number of transport time was 5.2 days. Details on transport times and temperatures are given in Table 24. In this table also the number of positives found for the artificially contaminated samples as well as for the naturally contaminated samples per laboratory are indicated. This was done to try to find a relation between transport times and/or transport temperatures and number of positives. Further details are discussed in Chapter 5.

*Table 24 Transport times and temperatures of the parcels*

| Lab code | Transport time (hours) | Number of hours between 0.5-5.0 °C | Number of hours between 5.5-10.0 °C | Number of hours between 10.5-15.0 °C | Number of hours between 15.5-20.0 °C | Number of positives artifically contaminated samples (max.120) | Number of positives naturally contaminated samples (max.120) |
|---|---|---|---|---|---|---|---|
| 1 | 97 | 5 (5%) | 46 (46%) | 27 (28%) | 19 (20%) | 6 | 26 |
| 2 | 50 | 10 (20%) | 40 (80%) | 0 | 0 | 100 | 8 |
| 3 | 159 | 103 (65%) | 56 (35%) | 0 | 0 | 95 | 44 |
| 4 | 72 | 31 (43%) | 41 (57%) | 0 | 0 | 118 | 20 |
| 5 | 69 | 26 (38%) | 43 (62%) | 0 | 0 | 114 | 114 |
| 7 | 98 | 43 (44%) | 55 (56%) | 0 | 0 | 37 | 18 |
| 8 | 229 | 123 (54%) | 68 (30%) | 28 (12%) | 10 (4%) | 38 | 68 |
| 9 | 242 | 40 (16%) | 101 (42%) | 101 (42%) | 0 | 98 | 105 |
| 11 | 22 | 2 (9%) | 20 (91%) | 0 | 0 | 36 | 27 |
| 12 | 73 | 10 (14%) | 44 (60%) | 19 (26%) | 0 | 35 | 34 |
| 13 | 248 | 59 (24%) | 95 (38%) | 28 (11%) | 66 (27%) | 8 | 99 |
| 14 | 44 | 18 (40%) | 24 (55%) | 2 (5%) | 0 | 93 | 110 |
| 15 | 167 | 44 (26%) | 54 (32%) | 69 (41%) | 0 | 0 | 12 |
| 16 | 244 | 3 (1%) | 156 (64%) | 85 (35%) | 0 | 6 | 34 |
| 18 | 98 | 59 (60%) | 39 (40%) | 0 | 0 | 102 | 40 |
| 19 | 23 | 9 (39%) | 14 (61%) | 0 | 0 | 120 | 120 |
| 20 | 191 | 16 (8%) | 128 (67%) | 47 (25%) | 0 | 27 | 34 |
| 21 | 70 | 45 (64%) | 25 (36%) | 0 | 0 | 113 | 92 |

# 5. Discussion

*Faecal samples*

To stabilise the micro-organisms (*Salmonella* and background flora) in the faeces (positive and negative for *Salmonella*) the faeces has been mixed with a peptone/glycerol solution and stored at -20 $^{o}$C. However, during transport, these samples will thaw and after receipt at the NRL they are frozen again. The effect of this freezing and thawing, as well as the effect of the peptone/glycerol solution on the different micro-organisms in the faeces is not fully understood and may result in different 'behaviour' of the strains than may be the case in 'fresh' routine samples.
Additional to earlier studies the MPN of *Salmonella* in naturally contaminated faeces was determined as well as the total bacterial count in both positive and negative chicken faeces samples. By determining the MPN, an indication of the number of *Salmonella* spp. in the naturally contaminated faeces is obtained. The total bacterial count was determined to built up history about the possible effect of the total number of competitive bacteria in the chicken faeces on the detection of *Salmonella*. For the calculation of the actual MPN the concentration of 1000 mg faeces in a total of 100 ml BPW was not used. The lowest dilution did not always gave positive results for all ten samples, while a next dilution did. This is probably caused by the overgrowth of competitive bacteria in the chicken faeces samples.

*Optimisation dissolving procedure of capsules in pre-enrichment medium BPW*

Complete dissolving of the gelatin capsules in the BPW is essential for the detection of *Salmonella* in the capsules. The dissolving of the capsules is influenced by the incubation time and temperature of the BPW and the thawing time and temperature of the chicken faeces. The results in this report showed an increase in positivity, when the BPW was pre-warmed at 37 $^{o}$C, the dissolving time of the capsules in the BPW was longer and the thawing of the chicken faeces was done overnight at +5 $^{o}$C. This all resulted in changes in the Standard Operation Procedure for the present study. To find out whether the improved SOP also resulted in improved results (being more positives) in the interlaboratory study, a comparison was made with three earlier studies. In Table 25 the number of positives found in studies IV to VII are summarised for the artificially contaminated samples when using MSRV. In this Table an obvious increase in the percentages of positives for the capsules containing *Salmonella* Enteritidis for study VII can be seen. It is very likely that this increase has been a result of the improved SOP.

*Table 25 Results of artificially contaminated faeces tested on MSRV of four studies*

| Study number | Year of study | STM 10 | STM 100 | SE 100 | SE 500 |
|---|---|---|---|---|---|
| IV | 1999 | 57 % | 80 % | 39 % | 55 % |
| V | 2000 | 55 % | 76 % | 23 % | 56 % |
| VI | 2002 | 36 % | 62 % | 32 % | 52 % |
| VII | 2003 | 56 % | 69 % | 64 % | 73 % |

*Control samples*

One laboratory (labcode 17) found *Salmonella* in the negative faeces control, due to a possible cross-contamination or mislabelling of the BPW jars. Three laboratories (labcodes 2, 9 and 16) found only one SPan 5 control sample positive (out of 2), and one laboratory (labcode 16) failed to test all STM10 and SE100 control capsules positive for all used medium combinations. Statistically it is possible that at low contamination levels (< 10 cfu/capsule) occasionally a negative capsule can be found. This may be the explanation for the negative results found with SPan 5. Statistically it is also possible that occasionally a STM10 capsule is found negative. However, it is not likely to find 2 or 3 STM10 capsules negative at the same time. Beside the negative results for the control samples, laboratory 16 also found many negative results with the artificially contaminated samples. A possible explanation could have been the long transport time of the samples (244 h) at elevated temperatures (5-15 $^{o}$C; see Table 24).

*Selective enrichment media for artificially contaminated samples*

Each laboratory was asked to test the media as mentioned in ISO 6579: 2002 (E) which prescribes the use of RVS and MKTTn as selective enrichment media. Since study IV also MSRV is tested in comparison with the earlier mentioned media. After the amended ISO 6579 was published in 2002, some media manufacturers produce MKTTn with a composition which is in accordance with the ISO. Not all laboratories used MKTTn with the composition according to ISO. Two laboratories used MKTTn with a pH outside the range as mentioned in the ISO. The influence of the deviating pH was not clear. It might have influenced the results of laboratory 18 (pH 8.6) when testing the STM capsules, but the effect was not found for the SE capsules. The results of laboratory 14 with a pH of the MKTTn medium of 7.6 were not deviating when MKTTn was compared with RVS and MSRV, irrespective of the capsules used.

The contrast results in p-values for all capsules and the SE capsules separately showed that with MSRV significant more positive results were found than with RVS for the artificially contaminated samples. Although also more positive isolations were found with MSRV in relation to MKTTn, this difference was not significant.

*Selective enrichment media for naturally contaminated samples*

In this study slightly more positive isolations were found with MKTTn in relation to MSRV and much more positive isolations in relation to RVS. The differences between MKTTn versus RVS and MSRV versus RVS were significant, but the difference between MKTTn and MSRVwas not significant. It seems therfore to be an advantage to use MSRV or MKTTn above RVS for the detection of *Salmonella* spp. in chicken faeces.

*Plating-out media*

The choice of the plating out media BGA or XLD did not influence the results of the artificially contaminated samples significantly, although for the MSRV and MKTTn slightly more positive isolations were found with XLD when compared to BGA. For the naturally contaminated samples more positive isolations were found with XLD and this difference was significant for all medium combinations together.

*PCR*

With the PCR method all control samples were tested correctly except for one laboratory (labcode 21). This laboratory not only tested the blank control capsules positive but also the procedure control (no capsule/no faeces) and the faeces control (only faeces negative for *Salmonella*). Cross contamination is probably the most plausible explanation here. In average over the six laboratories performing PCR, comparable results were found with the PCR and the bacteriological methods when testing the artificially contaminated samples. However, for the naturally contaminated samples, most laboratories found more samples positive with the PCR method than with the bacteriological methods. The unknown influence of freezing and thawing and the mixing of the faeces with the peptone/glycerol might play a role as well.

*Transport of the samples*

In the parcels containing the reference materials and the faeces samples also temperature recorders were included. The temperature was measured every hour during transport. Immediate after arrival at the NRL, the recorder was returned to the CRL-*Salmonella* where it was read with a computer device.
The transport time and temperature differed considerably among the different countries. Nine laboratories tested less than 50% positive for the artifically contaminated samples. Seven of these laboratories recorded a temperature during transport of more than 10.5 $^{o}$C.
The relation in the number of positives found for the naturally contaminated samples with the transport times and temperatures was less obvious. Eleven laboratories tested less than 50 positive samples with all medium combinations and only five of these laboratories recorded a temperature of more than 10.5 $^{o}$C

*Performance of laboratories*

Four laboratories (labcodes 10, 11, 12 and 16) did not test all six medium combinations, which made comparison of all results impossible. Seven laboratories (labcodes 1, 6, 7, 8, 13, 15 and 20) scored systematically below the average results of all laboratories for the artificially contaminated samples for all six medium combinations. Nine laboratories (labcodes 1, 2, 3, 4, 7, 15, 17, 18 and 20) scored systematically below the average results for the naturally contaminated samples. Four laboratories (labcodes 1, 7, 15 and 20) scored below the average results of all laboratories for the artificially as well as the naturally contaminated samples. In the performance of the bacteriological tests of three of these four laboratories (labcodes 7, 15 and 20) some minor technical deviations were noticed. These deviations were: the laboratories with labcode 7 and 15 used BPW with a deviating composition, both laboratories 15 and 20 used a ready-to-use medium for MKTTn from the same manufacturer and did not measure the pH before testing and furthermore, the laboratory with labcode 7 used plating-out media with a pH of 6.55 and 6.54 for respectively BGA (prescribed 7.0) and XLD (prescribed 7.4 ± 0.2). However, the influences of these technical deviations on the results are unknown.

# 6. Conclusions

- It was easier to isolate *Salmonella* from capsules with a high level of *Salmonella* Enteritidis (595 cfp/capsule), combined with (*Salmonella* negative) faeces than, in declining order, from capsules with a high level of *Salmonella* Typhimurium (96 cfp/capsule) or a low level of *Salmonella* Enteritidis (127 cfp/capsule), and from capsules with a low level of *Salmonella* Typhimurium (12 cfp/capsule).

- MSRV and MKTTn are sensitive media for the detection of *Salmonella* spp. in chicken faeces. For the naturally contaminated samples MKTTn showed slightly more positive results than MSRV and for the artificially contaminated samples MSRV showed slightly more positive results than MKTTn.

- Significantly more positive isolations were obtained by using XLD as the plating-out/isolation medium in comparison with the use of BGA except in combination with the selective enrichment in RVS concerning the artificially contaminated samples.

- The level of positive isolations was higher for the artificially contaminated samples than for the naturally contaminated samples.

- The dissolving procedure of the capsules in BPW and the thawing of the faeces was changed in this study. More positive isolations were obtained when the BPW was pre-warmed overnight at 37 $^{o}$C before adding the capsules to the BPW. Also more positive isolations were obtained when the thawing of the faeces was done overnight at +5 $^{o}$C and the dissolving time of the capsules was extended to 45 minutes before addition of the faeces.

- In comparison with earlier studies the optimised dissolving procedure seem to have had a positive effect on the number of positives detected with the SE capsules (both SE100 and SE500) in combination with *Salmonella* negative faeces.

- In this study the parcels containing the reference materials and the faeces samples also contained temperature recorders. These recorders gave important information about the temperature during transport. The combination of long transport time at elevated temperatures (> 10 $^{o}$C), seem to effect negatively the number of positive isolations in the artificially contaminated samples and to a lesser extent in the naturally contaminated samples.

- Four laboratories did not test all six medium combinations, which made comparison of all results impossible. Seven laboratories scored systematically below the average results of

all laboratories for the artificially contaminated samples for all six medium combinations. Nine laboratories scored systematically below the average results for the naturally contaminated samples. Four laboratories scored below the average results of all laboratories for the artificially as well as the naturally contaminated samples. In this study no further follow-up was undertaken for these laboratories. For future studies procedures will be set up to assist these laboratories in searching the possible cause of the poor performance by sending extra reference materials and/or faeces samples

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# Annex 1. Calculation of $T_2$

Calculation of $T_2$ in homogeneity studies

The variation between capsules of one batch of reference materials is calculated by means of the so-called $T_2$ statistic (Heisterkamp et al., 1993).

$$T_2 = \sum_i [ (z_i - z_+ / I)^2 / (z_+ / I) ]$$

where, $z_i$ = count of one capsule ($i$)
$z_+$ = sum of counts of all capsules
I = total number of capsules analysed

In case of a Poisson distribution $T_2$ follows a $\chi^2$ -distribution with (I-1) degrees of freedom. In this case, the expected $T_2$-value is the same as the number of degrees of freedom. Hence $T_2/(I-1)$ is expected to be equal to one. For the variation between capsules of one batch, the Poisson distribution is the theoretical smallest possible variation which could be achieved. However overdispersion is expected and $T_2/(I-1)$ will mostly be larger than 1 (Heisterkamp et al., 1993). The general accepted variation for a batch will therefore be $T_2/(I-1) \leq 2$.

# Annex 2. Results by laboratory, sample and medium combination

*Table 2.1 Number of positive isolations per laboratory with own medium combinations for all control samples*

| | Medium combination | | Capsules | | | |
|---|---|---|---|---|---|---|
| Labcode | Selective enrichment | Isolation medium | Blank (n=2) | SPan 5 (n=2) | STM 10 (n=3) | SE 100 (n=3) |
| 1 | RVS | BBLS | 0 | 2 | 3 | 3 |
| | MKTTn | BBLS | 0 | 2 | 3 | 3 |
| 5 | RVS | SMID | 0 | 2 | 3 | 3 |
| | MKTTn | SMID | 0 | 2 | 3 | 3 |
| | MSRV | SMID | 0 | 2 | 3 | 3 |
| 7 | Diasalm | BGA | 0 | 2 | 3 | 3 |
| | Diasalm | XLD | 0 | 2 | 3 | 3 |
| 8 | RVS | Rambach | 0 | 2 | 3 | 3 |
| | MKTTn | Rambach | 0 | 2 | 3 | 3 |
| | MSRV | Rambach | 0 | 2 | 3 | 3 |
| 9 | RVS | XLT4 | 0 | 2 | 3 | 3 |
| | MKTTn | XLT4 | 0 | 2 | 3 | 3 |
| | MSRV | XLT4 | 0 | 2 | 3 | 3 |
| 11 | RVS | XLT4 | 0 | 2 | 3 | 3 |
| | MKTTn | XLT4 | 0 | 2 | 3 | 3 |
| | MSRV | XLT4 | 0 | 2 | 3 | 3 |
| 12 | MKTTn | Rambach | 0 | 2 | 3 | 3 |
| | MSRV | Rambach | 0 | 2 | 3 | 3 |
| | TBG | XLD | 0 | 2 | 1 | 3 |
| | TBG | Rambach | 0 | 2 | 1 | 3 |
| 13 | RVS | Rambach | 0 | 2 | 3 | 3 |
| | MKTTn | Rambach | 0 | 2 | 3 | 3 |
| | MSRV | Rambach | 0 | 2 | 3 | 3 |
| 14 | RVS | Rambach | 0 | 2 | 3 | 3 |
| | MKTTn | Rambach | 0 | 2 | 3 | 3 |
| | MSRV | Rambach | 0 | 2 | 3 | 3 |
| | MK | Rambach | 0 | 2 | 3 | 3 |
| 15 | RVS | SMID | 0 | 2 | 3 | 3 |
| | MKTTn | SMID | 0 | 2 | 3 | 3 |
| | MSRV | SMID | 0 | 2 | 3 | 3 |
| 16 | Diasalm | BGA | 0 | 0 | 0 | 0 |
| | Diasalm | XLD | 0 | 1 | 3 | 2 |
| | Diasalm | Rambach | 0 | 1 | 0 | 1 |
| 19 | Diasalm | Rambach | 0 | 2 | 3 | 3 |
| 20 | RVS | XLT4 | 0 | 2 | 3 | 3 |
| | MKTTn | XLT4 | 0 | 2 | 3 | 3 |
| | MSRV | XLT4 | 0 | 2 | 3 | 3 |
| 21 | RVS | MLCB | 0 | 2 | 3 | 3 |
| | MKTTn | MLCB | 0 | 2 | 3 | 3 |
| | MSRV | MLCB | 0 | 2 | 3 | 3 |

*Table 2.2 Number of positive isolations per laboratory for STM 10 (n=5) with the addition of Salmonella negative faeces*

| | Labcode | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Medium Combination | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 |
| RVS/BGA | 0 | 4 | 3 | 4 | 5 | 1 | 2 | 0 | 3 | 3 | -- | 0 | 3 | 0 | 0 | 4 | 5 | 5 | 0 | 3 |
| RVS/XLD | 0 | 4 | 3 | 4 | 5 | 1 | 2 | 0 | 3 | -- | -- | 0 | 1 | 0 | 0 | 4 | 5 | 5 | 0 | 4 |
| MKTTn/BGA | 0 | 3 | 4 | 5 | 5 | 1 | 2 | 0 | 3 | 2 | -- | 0 | 3 | 0 | 0 | 5 | 0 | 5 | 1 | 5 |
| MKTTn/XLD | 0 | 4 | 4 | 5 | 5 | 1 | 1 | 0 | 3 | -- | 2 | 0 | 3 | 0 | 0 | 5 | 0 | 5 | 1 | 5 |
| MSRV/BGA | 0 | 4 | 4 | 5 | 4 | 3 | 2 | 0 | 1 | 1 | -- | 0 | 3 | 0 | -- | 5 | 5 | 5 | 0 | 5 |
| MSRV/XLD | 0 | 4 | 4 | 5 | 4 | 2 | 2 | 0 | 1 | -- | 5 | 0 | 3 | 0 | -- | 5 | 5 | 5 | 0 | 5 |
| | | | | | | | | | | | | | | | | | | | | |
| RVS/BBLS | 0 | | | | | | | | | | | | | | | | | | | |
| RVS/SMID | | | | | 5 | | | | | | | | | 0 | | | | | | |
| RVS/Rambach | | | | | | | | 0 | | | | 0 | 0 | | | | | | | |
| RVS/XLT4 | | | | | | | | | 3 | 3 | | | | | | | | | 0 | |
| RVS/MLCB | | | | | | | | | | | | | | | | | | | | 3 |
| | | | | | | | | | | | | | | | | | | | | |
| MKTTn/BBLS | 0 | | | | | | | | | | | | | | | | | | | |
| MKTTn/SMID | | | | | 5 | | | | | | | | | 0 | | | | | | |
| MKTTn/Ramb. | | | | | | | | 0 | | | 2 | 0 | 3 | | | | | | | |
| MKTTn/XLT4 | | | | | | | | | 3 | 2 | | | | | | | | | 1 | |
| MKTTn/MLCB | | | | | | | | | | | | | | | | | | | | 5 |
| | | | | | | | | | | | | | | | | | | | | |
| MSRV/SMID | | | | | 4 | | | | | | | | | 0 | | | | | | |
| MSRV/Ramb. | | | | | | | | 0 | | | 5 | 0 | 3 | | | | | | | |
| MSRV/XLT4 | | | | | | | | | 1 | 1 | | | | | | | | | 0 | |
| MSRV/MLCB | | | | | | | | | | | | | | | | | | | | 5 |
| | | | | | | | | | | | | | | | | | | | | |
| Diasalm/BGA | | | | | | | 2 | | | | | | | | 0 | | | | | |
| Diasalm/XLD | | | | | | | 2 | | | | | | | | 0 | | | | | |
| Diasalm/Ramb. | | | | | | | | | | | | | | | 0 | | | 5 | | |
| | | | | | | | | | | | | | | | | | | | | |
| TBG/XLD | | | | | | | | | | | 0 | | | | | | | | | |
| TBG/Rambach | | | | | | | | | | | 0 | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | | | |
| MK/Rambach | | | | | | | | | | | | | 1 | | | | | | | |
| | | | | | | | | | | | | | | | | | | | | |

*-- = not tested*

*Table 2.3 Number of positive isolations per laboratory for STM 100 (n=5) with the addition of Salmonella negative faeces*

| | Labcode | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Medium Combination | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 |
| RVS/BGA | 0 | 4 | 3 | 5 | 5 | 2 | 1 | 2 | 5 | 2 | -- | 1 | 4 | 0 | 1 | 5 | 5 | 5 | 0 | 4 |
| RVS/XLD | 0 | 5 | 2 | 5 | 5 | 2 | 1 | 2 | 5 | -- | -- | 1 | 2 | 0 | 0 | 5 | 5 | 5 | 0 | 4 |
| MKTTn/BGA | 0 | 5 | 3 | 5 | 5 | 3 | 2 | 1 | 5 | 4 | -- | 1 | 4 | 0 | 0 | 5 | 1 | 5 | 0 | 5 |
| MKTTn/XLD | 0 | 5 | 3 | 5 | 5 | 3 | 2 | 1 | 5 | -- | 4 | 1 | 4 | 0 | 0 | 5 | 1 | 5 | 0 | 5 |
| MSRV/BGA | 0 | 5 | 3 | 5 | 4 | 5 | 1 | 1 | 4 | 2 | -- | 1 | 5 | 0 | -- | 5 | 5 | 5 | 0 | 5 |
| MSRV/XLD | 0 | 5 | 3 | 5 | 5 | 5 | 2 | 1 | 4 | -- | 5 | 1 | 5 | 0 | -- | 5 | 5 | 5 | 1 | 5 |
| | | | | | | | | | | | | | | | | | | | | |
| RVS/BBLS | 0 | | | | | | | | | | | | | | | | | | | |
| RVS/SMID | | | | | 5 | | | | | | | | | 0 | | | | | | |
| RVS/Rambach | | | | | | | | 2 | | | | 1 | 0 | | | | | | | |
| RVS/XLT4 | | | | | | | | | 5 | 2 | | | | | | | | | 0 | |
| RVS/MLCB | | | | | | | | | | | | | | | | | | | | 4 |
| | | | | | | | | | | | | | | | | | | | | |
| MKTTn/BBLS | 0 | | | | | | | | | | | | | | | | | | | |
| MKTTn/SMID | | | | | 5 | | | | | | | | | 0 | | | | | | |
| MKTTn/Ramb. | | | | | | | | 2 | | | 4 | 1 | 4 | | | | | | | |
| MKTTn/XLT4 | | | | | | | | | 5 | 4 | | | | | | | | | 0 | |
| MKTTn/MLCB | | | | | | | | | | | | | | | | | | | | 5 |
| | | | | | | | | | | | | | | | | | | | | |
| MSRV/SMID | | | | | 5 | | | | | | | | | 0 | | | | | | |
| MSRV/Ramb. | | | | | | | | 1 | | | 5 | 1 | 5 | | | | | | | |
| MSRV/XLT4 | | | | | | | | | 4 | 2 | | | | | | | | | 0 | |
| MSRV/MLCB | | | | | | | | | | | | | | | | | | | | 5 |
| | | | | | | | | | | | | | | | | | | | | |
| Diasalm/BGA | | | | | | | 2 | | | | | | | | 0 | | | | | |
| Diasalm/XLD | | | | | | | 2 | | | | | | | | 0 | | | | | |
| Diasalm/Ramb. | | | | | | | | | | | | | | | 0 | | | 5 | | |
| | | | | | | | | | | | | | | | | | | | | |
| TBG/XLD | | | | | | | | | | | 1 | | | | | | | | | |
| TBG/Rambach | | | | | | | | | | | 1 | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | | | |
| MK/Rambach | | | | | | | | | | | | | 4 | | | | | | | |
| | | | | | | | | | | | | | | | | | | | | |

*-- = not tested*

*Table 2.4 Number of positive isolations per laboratory for SE 100 (n=5) with the addition of Salmonella negative faeces*

| | Labcode | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Medium Combination | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 |
| RVS/BGA | 1 | 3 | 5 | 5 | 4 | 1 | 1 | 0 | 5 | 1 | -- | 0 | 4 | 0 | 1 | 5 | 5 | 5 | 0 | 5 |
| RVS/XLD | 1 | 3 | 5 | 5 | 5 | 1 | 1 | 0 | 5 | -- | -- | 0 | 3 | 0 | 0 | 5 | 5 | 5 | 1 | 5 |
| MKTTn/BGA | 1 | 4 | 5 | 5 | 5 | 1 | 1 | 3 | 5 | 4 | -- | 0 | 4 | 0 | 0 | 5 | 5 | 5 | 2 | 5 |
| MKTTn/XLD | 1 | 4 | 5 | 5 | 5 | 1 | 1 | 3 | 5 | -- | 5 | 0 | 4 | 0 | 0 | 5 | 5 | 5 | 3 | 5 |
| MSRV/BGA | 1 | 4 | 4 | 5 | 4 | 3 | 1 | 3 | 4 | 2 | -- | 0 | 4 | 0 | -- | 5 | 5 | 5 | 1 | 5 |
| MSRV/XLD | 1 | 4 | 4 | 5 | 4 | 2 | 1 | 3 | 4 | -- | 4 | 0 | 4 | 0 | -- | 5 | 5 | 5 | 2 | 5 |
| | | | | | | | | | | | | | | | | | | | | |
| RVS/BBLS | 1 | | | | | | | | | | | | | | | | | | | |
| RVS/SMID | | | | | 4 | | | | | | | | | 0 | | | | | | |
| RVS/Rambach | | | | | | | | 0 | | | | 0 | 0 | | | | | | | |
| RVS/XLT4 | | | | | | | | | 5 | 1 | | | | | | | | | 1 | |
| RVS/MLCB | | | | | | | | | | | | | | | | | | | | 5 |
| | | | | | | | | | | | | | | | | | | | | |
| MKTTn/BBLS | 1 | | | | | | | | | | | | | | | | | | | |
| MKTTn/SMID | | | | | 5 | | | | | | | | | 0 | | | | | | |
| MKTTn/Ramb. | | | | | | | | 3 | | | 5 | 0 | 4 | | | | | | | |
| MKTTn/XLT4 | | | | | | | | | 5 | 4 | | | | | | | | | 3 | |
| MKTTn/MLCB | | | | | | | | | | | | | | | | | | | | 5 |
| | | | | | | | | | | | | | | | | | | | | |
| MSRV/SMID | | | | | 4 | | | | | | | | | 0 | | | | | | |
| MSRV/Ramb. | | | | | | | | 3 | | | 4 | 0 | 4 | | | | | | | |
| MSRV/XLT4 | | | | | | | | | 4 | 2 | | | | | | | | | 1 | |
| MSRV/MLCB | | | | | | | | | | | | | | | | | | | | 5 |
| | | | | | | | | | | | | | | | | | | | | |
| Diasalm/BGA | | | | | | | 1 | | | | | | | | 1 | | | | | |
| Diasalm/XLD | | | | | | | 1 | | | | | | | | 0 | | | | | |
| Diasalm/Ramb. | | | | | | | | | | | | | | | 1 | | | 5 | | |
| | | | | | | | | | | | | | | | | | | | | |
| TBG/XLD | | | | | | | | | | | 4 | | | | | | | | | |
| TBG/Rambach | | | | | | | | | | | 4 | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | | | |
| MK/Rambach | | | | | | | | | | | | | 5 | | | | | | | |
| | | | | | | | | | | | | | | | | | | | | |

*-- = not tested*

*Table 2.5 Number of positive isolations per laboratory for SE 500 (n=5) with the addition of Salmonella negative faeces*

| | Labcode | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Medium Combination | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 |
| RVS/BGA | 0 | 4 | 4 | 5 | 5 | 3 | 2 | 1 | 5 | 5 | -- | 0 | 5 | 0 | 2 | 5 | 5 | 5 | 1 | 4 |
| RVS/XLD | 0 | 4 | 4 | 5 | 5 | 3 | 2 | 1 | 5 | -- | -- | 0 | 4 | 0 | 0 | 5 | 5 | 5 | 3 | 4 |
| MKTTn/BGA | 0 | 5 | 5 | 5 | 5 | 3 | 2 | 2 | 5 | 5 | -- | 1 | 5 | 0 | 1 | 5 | 5 | 5 | 4 | 5 |
| MKTTn/XLD | 0 | 5 | 5 | 5 | 5 | 3 | 1 | 4 | 5 | -- | 5 | 1 | 5 | 0 | 1 | 5 | 5 | 5 | 3 | 5 |
| MSRV/BGA | 0 | 4 | 5 | 5 | 5 | 4 | 2 | 5 | 4 | 5 | -- | 0 | 5 | 0 | -- | 5 | 5 | 5 | 2 | 5 |
| MSRV/XLD | 0 | 4 | 5 | 5 | 5 | 4 | 2 | 5 | 4 | -- | 5 | 0 | 5 | 0 | -- | 5 | 5 | 5 | 2 | 5 |
| | | | | | | | | | | | | | | | | | | | | |
| RVS/BBLS | 0 | | | | | | | | | | | | | | | | | | | |
| RVS/SMID | | | | | 5 | | | | | | | | | 0 | | | | | | |
| RVS/Rambach | | | | | | | | 1 | | | | 0 | 1 | | | | | | | |
| RVS/XLT4 | | | | | | | | | 5 | 5 | | | | | | | | | 2 | |
| RVS/MLCB | | | | | | | | | | | | | | | | | | | | 4 |
| | | | | | | | | | | | | | | | | | | | | |
| MKTTn/BBLS | 0 | | | | | | | | | | | | | | | | | | | |
| MKTTn/SMID | | | | | 5 | | | | | | | | | 0 | | | | | | |
| MKTTn/Ramb. | | | | | | | | 4 | | | 5 | 1 | 5 | | | | | | | |
| MKTTn/XLT4 | | | | | | | | | 5 | 5 | | | | | | | | | 4 | |
| MKTTn/MLCB | | | | | | | | | | | | | | | | | | | | 5 |
| | | | | | | | | | | | | | | | | | | | | |
| MSRV/SMID | | | | | 5 | | | | | | | | | 0 | | | | | | |
| MSRV/Ramb. | | | | | | | | 5 | | | 5 | 0 | 5 | | | | | | | |
| MSRV/XLT4 | | | | | | | | | 4 | 5 | | | | | | | | | 2 | |
| MSRV/MLCB | | | | | | | | | | | | | | | | | | | | 5 |
| | | | | | | | | | | | | | | | | | | | | |
| Diasalm/BGA | | | | | | | 2 | | | | | | | | 0 | | | | | |
| Diasalm/XLD | | | | | | | 2 | | | | | | | | 0 | | | | | |
| Diasalm/Ramb. | | | | | | | | | | | | | | | 1 | | | 5 | | |
| | | | | | | | | | | | | | | | | | | | | |
| TBG/XLD | | | | | | | | | | | 5 | | | | | | | | | |
| TBG/Rambach | | | | | | | | | | | 5 | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | | | |
| MK/Rambach | | | | | | | | | | | | | 5 | | | | | | | |
| | | | | | | | | | | | | | | | | | | | | |

*-- = not tested*

# Annex 3. Information on the media used

*Table 3.1 Manufacturer of BPW*

| Labcode | Kind of medium | Name | Code | Batch |
|---|---|---|---|---|
| 1 | Dehydrated | Oxoid | CM 509 | 315195 |
| 2 | Dehydrated | Merck | 107228 | V 8000028/628 |
| 3 | Dehydrated | Oxoid | CM 509 | 267954 |
| 4 | Dehydrated | Merck | 107228 | VM 070928 |
| 5 | Ready-to-use | Biomerieux | 42042 | 774805701 |
| 6 | Dehydrated | Oxoid | CM 509 | B 258818 |
| 7 | Dehydrated | Merck | 107228 | VM 084928/323 |
| 8 | Dehydrated | Merck | 107228 | MO 84828/323 |
| 9 | Dehydrated | Oxoid | X 42227 B | 281147 |
| 11 | Ready-to-use | Oxoid | BO 01445 | 851667 |
| 12 | Dehydrated | Merck | 107228 | VM 084828 |
| 13 | Dehydrated | Merck | 107228 | VM 071028 |
| 14 | Dehydrated | AES Lab | AEB 140/302 | 307 140 |
| 15 | Dehydrated | Merck | 107228 | VM 084828/323 |
| 16 | Individual ingredients | -- | -- | -- |
| 17 | Individual ingredients | -- | -- | -- |
| 18 | Dehydrated | Oxoid | CM 509 | 305196 |
| 19 | Dehydrated | Merck | 107228 | VM 065128/312 |
| 20 | Dehydrated | Merck | 107228 | VM 994128 |
| 21 | Dehydrated | Oxoid | CM 509 | 306342 |

-- = *not reported*

*Table 3.2 Composition of BPW medium*

| Labcode | Peptone | Sodium chloride | Disodium phosphate | Potassium dihydrogen phosphate | pH after preparation | pH at day of use |
|---|---|---|---|---|---|---|
| ISO 6579 | 10.0 | 5.0 | 9.0 | 1.5 | -- | 7.0 ± 0.2 (25°C) |
| 1 | 10.0 | 5.0 | 3.5 | 1.5 | 7.11 (25°C) | -- |
| 2 | 10.0 | 5.0 | 5.0 | 5.0 | 7.2 (21°C) | 7.2 (21°C) |
| 3 | 10.0 | 5.0 | 3.5 | 1.5 | 7.20 (25°C) | 7.12 (25°C) |
| 4 | 10.0 | 5.0 | 9.0 | 1.5 | 7.2 (25°C) | 7.2 (26°C) |
| 5 | 10 | 5 | 9 | 1.5 | -- | -- |
| 6 | 10.0 | 5.0 | 3.5 | 1.5 | 7.2 (19.3°C) | 7.2 (24°C) |
| 7 | 10.0 | 5.0 | 9.0 | 1.5 | 7.28 (20°C) | 7.28 (26°C) |
| 8 | 10.0 | 5.0 | 9.0 | 1.5 | 7.3 (24°C) | 7.3 (24°C) |
| 9 | 10 | 5 | 3.5 | 1.5 | -- | -- |
| 11 | 10.0 | 5.0 | 3.5 | 1.5 | -- | -- |
| 12 | 10 | 5 | 10.5 → together | | 7.2 (25°C) | 7.2 (25°C) |
| 13 | 10 | 5 | 10.5 → together | | 7.3 (22.7°C) | 7.1 (27°C) |
| 14 | 10 | 5 | 3.5 | 1.5 | 7.1 (23°C) | -- |
| 15 | 10.0 | 5.0 | 9.0 | 1.5 | 7.1 (25°C) | 7.2 (25°C) |
| 16 | 10.0 | 5.0 | 9.0 | 1.5 | 7.0 (25°C) | 7.0 (25°C) |
| 17 | 10.0 | 5.0 | 4.5 | 1.5 | -- | 7.09 (33°C) |
| 18 | 10 | 5 | 3.5 | 1.5 | -- | -- |
| 19 | 10.0 | 5.0 | 5.0 | 5.0 | 7.2 (RT) | 7.3 (31°C) |
| 20 | 10.0 | 5.0 | 10.5 → together | | 7.2 (25°C) | 7.2 (25°C) |
| 21 | 10 | 5 | 3.5 | 1.5 | 7.1 (22°C) | -- |

*-- = not reported*

*Table 3.3 Manufacturer of RVS*

| Labcode | Kind of medium | Name | Code | Batch |
|---|---|---|---|---|
| 1 | Dehydrated | Oxoid | CM 866 | 284693/302 069 |
| 2 | Dehydrated | Merck | 107700 | VM 107700/335 |
| 3 | Dehydrated | Oxoid | CM 866 | 289297 |
| 4 | Dehydrated | Oxoid | CM 866 | 289297 |
| 5 | Ready-to-use | Oxoid | TV 5036E | 859014 |
| 6 | Dehydrated | Lab M | Lab 86 | 066736 |
| 7 | Dehydrated | Oxoid | CM 866 | 307226 |
| 8 | Dehydrated | Merck | 107700 | YM 964000/239 |
| 9 | Dehydrated | Lab M | Lab 86 | 069514 |
| 11 | Ready-to-use | Biorad | 355 5777 | 3G0135 |
| 12 | -- | -- | -- | -- |
| 13 | Dehydrated | Merck | 107700 | VL 785700/146 |
| 14 | Dehydrated | Oxoid | CM 866 | 307226 |
| 15 | Ready-to-use | Biorad | 55777 | 3G0135 |
| 16 | Individual ingredients | -- | -- | -- |
| 17 | Dehydrated | Oxoid | CM 866 | 295337 |
| 18 | Dehydrated | Oxoid | CM 866 | B 307226 |
| 19 | Dehydrated | Oxoid | CM 866 | B 307226 |
| 20 | Dehydrated | Oxoid | CM 866 | 302069 |
| 21 | -- | Merck | -- | 239 |

*-- = not reported*

*Table 3.4 Composition of RVS selective enrichment medium in g/l*

| Labcode | Soya peptone | NaCl | Potass.di hydrogen phosphate | Di potass. hydrogen phosphate | Mg chloride anhydrous | $MgCl.6H_2O$ | Malachite green | pH after preparation | pH at day of use |
|---|---|---|---|---|---|---|---|---|---|
| ISO 6579 | 5.0 | 8.0 | 1.4 | 0.2 | -- | 40.0 | 0.036 | -- | 5.2±0.2 (25 °C) |
| 1 | 4.5 | 7.2 | 1.26 | 0.18 | 13.58 | -- | 0.036 | 5.42 (25 °C) | -- |
| 2 | 4.5 | 7.2 | 1.26 | 0.18 | -- | 28.6 | 0.036 | 5.2 (21 °C) | 5.2 (21 °C) |
| 3 | 4.5 | 7.2 | 1.26 | 0.18 | 13.58 | -- | 0.036 | 5.33 (25 °C) | 5.33 (25 °C) |
| 4 | 4.5 | 7.2 | 1.26 | 0.18 | 13.52 | -- | 0.036 | 5.4 (25 °C) | 5.3 (20.7 °C) |
| 5 | 4.5 | 7.2 | 1.26 | 0.18 | 13.58 | -- | 0.036 | -- | -- |
| 6 | 4.5 | 7.2 | 1.26 | 0.18 | 13.58 | -- | 0.033 | 5.20 (25.2 °C) | 5.46 (25.3 °C) |
| 7 | 4.5 | 7.2 | 1.26 | 0.18 | 13.52 | -- | 0.036 | 5.52 (20 °C) | 5.44 (14 °C) |
| 8 | 4.5 | 8.0 | 0.6 | 0.4 | -- | 29.0 | 0.036 | 5.2 (24 °C) | 5.2 (24 °C) |
| 9 | 4.5 | 7.2 | 1.26 | 0.18 | 13.58 | -- | 0.033 | 5.13 (45 °C) | -- |
| 11 | 4.5 | 7.2 | 1.26 | 0.18 | -- | 28.6 | 0.036 | -- | -- |
| 12 | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| 13 | 4.5 | 8.0 | 0.6 | 0.4 | -- | 29.0 | 0.036 | 5.21 (25.5 °C) | 5.22 (20.8 °C) |
| 14 | 4.5 | 7.2 | 1.26 | 0.18 | 13.58 | -- | 0.036 | 5.4 (24.2 °C) | -- |
| 15 | 4.50 | 7.20 | 1.26 | 0.18 | 28.60 | -- | 0.036 | -- | -- |
| 16 | 5.0 | 8.0 | 1.4 | 0.2 | -- | 40.0 | 0.04 | 5.2 (25 °C) | 5.2 (25 °C) |
| 17 | 4.5 | 7.2 | 1.26 | 0.18 | 13.58 | -- | 0.036 | 5.44 (27.1 °C) | 5.57 (22.7 °C) |
| 18 | 4.5 | 7.2 | 1.26 | 0.18 | 13.58 | -- | 0.036 | 5.2 (20 °C) | -- |
| 19 | 4.5 | 7.2 | 1.26 | 0.18 | 13.58 | -- | 0.036 | 5.0 (RT) | 5.0 (24 °C) |
| 20 | 4.5 | 7.2 | 1.26 | 0.18 | 13.58 | -- | 0.036 | 5.4 (25 °C) | 5.4 (25 °C) |
| 21 | 4.5 | 8.0 | 0.6 | 0.4 | 29.0 | -- | 0.036 | 52 (22 °C) | -- |

*-- = not reported*

*Table 3.5 Manufacturer of MKTTn*

| Labcode | Kind of medium | Name | Code | Batch |
|---|---|---|---|---|
| 1 | Dehydrated | Oxoid | CM 1048 | 312201 |
| 2 | Dehydrated | Oxoid | CM 1048 | 302083 |
| 3 | Dehydrated | Oxoid | CM 1048 | 312201 |
| 4 | Dehydrated | Oxoid | CM 1048 | 312201 |
| 5 | Ready-to-use | Biorad | 3556140 | G 3175 |
| 6 | Dehydrated | Oxoid | CM 1048 | B 312201 |
| 7 | Dehydrated | Oxoid | CM 1048 | B 312201 |
| 8 | Ready-to-use | BioMerieux | REF 42114 | 776415001 |
| 9 | Ready-to-use | Biorad | 3556140 | G 3175 |
| 11 | Ready-to-use | Biorad | 3556140 | G 2275 |
| 12 | Dehydrated | Becton-Dick. | 218531 | 3030814 |
| 13 | Dehydrated | Biolife | 401745 | 3D2 501 |
| 14 | Dehydrated | Biokar | BK 135 HA | OM 922 |
| 15 | Ready-to-use | BioMerieux | 42114 | 777352301 |
| 16 | Individual ingredients | -- | -- | -- |
| 17 | Dehydrated | Oxoid | CM 1048 | 312201 |
| 18 | Individual ingredients | -- | -- | -- |
| 19 | Dehydrated | Oxoid | CM 343 | B 288779 |
| 20 | Ready-to-use | BioMerieux | 42114 | 777665001 |
| 21 | Dehydrated | Oxoid | CM 343 | 239428 |

*-- = not reported*

*Table 3.6 Composition (in g/L) and pH of MKTTn*

| Labcode | Meat extract | Casein | NaCl | $CaCO_3$ | $Na_2S_2O_3.5H_2O$ | Ox bile | Brilliant green | pH after preparation | pH at day of use |
|---|---|---|---|---|---|---|---|---|---|
| ISO 6579 | 4.3 | 8.6 | 2.6 | 38.7 | 47.8 | 4.78 | 0.0096 | -- | 8.2±0.2 (25 °C) |
| 1 | 4.3 | 8.6 | 2.6 | 38.7 | 47.8 | 4.78 | 0.0096 | 8.12 (25 °C) | 8.12 (25 °C) |
| 2 | 4.3 | 8.6 | 2.6 | 38.7 | 30.5* | 4.78 | 0.0096 | 8.2 (21 °C) | 8.2 (21 °C) |
| 3 | 4.3 | 8.6 | 2.6 | 38.7 | 47.8 | 4.78 | 0.0096 | 8.18 (25 °C) | 8.09 (25 °C) |
| 4 | 4.3 | 8.6 | 2.6 | 38.7 | 47.8 | 4.78 | 0.0096 | 8.0 (25 °C) | 8.2 (22.1 °C) |
| 5 | 2.3 | 7.0 | 2.5 | 25.0 | 40.7 | 4.75 | 0.0098 | -- | -- |
| 6 | 4.3 | 8.6 | 2.6 | 38.7 | 47.8 | 4.78 | 0.0096 | 8.30 (23.3 °C) | 8.23 (24.5 °C) |
| 7 | 4.3 | 8.6 | 2.6 | 38.7 | 47.8 | 4.78 | 0.0096 | 8.12 (25 °C) | 8.26 (14 °C) |
| 8 | 4.23 | 8.45 | 2.54 | 38.04 | 30.27 | 4.75 | 0.0095 | 8.0 (25 °C) | 7.3 (24 °C) |
| 9 | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| 11 | 4.2 | 8.4 | 2.5 | 37.8 | 46.6 | 4.7 | 0.0094 | -- | -- |
| 12 | 5 | -- | 3 | 45 | 38.1 | 4.7 | 0.0095 | 8.2 (16 °C) | 8.2 (16 °C) |
| 13 | 4.3 | 8.6 | 2.6 | 38.7 | 30.3 | 4.75 | 0.0095 | 8.13 (29 °C) | 8.19 (19 °C) |
| 14 | 4.23 | 8.45 | 2.54 | 38.04 | 30.27 | 4.75 | 0.0095 | 7.6 (23.8 °C) | -- |
| 15 | 4.23 | 8.45 | 2.54 | 38.04 | 30.27 | 4.75 | 0.0095 | -- | -- |
| 16 | 4.3 | 8.6 | 2.6 | 38.7 | 47.8 | 4.78 | 0.0096 | 8.2 (25 °C) | 8.2 (25 °C) |
| 17 | 4.3 | 8.6 | 2.6 | 38.7 | 30.5* | 4.78 | 0.0096 | 8.14 (26.5 °C) | 8.02 (22.9 °C) |
| 18 | 4.3 | 8.6 | 2.6 | 38.7 | 47.8 | 4.78 | 0.0096 | 8.6 (20 °C) | -- |
| 19 | 7.0** | 2.3# | 2.3 | 25.0 | 40.7 | 4.75 | 0.0096 | 8.2 (RT) | -- |
| 20 | 4.23 | 8.45 | 2.54 | 38.04 | 30.27 | 4.75 | 0.0095 | -- | -- |
| 21 | 7.0** | 2.3# | 2.3 | 25.0 | 40.7 | 4.75 | 0.0096 | -- | -- |

** anhydrous; ** tryptone; # peptone; -- = not reported*

*Table 3.7 Manufacturer of MSRV*

| Labcode | Kind of medium | Name | Code | Batch |
|---|---|---|---|---|
| 1 | Dehydrated | Oxoid | CM 910 | 281488 |
| 2 | Dehydrated | Oxoid | CM 910 | 302075 |
| 3 | Dehydrated | Oxoid | CM 910 | 308082 |
| 4 | Dehydrated | Oxoid | CM 910 | 298715 |
| 5 | Dehydrated | Oxoid | CM 910 | 298715 |
| 6 | Dehydrated | Oxoid | CM 910 | B 312950 |
| 7 | Dehydrated | Merck | 109878 | VM 963978 |
| 8 | Dehydrated | Biokar | BK 134 HA | 1 A 092 |
| 9 | Dehydrated | Oxoid | CM 910 | 292440 |
| 11 | Ready-to-use | Biorad | 355 6139 | G 1436 |
| 12 | Dehydrated | Merck | 109878 | VM 963978 |
| 13 | Dehydrated | Biolife | 401982 | 2G4602 |
| 14 | Dehydrated | AES Lab | AEB 140 672 | 116352 |
| 15 | Dehydrated | Merck | 109878 | VM 963978 |
| 16 | -- | -- | -- | -- |
| 17 | Dehydrated | Difco | R41-0011 | 2017008 |
| 18 | Dehydrated | Oxoid | CM 910 | 210783 |
| 19 | Dehydrated | Oxoid | CM 910 | B 312950 |
| 20 | Dehydrated | Difco | 218681 | 2017008 |
| 21 | Dehydrated | Oxoid | CM 910 | 312950 |

*-- = not reported*

*Table 3.8 Composition (in g/L) and pH of MSRV*

| Labcode | Tryp tose | Casein hydro lysate | NaCl | Potass. dihydrogen phosphate | $MgCl_2$ anhydrous | Malachite green oxalate | Agar | pH after preparation | pH at day of use |
|---|---|---|---|---|---|---|---|---|---|
| SOP | 4.59 | 4.59 | 7.34 | 1.47 | 10.93 | 0.037 | 2.7 | -- | 5.2±0.2 (25 °C) |
| 1 | 4.59 | 4.59 | 7.34 | 1.47 | 10.93 | 0.037 | 2.7 | 5.48 (25 °C) | 5.48 (25 °C) |
| 2 | 4.59 | 4.59 | 7.34 | 1.47 | 10.93 | 0.037 | 2.7 | 5.4 (21 °C) | 5.4 (21 °C) |
| 3 | 4.59 | 4.59 | 7.34 | 1.47 | 10.93 | 0.037 | 2.7 | 5.5 (25 °C) | 5.48 (25 °C) |
| 4 | 4.59 | 4.59 | 7.34 | 1.47 | 10.93 | 0.037 | 2.7 | 5.4 (25 °C) | 5.3 (20.9°C) |
| 5 | 4.59 | 4.59 | 7.34 | 1.47 | 10.93 | 0.037 | 2.7 | 5.4 (20 °C) | 5.4 (20 °C) |
| 6 | 4.59 | 4.59 | 7.34 | 1.47 | 10.93 | 0.037 | 2.7 | 5.2 (25 °C) | 5.1 (28 °C) |
| 7 | 4.59 | 4.59 | 7.34 | 1.47 | 10.93 | 0.037 | 2.7 | 5.48 (50 °C) | -- |
| 8 | 4.59 | 4.59 | 7.34 | 1.47 | 10.93 | 0.037 | 2.7 | 5.2 (24 °C) | 5.2 (24 °C) |
| 9 | 4.59 | 4.59 | 7.34 | 1.47 | 10.93 | 0.037 | 2.7 | 5.42 (25 °C) | -- |
| 11 | 9.2 → | together | 7.34 | 1.47 | 10.93 | 0.037 | 2.7 | -- | -- |
| 12 | 4.59 | 4.59 | 7.34 | 1.47 | 10.93 | 0.037 | 2.7 | 5.7 (RT) | 5.7 (RT) |
| 13 | 4.59 | 4.59 | 7.34 | 1.47 | 10.93 | 0.037 | 2.7 | 5.43 (22.7°C) | 5.54 (23 °C) |
| 14 | 4.59 | 4.59 | 7.34 | 1.47 | 10.93 | 0.037 | 2.7 | 5.6 (22.5°C) | -- |
| 15 | 4.59 | 4.59 | 7.34 | 1.47 | 10.93 | 0.037 | 2.7 | 5.6 (25 °C) | -- |
| 16 | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| 17 | 4.59 | 4.59 | 7.34 | 1.47 | 10.93 | 0.037 | 2.7 | 5.19 (32.4°C) | -- |
| 18 | 4.59 | 4.59 | 7.34 | 1.47 | 10.93 | 0.037 | 2.7 | 5.36 (20 °C) | -- |
| 19 | 4.59 | 4.59 | 7.34 | 1.47 | 10.93 | 0.037 | 2.7 | 5.08 (RT) | -- |
| 20 | 4.59 | 4.59 | 7.34 | 1.47 | 10.93 | 0.037 | 2.7 | 5.1 (25 °C) | -- |
| 21 | 4.59 | 4.59 | 7.34 | 1.47 | 10.93 | 0.037 | 2.7 | 5.2 (42 °C) | -- |

*-- = not reported*

*Table 3.9 Manufacturer of own selective medium*

| Labcode | Kind of medium | Name Medium | Manufacturer | Code number | Batch | pH after preparation | pH at day of use |
|---|---|---|---|---|---|---|---|
| 7 | Dehydrated | Diassalm | Lab M | Lab 537 | 069192 | 5.36 (50.0 °C) | -- |
| 12 | Dehydrated | TBG | Merck | 105178 | VM 019778 | 7.2 (26 °C) | 7.6 (23 °C) |
| 14 | Dehydrated | MK | AES Lab | 140 702 | 240 435 | 8 (23.7 °C) | -- |
| 16 | Dehydrated | Diassalm | Lab M | Lab 537 | 057619 | 5.5 (25 °C) | 5.5 (25 °C) |
| 19 | Dehydrated | Diassalm | Merck | 109803 | VM 938703 | 6.2 (RT) | -- |

*-- = not reported*

*Table 3.10 Manufacturer of BGA*

| Labcode | Kind of medium | Manu facturer | Code number | Batch number | pH after preparation | pH at day of use |
|---|---|---|---|---|---|---|
| 1 | Dehydrated | Merck | 10747 | VM 55547313 | 6.95 (25 °C) | -- |
| 2 | Dehydrated | Merck | 107237 | VM 003637 | 6.9 (21 °C) | 6.9 (21 °C) |
| 3 | Dehydrated | Oxoid | CM 0263 | 290577 | 6.86 (25 °C) | 6.83 (25 °C) |
| 4 | Dehydrated | Oxoid | CM 329 | 291982 | 7.0 (20.0 °C) | 7.0 (21.7 °C) |
| 5 | Ready-to-use | Biomerieux | 43588 | 779667601 | -- | -- |
| 6 | Dehydrated | Oxoid | CM 329 | B 256433 | 6.89 (24.5 °C) | 6.92 (24.7 °C) |
| 7 | Dehydrated | Oxoid | CM 329 | B 291982 | 6.55 (45 °C) | -- |
| 8 | Dehydrated | Biolife | 401255 | 3B2801 | 6.9 (24 °C) | 6.9 (24 °C) |
| 9 | Dehydrated | Biogenetics | BM 730 | 3M 1403 | 6.97 (45 °C) | -- |
| 11 | Ready-to-use | Oxoid | PO 5033 A | 859595 | -- | -- |
| 13 | Dehydrated | Merck | 110747 | VL 769147 142 | 7.04 (23.8 °C) | 7.02 (23.8 °C) |
| 14 | Dehydrated | AES Lab | AEB 151 492 | 321 135 | 6.9 (29.8 °C) | -- |
| 15 | Dehydrated | Merck | 110747 | VM 136947 338 | 6.9 (25 °C) | -- |
| 16 | Individual ingredients | -- | -- | -- | 6.9 (25 °C) | 6.9 (25 °C) |
| 17 | Dehydrated | Oxoid | CM 329 | -- | -- | -- |
| 18 | Dehydrated | Oxoid | CM 329 | 301648 | 7.1 (20 °C) | -- |
| 19 | Dehydrated | Oxoid | CM 329 | 286214 | 7.26 (RT) | -- |
| 20 | Dehydrated | Merck | 107237 | VM 003637 | 6.9 (25 °C) | -- |
| 21 | Dehydrated | Lab M | Lab 34 | 060245 | 6.9 (50 °C) | -- |

*-- = not reported*

*Table 3.11 Composition of BGA*

| Labcode | Peptone | Yeast extract | Lactose | Sucrose/ saccharose | NaCl | Phenol red |
|---|---|---|---|---|---|---|
| ISO 6579 (1993) | 10.0 | 3.0 | 10.0 | 10.0 | -- | 0.09 |
| 1 | 10.0 | 3.0 | 10.0 | 10.0 | -- | 0.09 |
| 2 | 10.0 | -- | 10.0 | 10.0 | 3.0 | 0.08 |
| 3 | 10.0 | 3.0 | 10.0 | 10.0 | 5.0 | 0.08 |
| 4 | 10.0 | 3.0 | 10.0 | 10.0 | -- | 0.09 |
| 5 | 10 | 3 | 10 | 10 | -- | 0.09 |
| 6 | 10.0 | 3.0 | 10.0 | 10.0 | -- | 0.09 |
| 7 | 10.0 | 3.0 | 10.0 | 10.0 | -- | 0.09 |
| 8 | 10.0 | 3.0 | 10.0 | 10.0 | 5.0 | 0.08 |
| 9 | 10.0 | 3 | 10 | 10 | 5 | 0.08 |
| 11 | 10.0 | 3.0 | 10.0 | 10.0 | -- | 0.09 |
| 13 | 10.0 | 3.0 | 10.0 | 10.0 | -- | 0.09 |
| 14 | 10 | 3 | 10 | 10 | -- | 0.09 |
| 15 | 10 | 3 | 10 | 10 | -- | 0.09 |
| 16 | 11.1 | 3.3 | 11.1 | 11.1 | -- | -- |
| 17 | 10.0 | 3.0 | 10.0 | 10.0 | 5.0 | 0.08 |
| 18 | 10.0 | 3.0 | 10.0 | 10.0 | 5.0 | 0.08 |
| 19 | 10.0 | 3.0 | 10.0 | 10.0 | 5.0 | 0.08 |
| 20 | 10.0 | -- | 10.0 | 10.0 | 3.0 | 0.08 |
| 21 | 10.0 | 3.0 | 10.0 | 10.0 | -- | 0.09 |

| Labcode | Brilliant green | Agar | Disodium Hydrogen Phosphate | Sodium dihydrogen phosphate | Beef extract | Meat extract |
|---|---|---|---|---|---|---|
| ISO 6579 (1993) | 0.005 | 12 - 18 | 1.0 | 0.6 | -- | 5.0 |
| 1 | 0.0047 | 12.0 | 1.0 | 1.0 | 5.0 | -- |
| 2 | 0.125 | 12.0 | 2.0 | -- | -- | 5.0 |
| 3 | 0.0125 | 12.0 | -- | -- | -- | -- |
| 4 | 0.0047 | 12.0 | 1.0 | 1.0 | 5.0 | |
| 5 | 0.005 | 15 | 1.0 | 0.6 | 5.0 | -- |
| 6 | 0.0047 | 12.0 | 1.0 | 1.0 | 5.0 | -- |
| 7 | 0.0047 | 12.0 | 1.0 | 1.0 | 5.0 | -- |
| 8 | 0.0125 | 20.0 | -- | -- | -- | -- |
| 9 | 0.0125 | 20 | -- | -- | -- | -- |
| 11 | 0.070 | 12 | 1.0 | 0.6 | 5.0 | -- |
| 13 | 0.0047 | 12.0 | 1.0 | 1.0 | 5.0 | -- |
| 14 | 0.0047 | 12 | 1 | 0.6 | -- | 5 |
| 15 | 0.0047 | 12 | 1.0 | 0.6 | -- | -- |
| 16 | -- | -- | 1.1 | 0.7 | | 5.5 |
| 17 | 0.0047 | 12.0 | 1.0 | 1.0 | 5.0 | -- |
| 18 | 0.0125 | 12.0 | -- | -- | -- | -- |
| 19 | 0.0125 | 12.0 | -- | -- | -- | -- |
| 20 | 0.125 | 12.0 | 2.0 | -- | -- | 5.0 |
| 21 | -- | -- | 1.0 | 0.6 | 5.0 | -- |

*-- = not reported*

*Table 3.12 Manufacturer and pH of XLD*

| Labcode | Kind of medium | Manu facturer | Code number | Batch number | pH after preparation | pH at day of use |
|---|---|---|---|---|---|---|
| ISO | -- | -- | -- | -- | 7.4 ± 0.2 (25 °C) | 7.4 ± 0.2 (25 °C) |
| 1 | Dehydrated | Difco | 0788-17 | 13DKYR | 7.36 (25 °C) | -- |
| 2 | Dehydrated | Merck | 1.05287 | VM 126887 | 7.4 (21 °C) | 7.4 (21 °C) |
| 3 | Dehydrated | Lab M | Lab 32 | 065986 | 7.41 (25 °C) | 7.47 (25 °C) |
| 4 | Dehydrated | Oxoid | CM 469 | 279293 | 7.5-7.6 (25 °C) | 7.4-7.5 (25 °C) |
| 5 | Ready-to-use | Biomerieux | 43563 | 779248801 | -- | -- |
| 6 | Dehydrated | Oxoid | CM 469 | B 256295 | 7.4 (24 °C) | 7.42 (24.5 °C) |
| 7 | Dehydrated | Oxoid | CM 469 | B 311435 | 6.54 (50 °C) | -- |
| 8 | Dehydrated | Merck | 1.05287 | YM12688 7 331 | 7.4 (24 °C) | 7.4 (24 °C) |
| 9 | Ready-to-use | Becton Dickinson | -- | 3260964 | -- | -- |
| 11 | -- | -- | -- | -- | -- | -- |
| 12 | Dehydrated | Merck | 1.05287 | VK539687 | 7.4 (RT) | 7.4 (RT) |
| 13 | Dehydrated | Merck | 1.05287 | VM 920587226 | 7.5 (26 °C) | 7.66 (23.5 °C) |
| 14 | Dehydrated | AES Lab | AEB 523 409 | 328 808 | -- | -- |
| 15 | Dehydrated | Biorad | 69124 | 2F0188 | 7.4 (25 °C) | -- |
| 16 | Dehydrated | Merck | 1.05287 | V343487 | 7.4 (25 °C) | 7.4 (25 °C) |
| 17 | Dehydrated | Oxoid | CM 469 | 288994 + 307051 | 7.44 (25.5 °C) | 7.40 (37.5 °C) |
| 18 | Dehydrated | Lab M | Lab 32 | 071026 | 7.4 (20 °C) | -- |
| 19 | Dehydrated | Oxoid | CM 469 | 307051 | 7.37 (RT) | -- |
| 20 | Dehydrated | Difco | 278850 | 2288259 | 7.2 (25 °C) | -- |
| 21 | Dehydrated | Difco | -- | B DKYR | 7.3 (50 °C) | -- |

*-- = not reported*

*Table 3.13 Composition of XLD in g/L*

| Labcode | Xylose | L-lysine | Lactose | Sucrose | NaCl | Yeast extract |
|---|---|---|---|---|---|---|
| ISO | 3.75 | 5.0 | 7.5 | 7.5 | 5.0 | 3.0 |
| 1 | 3.75 | 5.0 | 7.5 | -- | 5.0 | 3.0 |
| 2 | 3.75 | 5.0 | 7.5 | -- | 5.0 | 3.0 |
| 3 | 3.75 | 5.0 | 7.5 | 7.5 | 5.0 | 3.0 |
| 4 | 3.74 | 5.0 | 7.5 | 7.5 | 5.0 | 3.0 |
| 5 | 3.5 | 5 | 7.5 | -- | 5 | 3 |
| 6 | 3.75 | 5.0 | 7.5 | 7.5 | 5.0 | 3.0 |
| 7 | 3.75 | 5.0 | 7.5 | 7.5 | 5.0 | 3.0 |
| 8 | 3.75 | 5 | 7.5 | 7.5 | 5.0 | 3.0 |
| 9 | -- | -- | -- | -- | -- | -- |
| 11 | -- | -- | -- | -- | -- | -- |
| 12 | 3.75 | 5 | 7.5 | 7.5 | 5.0 | 3.0 |
| 13 | 3.75 | 5 | 7.5 | 7.5 | 5.0 | 3.0 |
| 14 | -- | -- | -- | -- | -- | - |
| 15 | 3.5 | 5.0 | 7.5 | -- | 5.0 | 3.0 |
| 16 | 3.75 | 5 | 7.5 | 7.5 | 5.0 | 3.0 |
| 17 | 3.75 | 5.0 | 7.5 | 7.5 | 5.0 | 3.0 |
| 18 | 3.75 | 5.0 | 7.5 | 7.5 | 5.0 | 3.0 |
| 19 | 3.75 | 5.0 | 7.5 | 7.5 | 5.0 | 3.0 |
| 20 | 3.75 | 5.0 | 7.5 | -- | 5.0 | 3.0 |
| 21 | 7.5 | 5.0 | -- | -- | 5.0 | 3.0 |

| Labcode | Phenol red | Agar | Sodium desoxycholate | Sodium thiosulphate | Ferric.amm. citrate | Saccharose |
|---|---|---|---|---|---|---|
| ISO | 0.08 | 9 – 18 | 1.0 | 6.8 | 0.8 | -- |
| 1 | 0.08 | 15.0 | 2.5 | 6.8 | 0.8 | 7.5 |
| 2 | 0.08 | 14.5 | 1.0 | 6.8 | 0.8 | 7.5 |
| 3 | 0.08 | 13.0 | 1.0 | 6.8 | 0.8 | -- |
| 4 | 0.08 | 12.5 | 1.0 | 6.8 | 0.8 | -- |
| 5 | 0.08 | 13.5 | 2.5 | 6.8 | 0.8 | 7.5 |
| 6 | 0.08 | 12.5 | 1.0 | 6.8 | 0.8 | -- |
| 7 | 0.08 | 12.5 | 1.0 | 6.8 | 0.8 | -- |
| 8 | 0.08 | 14.5 | 1.0 | 6.8 | 0.8 | -- |
| 9 | -- | -- | -- | -- | -- | -- |
| 11 | -- | -- | -- | -- | -- | -- |
| 12 | 0.08 | 14.5 | 1.0 | 6.8 | 0.8 | -- |
| 13 | 0.08 | 14.5 | 1.0 | 6.8 | 0.8 | -- |
| 14 | -- | -- | -- | -- | -- | -- |
| 15 | 0.08 | 13.5 | 2.5 | 6.8 | 0.8 | 7.5 |
| 16 | 0.08 | 14.5 | 1.0 | 6.8 | 0.8 | -- |
| 17 | 0.08 | 12.5 | 1.0 | 6.8 | 0.8 | -- |
| 18 | 0.08 | 13.0 | 1.0 | 6.8 | 0.8 | -- |
| 19 | 0.08 | 12.5 | 1.0 | 6.8 | 0.8 | -- |
| 20 | 0.08 | 15.0 | 2.5 | 6.8 | 0.8 | 7.5 |
| 21 | 0.08 | 15.0 | 2.5 | 6.8 | 0.8 | 7.5 |

*-- = not reported*

*Table 3.14 Manufacturer and pH of own isolation medium*

| Labcode | Kind of medium | Manu facturer | Code number | Batch number | pH after preparation | pH at day of use |
|---|---|---|---|---|---|---|
| 1 | BBLS (individual ingredients) | Difco | 0044 | -- | 7.33 (25 $^{o}$C) | -- |
| 5 | SMID (ready-to-use) | Biomerieux | 43291 | 779507201 | -- | -- |
| 8 | Rambach (dehydrated) | Merck | 1.07500 | OC 3090879 | 7.2 (24 $^{o}$C) | 7.2 (24 $^{o}$C) |
| 9 | XLT4 (dehydrated) | Difco | 223420 | 2326427 | 7.36 (45 $^{o}$C) | -- |
| 11 | XLT4 (ready-to-use) | Biorad | 356 3654 | 0118 B | -- | -- |
| 12 | Rambach (ready-to-use) | Merck | 15999 | 30602 | -- | 7.1 (RT) |
| 13 | Rambch (dehydrated) | Merck | 1.07500 | 00308001 | 7.2 (23.2 $^{o}$C) | 7.15 (23.8 $^{o}$C) |
| 14 | Rambach (dehydrated) | Merck | 1.07500 | OC 285636 | 7.3 (23.9 $^{o}$C) | -- |
| 15 | SMID (ready-to-use) | Biomerieux | 43291 | 779696701 | -- | -- |
| 16 | Rambach (dehydrated) | Merck | 1.07500 | 29079 | 7.3 (25 $^{o}$C) | 7.3 (25 $^{o}$C) |
| 19 | Rambach (dehydrated) | Merck | 1.07500 | 00309089 | 7.02 (RT) | -- |
| 20 | XLT4 (dehydrated) | Difco | 223420 | 3069737 | 7.4 (25 $^{o}$C) | -- |
| 21 | MCLB (dehydrated) | Oxoid | CM 783 | B 257055 | 6.9 (50 $^{o}$C) | -- |

*-- = not reported*

*Table 3.15 Manufacturer and pH of nutrient agar*

| Labcode | Name agar | Kind of medium | Manufact. | Code | Batch | pH at day of use |
|---|---|---|---|---|---|---|
| 2 | Gassner-agar | Dehydrated | Merck | 1.01282 | VM 981 582 245 | 7.2 (21 °C) |
| 4 | Colombia agar + 5% cattle blood | Raedy-to-use | SSI | 18210 | 190903 | 7.3 (25 °C) |
| 6 | Nutrient agar | Dehydrated | BSL Global | KM 1073 | B 00626 | 7.28 (23 °C) |
| 8 | Drigalski agar | Individual ingredients | -- | -- | -- | 7.6 (24 °C) |
| 9 | Nutrient agar | Dehydrated | Biogenetics | BM 347 | OL 2402 | 7.9 (45 °C) |
| 18 | Bromcresol lact.agar | Individual ingredients | -- | -- | -- | 7.2 (20 °C) |
| 19 | Nutrient agar | Dehydrated | Oxoid | CM3 | 288793 | 7.2 (RT) |
| 20 | Nutrient agar | Dehydrated | Difco | 21300 | 2043004 | 6.8 (25 °C) |

*-- = not reported*

*Table 3.16 Manufacturer and pH of TSI*

| Labcode | Name | Code number | Batch number | pH of medium |
|---|---|---|---|---|
| 1 | Difco | 0265 | 0053002 | 7.08 (25 °C) |
| 3 | Merck | 1.03915 | VM 010515311 | 7.42 (25 °C) |
| 6 | Merck | 1.03915 | VL 724615/132 | 7.4 (24.2 °C) |
| 8 | Biolife | 402141 | 3F 5301 | 7.3 (24 °C) |
| 9 | Lab M | Lab 59 | 063865 | 7.25 (45 °C) |
| 11 | Oxoid | CM 277 | B 298602 | 7.4 (--) |
| 13 | Merck | 1.03915 | V 381515 932 | 7.5 (25 °C) |
| 15 | Difco | 226540 | 2303681 | 7.4 (25 °C) |
| 16 | Home-made | -- | -- | 7.4 (25 °C) |
| 17 | Individual ingredients | -- | -- | -- |
| 18 | BBL | 11749 | 1000C2D KGV | 7.3 (20 °C) |
| 20 | Merck | 1.03915 | VM 010515311 | 7.3 (25 °C) |
| 21 | MAST | DM 224 | 109079/105559 | 7.4 (50 °C) |

*-- = not reported*

*Table 3.17 Manufacturer and pH of urea agar*

| Labcode | Name | Code number | Batch number | pH of medium |
|---|---|---|---|---|
| 1 | Oxoid | CM53 + SR20 | 271712 + 03051501 | 6.7 (25 °C) |
| 3 | Oxoid | CM 53 | 285572 | 6.82 (25 °C) |
| 6 | Merck | 1.08492 | VL 864392/214 | 6.8 (25 °C) |
| 7 | Kohns Media-Mast | DM 138-1A | 142567/139056 | 6.5 (50 °C) |
| 8 | Home-made | -- | -- | 6.8 (24 °C) |
| 9 | Oxoid | CM 53 | 269475 | 6.71 (45 °C) |
| 11 | Oxoid | TV 5007N | 858571 | -- |
| 13 | Merck | 1.08492 | VL 864392 217 | 6.77 (24.9 °C) |
| 14 | AES Lab | 111545 | 303 528 | -- |
| 15 | Difco | 228320 | 0313008 | 6.8 (25 °C) |
| 16 | Home-made | -- | -- | 6.8 (25 °C) |
| 20 | Merck | 1.08492 | V 316192911 | 6.8 (25 °C) |

*-- = not reported*

*Table 3.18 Manufacturer and pH of LDC*

| Labcode | Name | Code number | Batch number | pH of medium |
|---|---|---|---|---|
| 1 | Home-made | -- | -- | 6.8 (25 °C) |
| 3 | Difco + Calbiochem | 287220 + 4400 | 1288012 + B 43743 | 6.80 (25 °C) |
| 6 | Fluka | 62895 | 478831/140302 | 6.5 (25 °C) |
| 8 | Home-made | -- | -- | 6.7 (24 °C) |
| 9 | Difco | 211759 | 1341012 | 6.67 (45 °C) |
| 11 | Oxoid | TV 5028 N | 856650 | -- |
| 13 | Home-made | -- | -- | 7.0 (25 °C) |
| 14 | Difco | 211 759 | 124 1012 | 6.7 (23.8 °C) |
| 15 | Difco | 221520 | 1016003 | 6.8 (25 °C) |
| 16 | Home-made | -- | -- | 6.8 (25 °C) |
| 17 | Individual ingredients | -- | -- | 6.69 (20.7 °C) |
| 18 | BBL | 11430 | 1000IlDMZ | 6.0 (20 °C) |
| 20 | Difco | 211759 | 141101 | 6.6 (25 °C) |

*-- = not reported*

# Annex 4. Results of PCR detection

*Table 4.1 PCR results of artificially contaminated samples of laboratories 2, 3, 8, 18 and 21*

| | Lab 2 | | Lab 3 | | Lab 8 | | Lab 18 | | Lab 21 | |
|---|---|---|---|---|---|---|---|---|---|---|
| Number | BAC | PCR | BAC | PCR | BAC | PCR | BAC | PCR | BAC | PCR |
| 1 | + | + | + | - | - | + | + | - | + | + |
| 2* | - | - | - | - | - | - | - | - | - | - |
| 3 | + | + | + | + | - | + | + | + | + | + |
| 4 | + | + | + | I | + | + | + | - | + | + |
| 5 | + | + | - | + | - | + | + | - | + | + |
| 6 | + | + | + | + | + | + | + | - | + | + |
| 7 | + | + | + | - | + | + | + | - | + | + |
| 8* | - | - | - | - | - | - | - | - | - | - |
| 9 | + | + | + | - | + | + | + | - | + | + |
| 10 | - | + | + | - | + | + | + | - | + | + |
| 11 | - | + | + | - | - | + | + | + | + | - |
| 12* | - | - | - | - | - | - | - | - | - | - |
| 13 | + | + | + | - | + | + | + | - | + | - |
| 14 | + | + | + | - | - | + | + | - | + | + |
| 15 | + | + | + | + | + | + | + | + | + | + |
| 16* | - | - | - | - | - | - | - | - | - | + |
| 17 | + | + | + | + | - | NT | + | + | + | + |
| 18 | + | + | + | - | - | + | + | - | + | + |
| 19 | + | + | + | - | + | + | + | - | + | + |
| 20 | + | + | - | - | - | + | + | - | + | + |
| 21* | - | - | - | - | - | - | - | - | - | - |
| 22 | + | + | + | I | - | + | + | - | + | - |
| 23 | + | + | + | - | + | + | + | - | + | + |
| 24 | + | + | + | - | + | + | + | - | + | + |
| 25 | + | + | + | + | + | + | + | - | + | + |
| Total pos. | 18 | 20 | 18 | 6 | 11 | 19 | 20 | 4 | 20 | 18 |

*I = Inhibition of PCR reaction; NT = Not tested; BAC = Bacteriology; * = Blank capsules*
*+ = positive; - = negative*

*Table 4.2 PCR results of artificially contaminated samples of laboratory 20*

| | Labcode 20 | | | |
|---|---|---|---|---|
| Number | BAC | PCR - A | PCR - B | PCR - C |
| 1 | - | - | - | - |
| 2* | - | - | - | - |
| 3 | - | - | + | - |
| 4 | + | + | + | + |
| 5 | - | - | - | - |
| 6 | + | + | + | + |
| 7 | + | + | + | + |
| 8* | - | - | - | - |
| 9 | + | + | + | + |
| 10 | + | + | + | + |
| 11 | - | - | - | - |
| 12* | - | - | - | - |
| 13 | + | + | + | + |
| 14 | - | - | - | - |
| 15 | - | - | - | - |
| 16* | - | - | - | - |
| 17 | + | - | - | - |
| 18 | - | - | - | - |
| 19 | - | + | + | - |
| 20 | - | - | - | - |
| 21* | - | - | - | - |
| 22 | + | + | + | + |
| 23 | - | - | - | - |
| 24 | + | + | + | + |
| 25 | - | - | + | - |
| Total pos. | 9 | 9 | 11 | 8 |

*BAC = Bacteriology; * = Blank capsules; + = positive; - = negative*

*Table 4.3 PCR results of naturally contaminated samples of labs 2, 3, 8, 18 and 21*

| | Lab 2 | | Lab 3 | | Lab 8 | | Lab 18 | | Lab 21 | |
|---|---|---|---|---|---|---|---|---|---|---|
| Number | BAC | PCR | BAC | PCR | BAC | PCR | BAC | PCR | BAC | PCR |
| N1 | - | + | + | + | + | + | - | - | - | + |
| N2 | - | + | + | + | - | + | + | - | - | + |
| N3 | - | + | + | + | + | + | + | - | - | + |
| N4 | + | + | - | + | + | + | - | - | + | + |
| N5 | - | + | - | + | + | + | + | - | + | + |
| N6 | - | + | - | + | + | + | + | - | + | + |
| N7 | + | + | + | + | + | + | + | - | + | + |
| N8 | - | + | + | + | + | + | + | - | + | + |
| N9 | - | + | + | + | + | + | - | - | + | + |
| N10 | - | + | + | + | + | + | - | - | + | + |
| N11 | - | + | - | + | + | + | + | - | + | + |
| N12 | - | + | + | + | + | + | + | - | + | + |
| N13 | - | + | + | + | + | + | - | - | + | + |
| N14 | - | + | + | + | + | + | - | - | + | + |
| N15 | - | + | + | + | + | + | - | - | + | + |
| N16 | - | + | - | + | - | + | - | - | + | + |
| N17 | - | + | + | + | + | + | - | - | + | + |
| N18 | - | + | + | + | + | + | + | - | + | + |
| N19 | - | + | + | + | + | + | + | - | + | + |
| N20 | - | + | + | + | + | + | - | - | + | + |
| Total pos. | 2 | 20 | 15 | 20 | 18 | 20 | 10 | 0 | 17 | 20 |

*BAC = Bacteriology; + = positive; - = negative*

*Table 4.4 PCR results of naturally contaminated samples of laboratoy 20*

| | Labcode 20 | | | |
|---|---|---|---|---|
| Number | BAC | PCR - A | PCR - B | PCR - C |
| N1 | - | + | - | - |
| N2 | + | + | + | + |
| N3 | + | + | + | + |
| N4 | - | + | + | - |
| N5 | - | + | + | - |
| N6 | + | + | + | + |
| N7 | + | + | - | + |
| N8 | + | + | + | + |
| N9 | - | + | + | - |
| N10 | + | + | + | + |
| N11 | - | + | + | - |
| N12 | - | + | + | - |
| N13 | + | + | + | + |
| N14 | - | + | + | - |
| N15 | - | + | + | - |
| N16 | - | + | + | - |
| N17 | + | + | + | + |
| N18 | + | + | + | + |
| N19 | - | + | + | - |
| N20 | + | + | + | + |
| Total pos. | 10 | 20 | 18 | 10 |

*BAC = Bacteriology; + = positive; - = negative*

*Table 4.5 PCR results of control samples samples of laboratories 2, 3, 5, 8 and 18*

| | Lab 2 | | Lab 3 | | Lab 5 | | Lab 8 | | Lab 18 | |
|---|---|---|---|---|---|---|---|---|---|---|
| Number | BAC | PCR | BAC | PCR | BAC | PCR | BAC | PCR | BAC | PCR |
| C1 | - | - | - | - | - | - | - | - | - | - |
| C2 | + | + | + | + | + | + | + | + | + | + |
| C3 | - | - | - | - | - | - | - | - | - | - |
| C4 | + | + | + | + | + | + | + | + | + | + |
| C5 | + | + | + | + | + | + | + | + | + | + |
| C6 | + | + | + | + | + | + | + | + | + | + |
| C7 | + | + | + | + | + | + | + | + | + | + |
| C8 | + | + | + | + | + | + | + | + | + | + |
| C9 | + | + | + | + | + | + | + | + | + | + |
| C10 | + | + | + | + | + | + | + | + | + | + |
| C11 | - | - | - | - | - | - | - | - | - | - |
| C12 | - | - | - | - | - | - | - | - | - | - |
| Total pos. | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 |

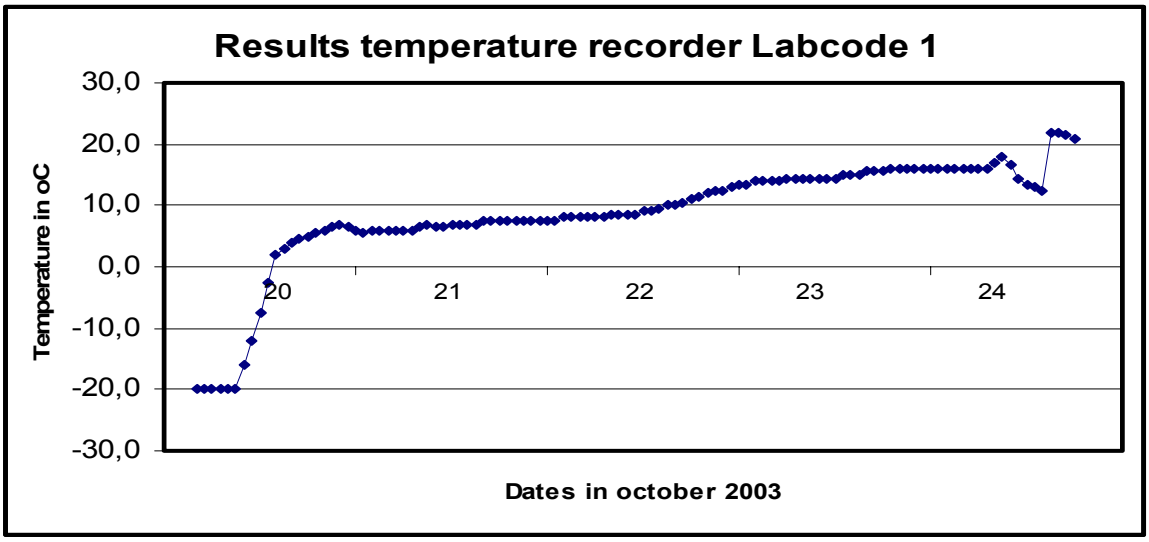
*BAC = Bacteriology; + = positive; - = negative*

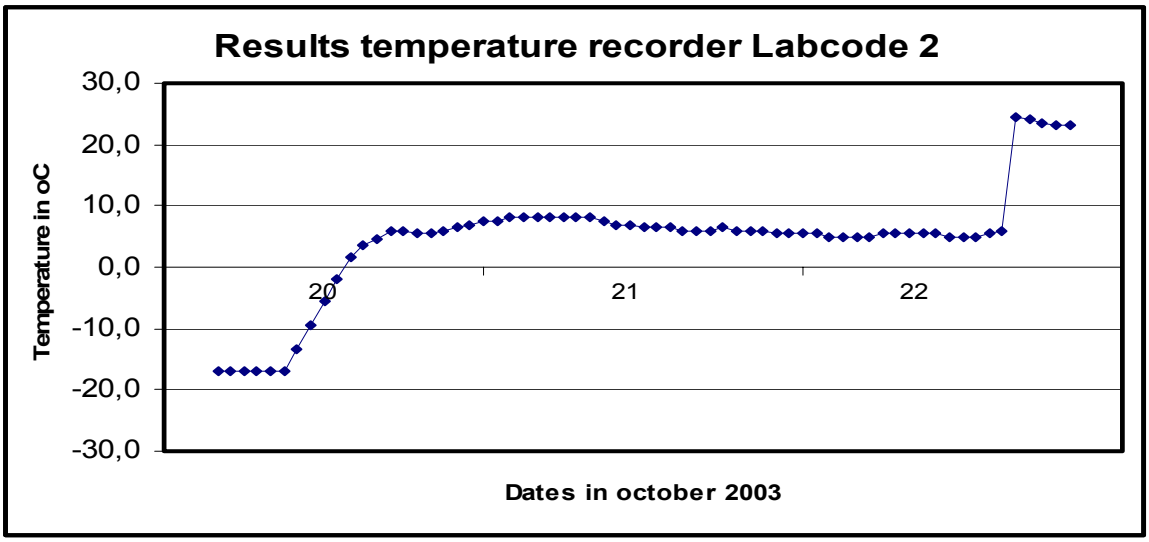
*Table 4.6 PCR results of control samples samples of laboratories 20 and 21*

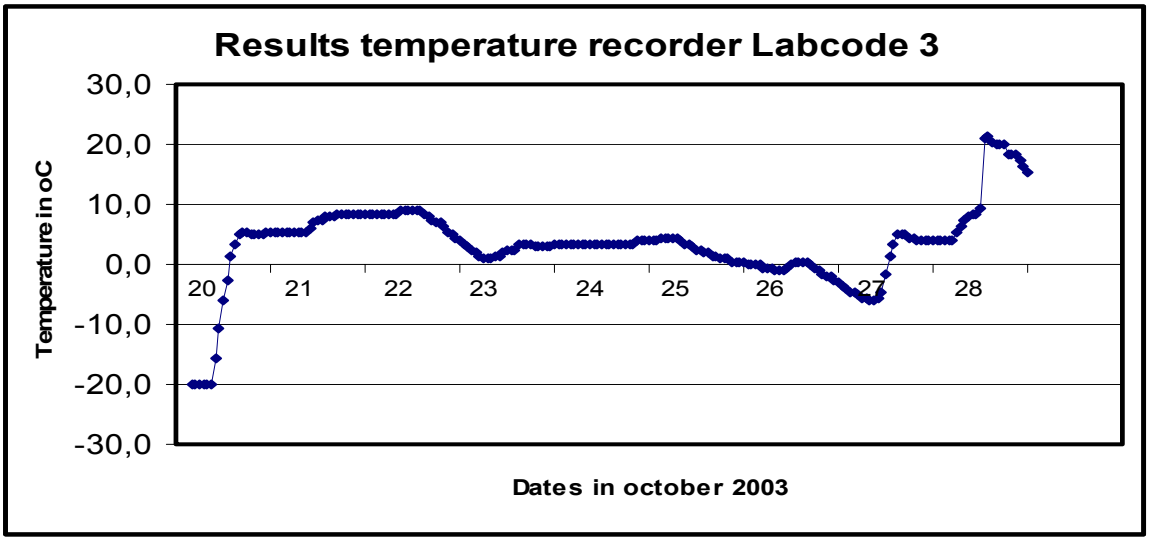
| | Lab 20A | | Lab 20B | | Lab 20C | | Lab 21 | |
|---|---|---|---|---|---|---|---|---|
| Number | BAC | PCR | BAC | PCR | BAC | PCR | BAC | PCR |
| C1 | - | - | - | - | - | - | - | + |
| C2 | + | + | + | + | + | + | + | + |
| C3 | - | - | - | - | - | - | - | + |
| C4 | + | + | + | + | + | + | + | + |
| C5 | + | + | + | + | + | + | + | + |
| C6 | + | + | + | + | + | + | + | + |
| C7 | + | + | + | + | + | + | + | + |
| C8 | + | + | + | + | + | + | + | + |
| C9 | + | + | + | + | + | + | + | + |
| C10 | + | + | + | + | + | + | + | + |
| C11 | - | - | - | - | - | - | - | + |
| C12 | - | - | - | - | - | - | - | + |
| Total pos. | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 12 |

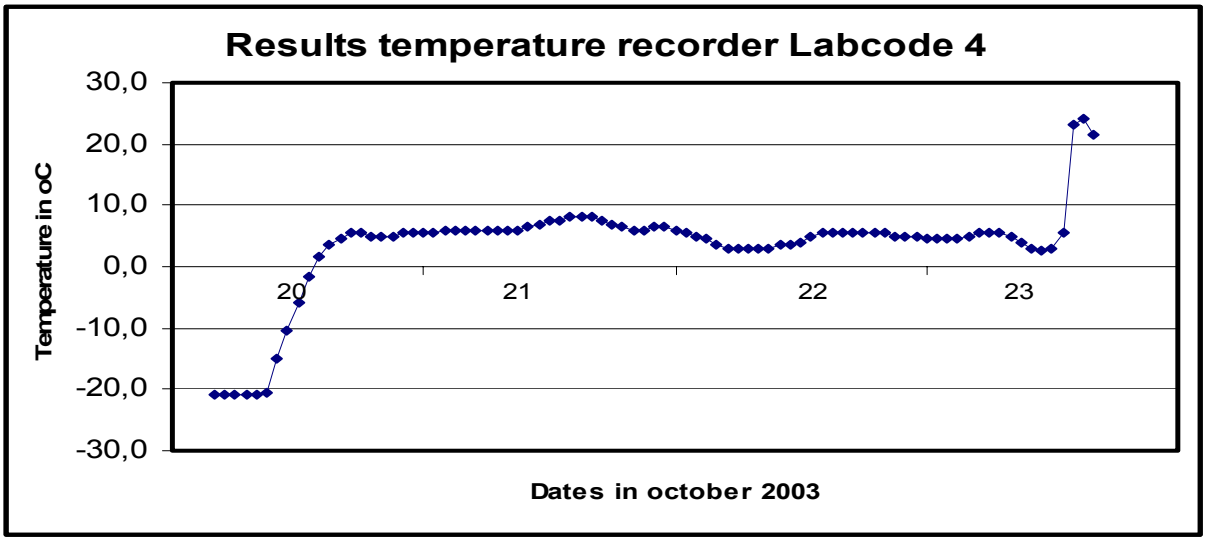
*BAC = Bacteriology; + = positive; - = negative*

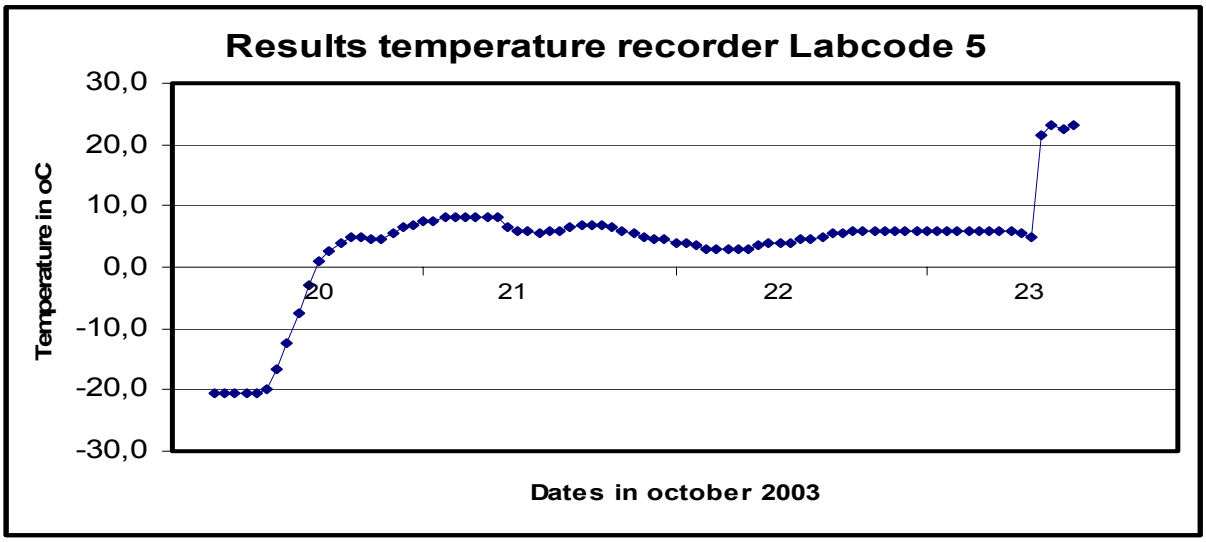
# Annex 5. Temperature recording




Results temperature recorder Labcode 4
30,0
20,0
10,0
0,0
-10,0
-20,0
-30,0
Temperature in oC
20
21
22
23
Dates in october 2003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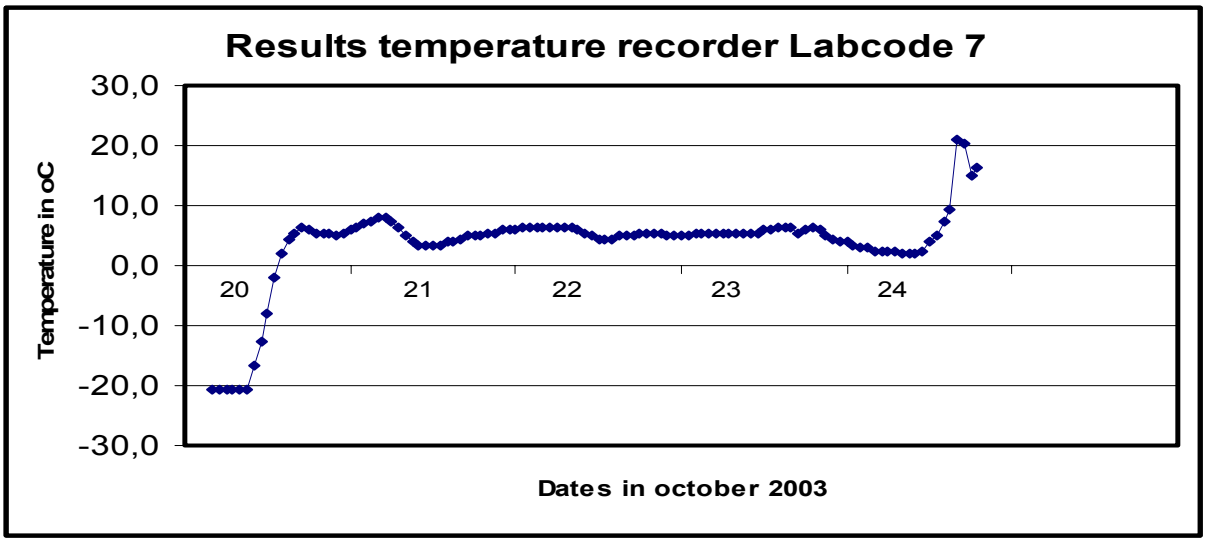
Dates in october 2003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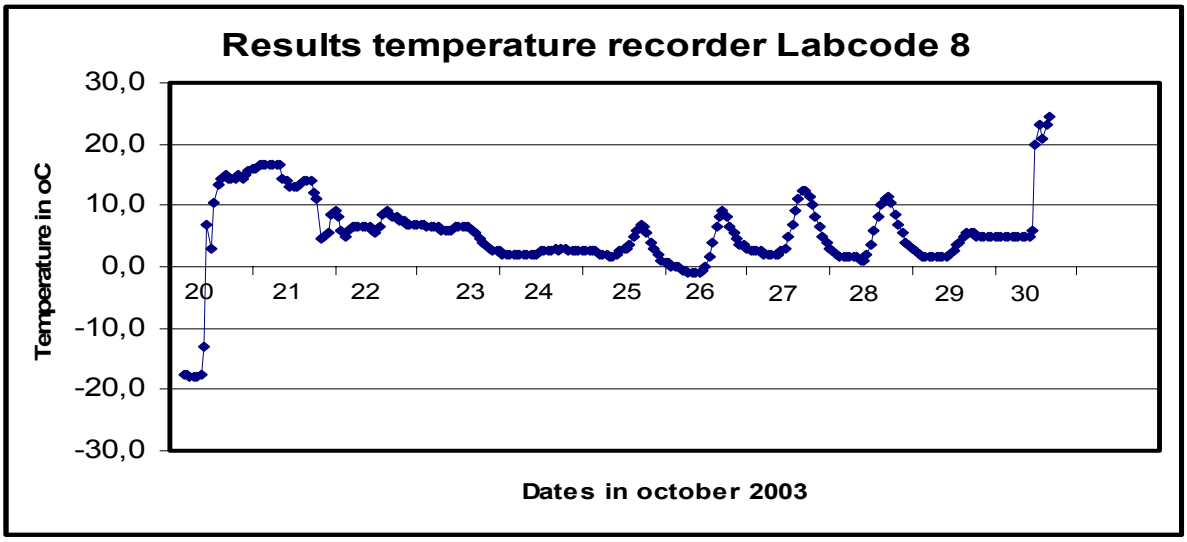
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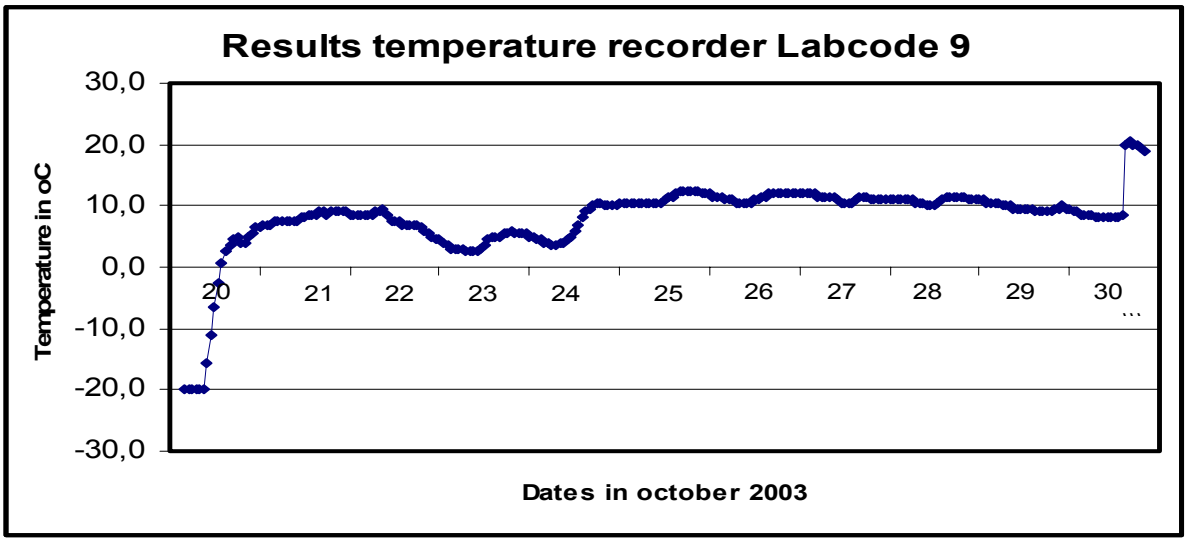
Dates in october 2003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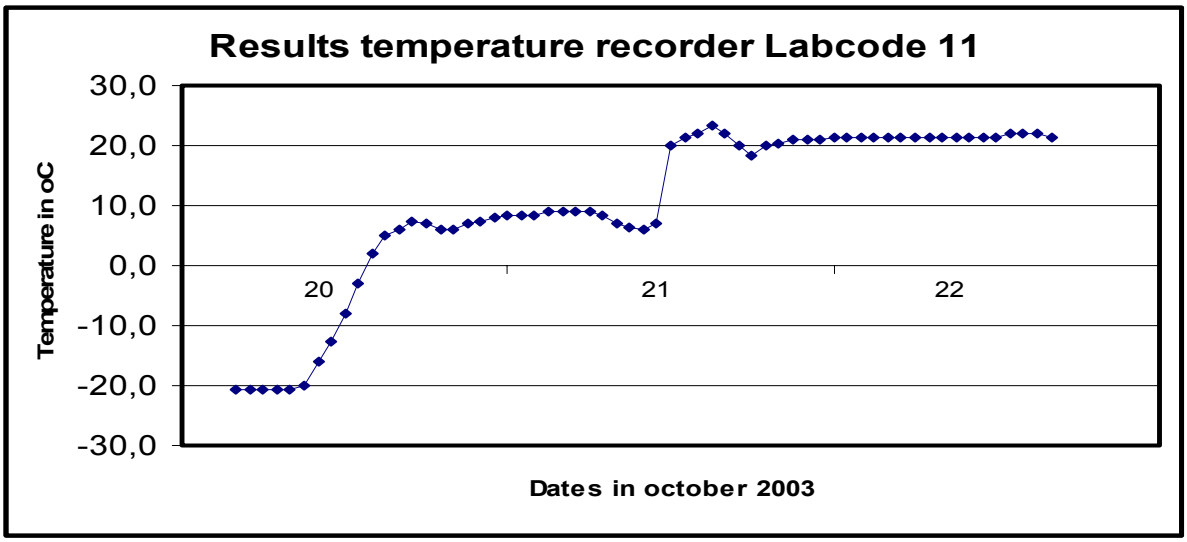
Dates in october 2003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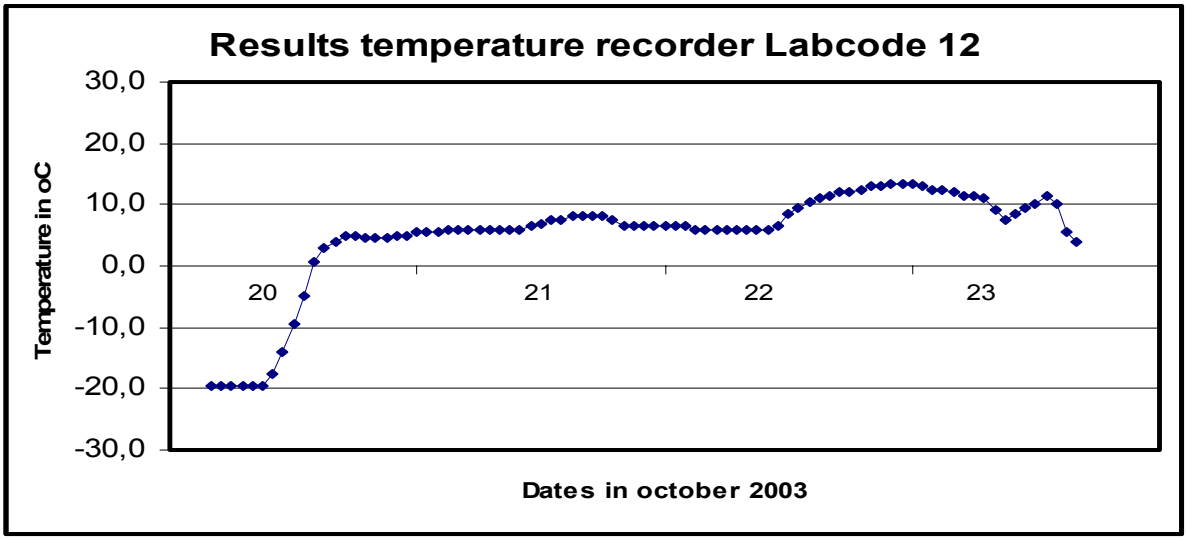
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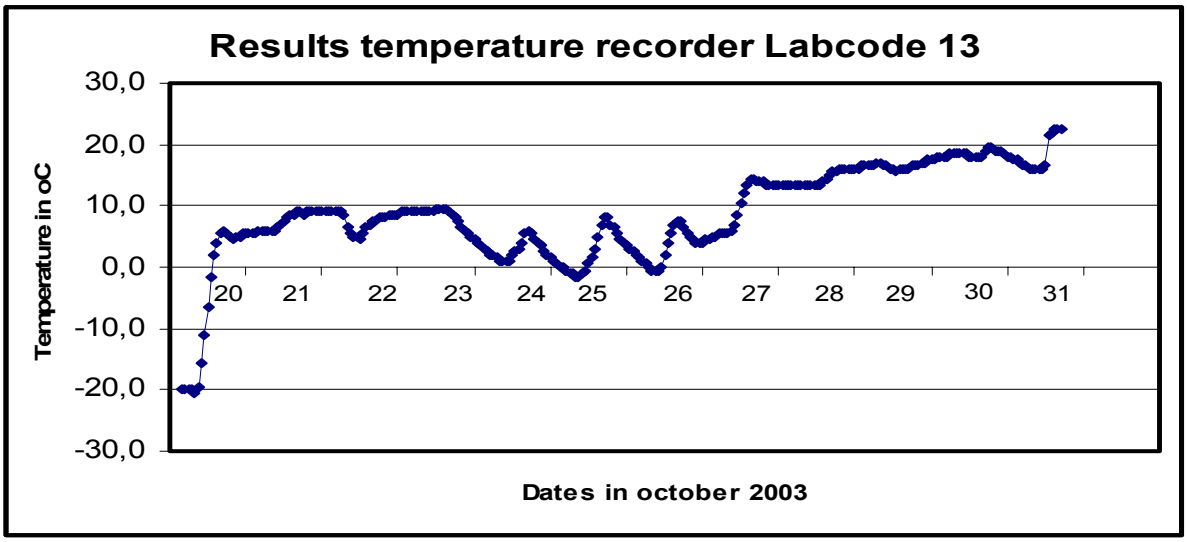
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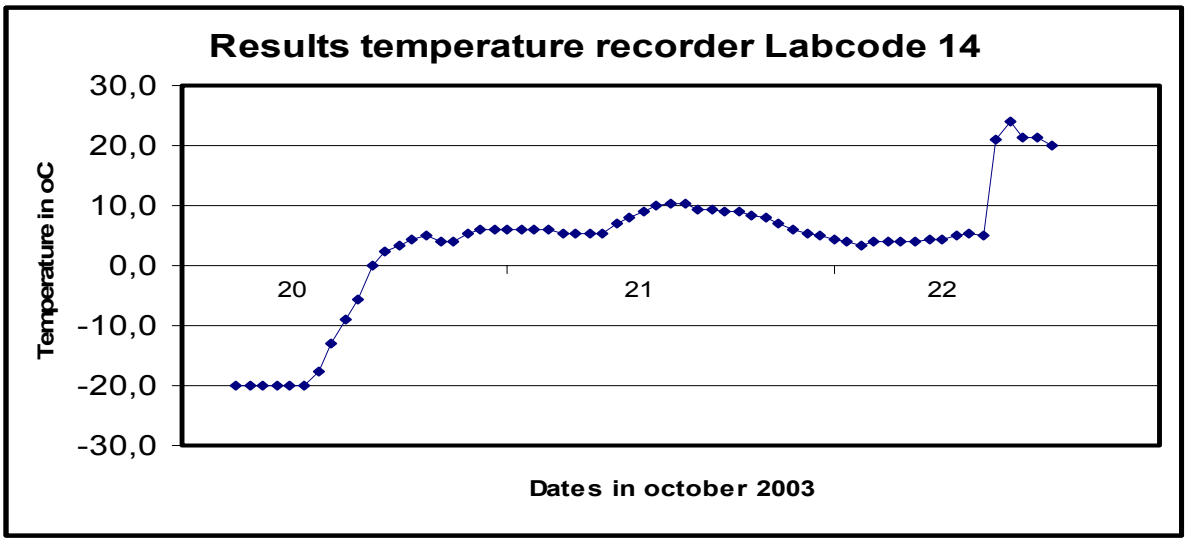
Dates in october 2003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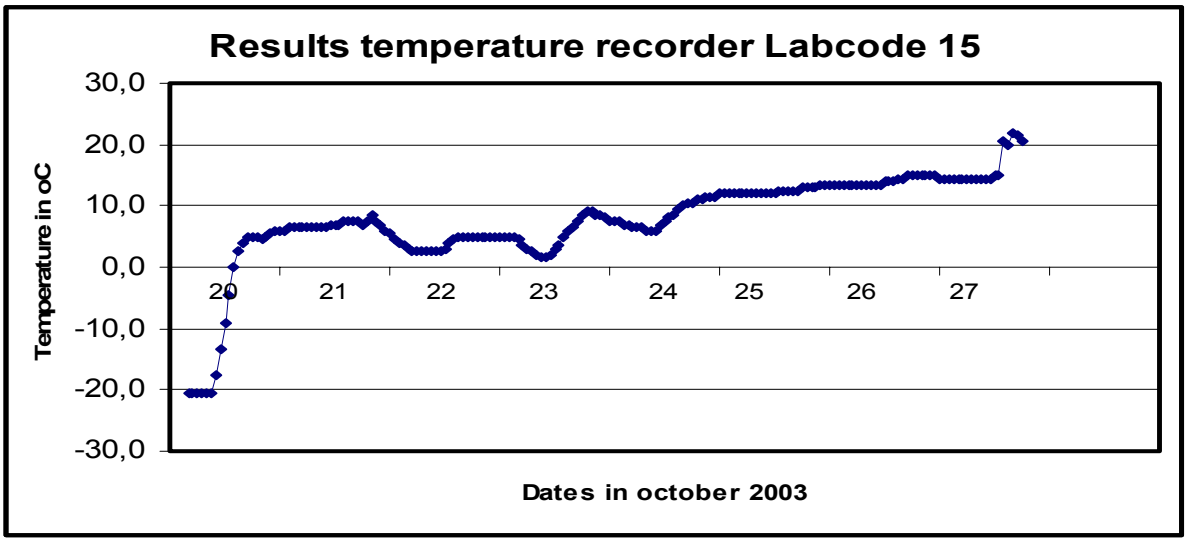
Dates in october 2003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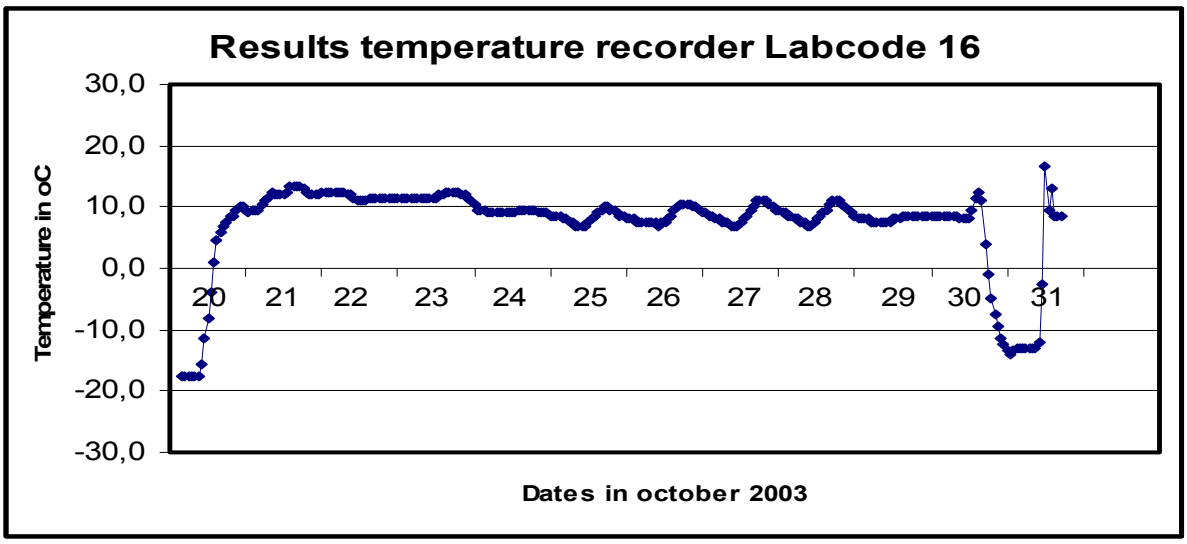
Dates in october 2003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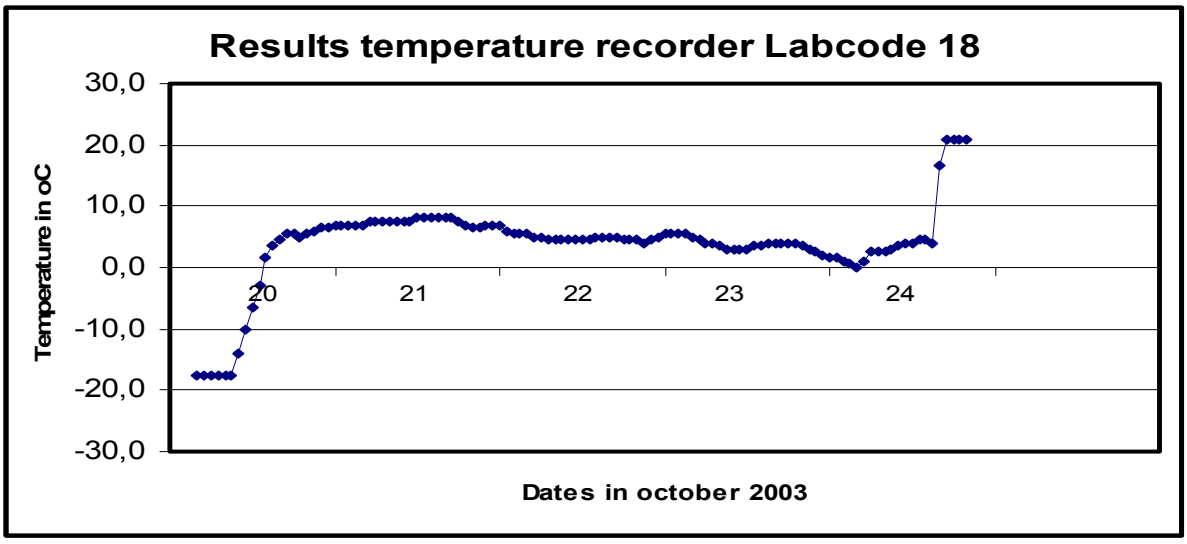
Dates in october 2003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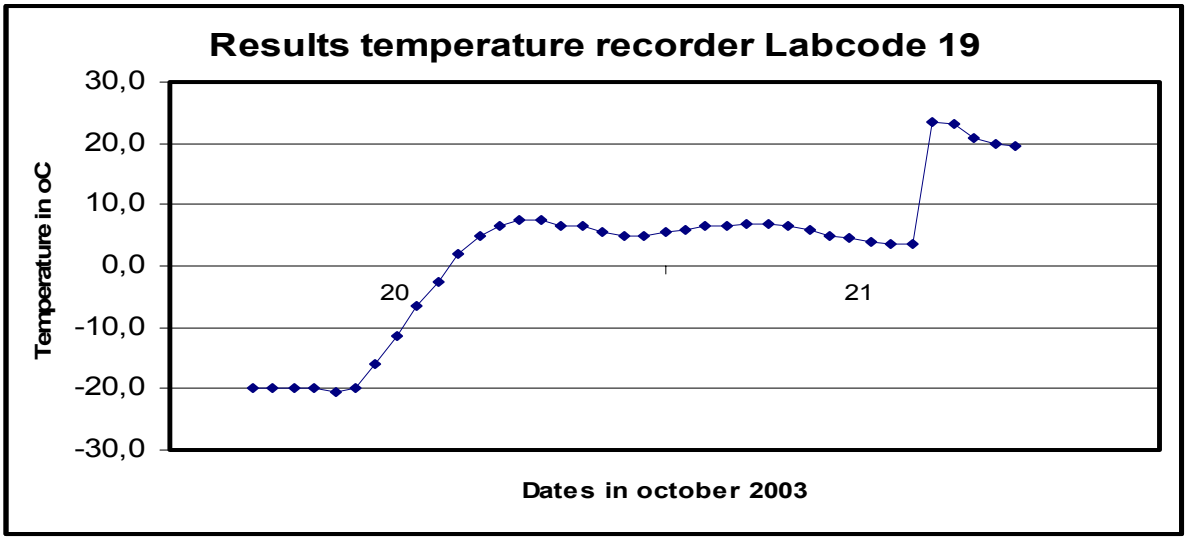
Dates in october 2003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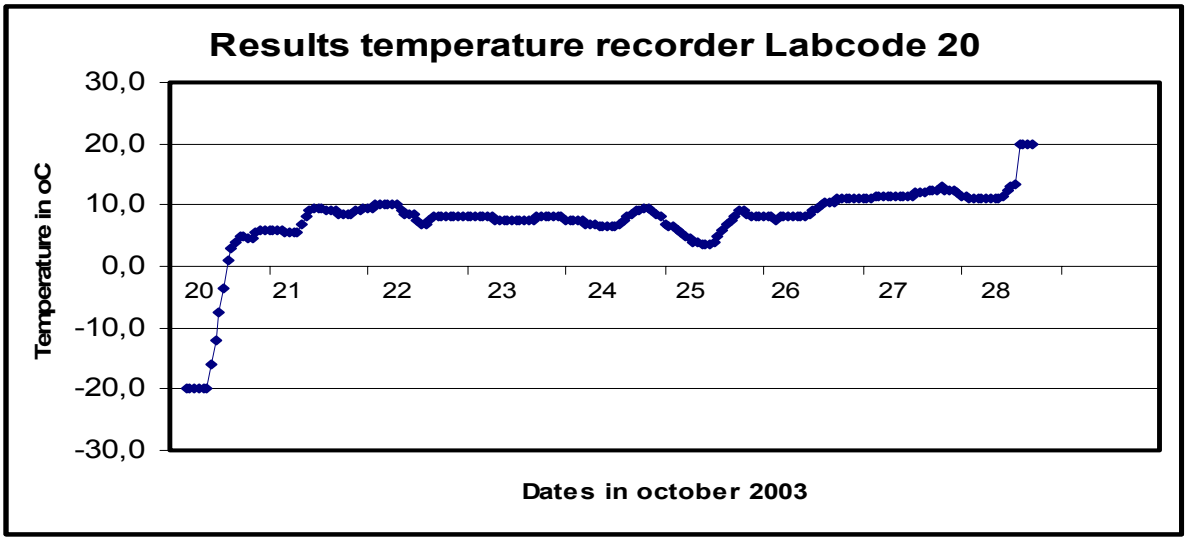
Dates in october 2003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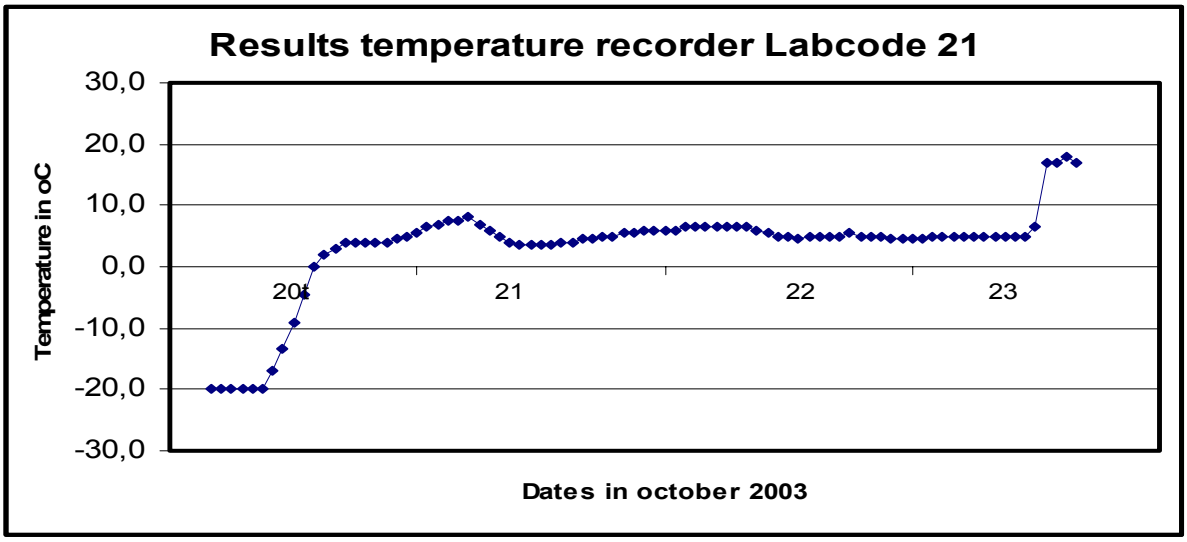
Dates in october 2003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Dates in october 2003

# Annex 6. Protocol

## BACTERIOLOGICAL COLLABORATIVE STUDY VII (2003) ON THE DETECTION OF *SALMONELLA* spp. organised by CRL-*Salmonella*

### Introduction

This 7th collaborative study on the detection of *Salmonella* spp. amongst the National Reference Laboratories (NRLs) in the EU, will have a similar set-up as the 6th study.
At the workshop of May 2003 it was discussed whether the 7th study should be 'simplified' by decreasing the number of methods or whether again the (new) ISO 6579 (2002) should be tested as well. At the same workshop it was also agreed that the CRL and NRLs would like to have a standard (ISO) method for the detection of *Salmonella* spp. in poultry faeces. For this latter it would be necessary to show to the relevant ISO committee that a suggested new 'standard' method would perform better than the ISO procedure. At the workshop it was suggested that replacing a selective broth in the ISO procedure by a semi-solid medium would lead to a higher positivity rate of *Salmonella* spp. in faeces. In the collaborative studies of the CRL good experiences have been obtained with the semi-solid medium MSRV (Modified Semi Solid Rappaport Vassiliadis).
In the 6th collaborative study the performance of the new ISO 6579 (2002) was tested beside MSRV. However, in this study, not all laboratories were able to prepare the MKTTn medium (Muller Kauffmann Tetra Thionate with novobiocin) in accordance with the prescription as given in ISO. It was therefore decided to repeat the set-up of the 6th study in the 7th study. Thus testing the complete ISO 6579 (2002) beside the procedure with MSRV.
Furthermore laboratories who are interested can also perform PCR on the samples and/or use additional methods (routinely) used in their laboratories.

Still there are some (small) differences between study 6 and 7.
Again reference materials (RMs) and poultry faeces will be used. The RMs consist of gelatin capsules containing sublethally injured *Salmonella* Typhimurium (STM), *Salmonella* Enteritidis (SE) or *Salmonella* Panama (SPan).
Each laboratory will again examine 25 faeces samples (**10 g each** and negative for *Salmonella* spp.) in combination with a capsule containing STM or SE and 10 control samples (no faeces added to the capsule). Next to the capsules, again 20 faeces samples

which are naturally contaminated with *Salmonella* will be examined (no addition of capsules), but **in this study 10 g portions of faeces should be analysed instead of 25 g.** This was chosen to come to more uniformity in the study with the 'spiked' samples (faeces 'spiked' with capsules). Furthermore, the use of only 10 g portions of faeces might lead to less confusion in the amount of buffered peptone water (BPW) to be used for pre-enrichment. According to ISO 6579 a 1/10 dilution of the sample in BPW should be achieved. In former collaborative studies this 'rule' was not always followed (10 g of faeces was added to 225 ml BPW). As in this 7th we would like to follow the ISO as strictly as possible, we would also like to introduce this 'rule'. Meaning that **10 g of faeces should be added to 90 ml BPW**.
Furthermore, at the CRL we have performed some experiments, to try to find the most optimal procedure for dissolving the capsules in the presence of chicken faeces. Complete dissolution of the capsules is essential to bring all *Salmonella* bacteria in solution and give them a change to grow. The results of these experiments have lead to some improvements in the procedure for dissolving the capsules (see SOP Bacteriological collaborative study VII (2003)).
Finally, to obtain more detailed information on the temperatures and times during transport of the samples we will include an electronic temperature recorder in the parcel. The amount of materials can not be sent in one parcel and will be divided over three parcels (one containing capsules, one containing *Salmonella* negative faeces and one containing *Salmonella* positive faeces). We will include only one recorder and only in the parcel containing the capsules. The recorder will be packed in a plastic bag, which will also contain your labcode. **You are urgently requested to return this complete plastic bag with recorder and labcode to the CRL-*Salmonella*, immediately after receipt of the parcel**. For this purpose a return envelope with a preprinted address label of the CRL-Salmonella has been included.

**Objectives**

The main objective of the seventh bacteriological collaborative study is to evaluate the results of the detection of different contamination levels of *Salmonella* in the presence of competitive micro-organisms, using different methods, among and within the NRLs.

**Outline of the study**

Each participant will receive 3 parcels containing:

Parcel 1:
- 25 numbered vials; each containing one *Salmonella* Typhimurium, one *Salmonella* Enteritidis or blank capsule (numbered 1-25);
- 10 control vials; each containing one capsule with or without *Salmonella* (numbered C1-C10).

This parcel will contain the small electronic temperature recorder in a plastic bag with your labcode. This recorder (in the plastic bag) should be returned to the CRL-*Salmonella* as soon as possible.

Parcel 2:
- 300 g of (frozen) poultry faeces (free from *Salmonella*).

Parcel 3:
- 300 g of naturally contaminated (with *Salmonella*) (frozen) faeces.

The performance of the study will be in week 45 (starting on 3 November 2003).

The documents necessary for performing the study are:

- Protocol Bacteriological Collaborative Study VII (2003), on the detection of *Salmonella* spp.;
- SOP Bacteriological Collaborative Study VII (2003), on the detection of *Salmonella* spp.
- Test Report Bacteriological Collaborative Study VII (2003), on the detection of *Salmonella* spp.;
- ISO 6579 (2002). Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella* spp.;
- Newsletter CRL-*Salmonella*, Vol. 5, No. 2, June 1999 (for preparation of MSRV). See also on our website.

All data will be reported in the test report and send to the CRL-*Salmonella* and will be used for (statistical) analysis.

The media used for the collaborative study will not be supplied by the CRL.

## Time table of bacteriological collaborative study VII (2003)

| Week | Date | Topic |
|---|---|---|
| 41 | 6 – 10 October | Mailing of the protocol, standard operating procedure and test report to the NRLs |
| 43 + 44 | 20 – 31 October | Mailing of the parcels to the NRLs.<br>Immediately after arrival of the parcels at the laboratory:<br>- Check for any serious damages (**do not accept damaged packages)**;<br>- Check for completeness;<br>- Remove the electronic temperature recorder from the parcel (leave it in the plastic bag with labcode) and **return it to CRL-*Salmonella* using the return envelope**;<br>- **store all materials at -20°C ± 5°C**.<br>If you did not receive the parcel at 31 October, do contact the CRL immediately.<br><br>Preparation of:<br>1. Non selective pre-enrichment medium (see SOP 6.1)<br>2. Selective enrichment media (see SOP 6.2)<br>3. Solid selective plating media (see SOP 6.3)<br>4. Confirmation media (see SOP 6.4) |
| 45 | 3 - 7 November | Performance of the study, following the instructions as given in the protocol and the SOP of study VII (2003). |
| 47 | 17 - 21 November | Completion of the test report and faxing or e-mailing it to the CRL. The original test report will be sent to CRL. |
| 48 | 24-28 November | Checking the results by the National Reference Laboratories. |

If you have questions or remarks about the bacteriological collaborative study please contact:

Hans Korver (research assistant CRL-*Salmonella*)
P.O. Box 1
3720 BA Bilthoven
The Netherlands
tel. number: + 31 30 274 4263
fax. number: + 31 30 274 4434
e-mail: Hans.Korver@rivm.nl

# Annex 7. Standard Operation Procedure

## BACTERIOLOGICAL COLLABORATIVE STUDY VII (2003) ON THE DETECTION OF *SALMONELLA* spp. organised by CRL-*Salmonella*

### 1 Scope and field of application

This standard operating procedure (SOP) describes the procedure for the detection of *Salmonella* in the presence of competitive micro-organisms. For this purpose Reference Materials (RMs) containing sublethally injured *Salmonella* Typhimurium (STM), *Salmonella* Enteritidis (SE) or *Salmonella* Panama (SPan) as prepared by the Community Reference Laboratory for *Salmonella* (CRL) are used. Furthermore poultry faeces is used. The application of this SOP is limited to the bacteriological collaborative study for *Salmonella* described in this SOP.

### 2 References

International Standard – ISO 6579: 2002(E)
Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella* spp.

Beckers, H.J., Van Leusden, F.M., Meijssen, M.J.M., Kampelmacher, E.H. 1985.
Reference material for the evaluation of a standard method for the detection of *Salmonella* in foods and feeding stuffs. J. Appl. Bacteriol., 59, 507-512.

## 3 Definitions

For the purpose of this SOP, the following definitions apply:

- *Salmonella*: micro-organisms which form typical colonies on isolation media for *Salmonella* and which display the serological and/or biochemical reactions described when tests are carried out in accordance with this SOP.
- *Detection of Salmonella*: detection of *Salmonella* from reference materials in the presence of competitive organisms, when the test is carried out in accordance with this SOP.
- *Reference Material*: a gelatine capsule containing a quantified amount artificially contaminated spray dried milk.

## 4 Principle

The detection of *Salmonella* involves the following stages:

a) Pre-enrichment
b) Selective enrichment
c) Isolation
d) Confirmation of typical colonies as *Salmonella*.

## 5 List of abbreviations

| | |
|---|---|
| BGA | phenol red/Brilliant Green Agar |
| BPW | Buffered Peptone Water |
| MKTTn | Muller Kaufmann Tetrathionate novobiocin broth |
| MSRV | Modified semi-solid Rappaport Vassiliadis medium |
| RM | Reference Material |
| RVS | Rappaport Vassiliadis mediium with Soya |
| SOP | Standard Operating Procedure |
| TSI | Triple sugar/iron agar |
| UA | Urea Agar |
| XLD | Xylose Lysine Deoxycholate agar |

# 6 Culture media

Composition and preparation of the media and reagents are described in Annex B of the ISO 6579: 2002(E). Complete ready-to-use media or dehydrated media are also allowed to be used, as long as the composition is in accordance with the information given below. Control the quality of the media before use.

## 6.1 Non selective pre-enrichment medium

- Buffered Peptone water (BPW) (Annex B.1)

Mind to distribute the BPW in portions of **90 ml** into suitable flasks before sterilisation.

## 6.2 Selective enrichment medium

- Rappaport Vassiliadis medium with soya (RVS broth) (Annex B.2)
- Muller Kauffmann tetrathionate-novobiocin broth (MKTTn) (Annex B.3)
- Modified Semi solid Rappaport Vassiliadis (MSRV) (Newsletter, Vol.5, No.2, June 1999)

  This medium must be boiled to dissolve (instructions manufacturer). After boiling the medium must be transparent blue. After cooling down to 50 °C the supplement or the novobiocine has to be added. The final concentration of the novobiocine in the medium should be 0.01 g/l. Plates should be poured with a volume of 15 to 20 ml.
- Selective enrichment medium routinely used in your laboratory (optional)

## 6.3 Solid selective media for first and second isolation

- Phenol red/brilliant green agar (Annex B.4, ISO 6579: 1993)

  The medium must be boiled to dissolve (instructions manufacturer). After boiling the medium must be transparent red.

  Plates should be poured with a volume of 30-40 ml (140 mm-plates).
- Xylose-Lysine-Desoxycholate (Annex B.4)

  This medium must be boiled to dissolve (instructions manufacturer). After boiling the medium must be transparent red. Plates should be poured with a volume of 30-40 ml (140 mm-plates).
- Third medium (optionally) (Paragraph 4.4)

### 6.4 Confirmation media

Biochemical confirmation

- Triple sugar/iron agar (TSI agar) (Annex B.6)
- Urea agar (Annex B.7)
- l-Lysine decarboxylation medium (Annex B.8)
- Nutrient agar (optional) (Annex B.5)

## 7 Apparatus and glassware

The usual microbiological laboratory equipment. If requested, note specifications of the apparatus and glassware on the test report.

### 7.1 Apparatus

- Oven (for dry sterilisation) or autoclave (for wet sterilisation);
- Incubator, capable of operating at 37 °C ± 1 °C;
- Water bath, capable of operating at 41,5 °C ± 1 °C or incubator, capable of operating at 41,5 °C ± 1 °C;
- Water bath, capable of operating at 37 °C ± 1 °C;
- Loops;
- pH-meter; having an accuracy of calibration of ± 0.1 pH unit at 25 °C.

### 7.2 Glassware

- Culture bottles or jars with nominal capacity of 200 ml;
- Culture tubes with approximate sizes: 8 mm in diameter and 160 mm in length;
- Micro-pipettes; nominal capacity 0.1 ml;
- Petri dishes; small size (diameter 90 mm to 100 mm) and/or large size (diameter 140 mm).

# 8 Procedure

## 8.1 Prewarming BPW and thawing faeces

Take the frozen faeces out of the freezer at the end of the day before you start testing and thaw the portions frozen faeces in the closed container **overnight at 5 °C.**
Label 25 jars containing 90 ml of BPW from 1 to 25. For the naturally contaminated samples number 20 jars of BPW from N1 to N20. Also label 12 jars of BPW from C1 to C12 (control capsules). One jar is a procedure control (= C11) to which no capsule or faeces is added and one jar is a negative faeces control to which only 10 gr. faeces is added (= C12). These control jars should further be handled in the same way as the other jars. Place all jars **overnight** at 37 °C (± 1 °C). Also place some extra non-labelled jars containing 90 ml of BPW at 37 °C in case some jars might have been contaminated. Record in the test report (page 2) the requested data of BPW.

## 8.2 Pre-enrichment

Take the numbered vials with the *Salmonella* capsules and the control capsules out of the freezer one hour before they are added to the BPW, to allow them to equilibrate to room temperature.
Shortly before adding the capsules, take the jars with BPW from the 37 °C incubator and inspect them for visual growth. Discard infected jars.
Add to 35 labelled jars a gelatine capsule from the vial with the corresponding label number. Do not open the gelatine capsule and do not shake the BPW to dissolve the capsule more rapidly. Place the jars with the capsules in the 37 °C incubator for **45 minutes** for dissolving of the capsules. Record the temperature and time at the start and at the end of this period in the test report (page 3). After 45 minutes add the thawed faeces to the jars according to the following scheme:

- **Add 10 grams of faeces from portion 2 (number of parcel ) to jars labelled 1-25 and C12,**
- **Add no faeces to jars labelled C1 - C11,**
- **Add 10 grams of faeces from portion 3 (number of parcel) to jars labelled N1-N20.**

***Do not shake the jars after adding the faeces.***

Place all jars in the 37 °C (± 1 °C) incubator for 16 h to 20 h. Record the temperature and time at the start and at the end of the incubation period and other requested data on page 3 of the test report.
If PCR is performed, fill in all requested data in the test report page 21. Results of PCR can be written in the test report Table 5 (page 34).

## 8.3 Selective enrichment

Allow the selective enrichment broths to equilibrate to room temperature, if they were stored at a lower temperature. Dry the surface of the MSRV plates in a Laminair Air Flow cabinet. Record (page 4-11) the requested data of the selective enrichment broths and MSRV plates in the test report. Label 25 jars/tubes/plates of each selective enrichment broth from 1 to 25. Also label 20 selective enrichment jars/tubes/plates from N1 to N20 and 12 jars/tubes/plates from C1 to C12. All selective media are incubated for 24 h and later on for another 24 h.

After equilibration:

- Transfer 0.1 ml of homogenised BPW culture to each tube containing 10 ml RVS medium. Incubate at 41.5 °C ± 1 °C for 24 h ± 3 h and later on another 24 h ± 3 h;
- Transfer 1 ml of homogenised BPW culture to each tube containing 10 ml MKTTn medium. Incubate at 37°C ± 1 °C for 24 h ± 3 h and later on another 24 h ± 3 h;
- Inoculate the MSRV plates with three drops of BPW culture, with a total volume of 0.1 ml. Incubate **(not upside down)** at 41.5 °C ± 1 °C for 24 h ± 3 h and later on another 24 h ± 3 h;
- Inoculate the routinely used selective medium/media (other than those mentioned above), with the corresponding BPW culture (note the inoculation volume of BPW used and the volume of the selective medium/media on test report). Incubate at the temperature routinely used.

Place the jars/tubes in the appropriate incubator(s)/waterbath(s) and record the temperature and time for the different enrichment media at the start and at the end of the incubation period and other requested data in the test report (page 4-11).

## 8.4 Isolation media (first and second isolation)

*Note:*
In the case that you do not have large dishes (140 mm) at your disposal use two small (90-100 mm) dishes, one after the other, using the same loop.
Record in the test report (page 12-14) the requested data of the isolation media used. Label 25 large Petri dishes of the isolation media from 1 to 25, label 20 large Petri dishes from N1 to N20 and label 12 large Petri dishes from C1 to C12.

First isolation after 24 h
*Inoculation*:
Inoculate, by means of a loop, from all selective enrichment cultures and from suspect MSRV plates, the surface of an isolation medium in a large size Petri dish with the corresponding label number. The following isolation media will be used:

1) Phenol red/brilliant green agar BGA)
   Place the Petri dishes with the bottom up in the incubator set at 37 °C ± 1 °C (record the temperature and time at the start and at the end of the incubation and other requested data in test report, page 12-13).

2) Xylose Lysine Desoxycholate agar (XLD)
   Place the Petri dishes with the bottom up in the incubator set at 37 °C (note the temperature and time at the start and at the end of the incubation and other requested data on test report, page 14-15).

3) Optionally: selective isolation medium/media routinely used in your laboratory. Only if media used are different from those mentioned above.
   Incubate the medium/media at the temperature routinely used (record temperature and time and other requested data in test report, page 16-17).

After incubation for 24 h ± 3 h, examine the Petri dishes for the presence of typical colonies of *Salmonella*.

**Second isolation after 48 h**
After a total incubation time of 48 h ± 3h of the selective enrichment media, repeat the procedure described above (**First isolation after 24 h**).

## 8.5 Confirmation of colonies from first and second isolation

For confirmation take from each Petri dish of each selective medium at least 1 colony considered to be typical or suspect (only use isolated colonies). Store plates at 5 $^{o}$C ± 3 $^{o}$C.
Before biochemical confirmation, optionally, streak the typical colonies onto the surface of nutrient agar plates with the corresponding label numbers, in a manner which allows to develop well isolated colonies. Record on test report (page 18-19) the requested data of the nutrient agar. Incubate the inoculated plates at 37 °C ± 1 °C for 24 h ± 3 h.
If the selected colony is not confirmed as *Salmonella*, test at maximum another 5 typical colonies from the original isolation medium (stored at 5 °C). Report the number of colonies tested and the number of colonies confirmed as *Salmonella* for each dish in Table 1 (isolation using RVS), Table 2 (isolation using MKTTn), Table 3 (isolation using MSRV) and Table 4 (isolation using own enrichment) on test report page 22-33. For the results of detection of *Salmonella* using PCR fill in Table 4 on test report page 34.

**Biochemical confirmation**
By means of a loop, inoculate the media specified below with the colony selected as described above (either directly from the isolation medium, or from nutrient agar). For each of the mentioned media follow the instructions in 9.5 of ISO 6579 (2002). Optionally inoculate other media which are routinely used for biochemical confirmation. Record in test report (page 20) the requested data of the media.

- TSI agar
- Urea agar
- l-Lysine decarboxylation medium

**Interpretation of the biochemical tests**
*Salmonella* generally show the reactions given in Table 1 of ISO 6579:2002(E) on page 9).

- TSI agar:
  *Butt:* -yellow by fermentation of glucose;
  -black by formation of hydrogen sulfide; and
  -bubbles or cracks due to gas formation from glucose;
  *Slant:* -red or unchanged
- Urea agar: yellow;
- l-Lysine decarboxylation medium: coloured purple.

# 9 Test report

The test report will contain all information, that might influence the results and is not mentioned in this SOP. Some incidents or deviations from the specified procedures will also be recorded. The test report will include the names of the persons, who are carrying out the work and will be signed by these persons.

**Scheme of bacteriological collaborative study VII (2003) on detection of *Salmonella* spp.**

| Day | Topic | Description |
|---|---|---|
| 1 | **Prewarming BPW**<br><br>**Thawing faeces** | Place at the end of the day sufficient jars, each containing 90 ml BPW, at 37 °C ± 1 °C.<br>Place at the end of the day the chicken faeces in the closed containers at 5 °C ± 3 °C |
| 2 | **Pre-enrichment** | 1 capsule to 90 ml (prewarmed) BPW<br>Do not shake<br>45 min. at 37 °C ± 1 °C<br>Add 10 g faeces (thawed at 5 °C) to BPW<br>Incubate 16-20 h at 37 °C ± 1 °C |
| 3 | **Selective enrichment** | 0,1 ml BPW culture in 10 ml RVS, incubate at (41.5 ± 1) °C for (24 ± 3) h<br>1 ml BPW culture in 10 ml MKTTn, incubate at (37 ± 1) °C for (24 ± 3) h<br>0,1 ml BPW culture on MSRV plate, incubate at (41.5 ± 1) °C for (24 ± 3) h<br>Other selective enrichment medi(um)(a) |
| 4 | **First isolation after 24 h** | Inoculate from RVS, MKTTn, suspect MSRV plates and other medi(um)(a)<br>➢ phenol red/brilliant green agar, incubate at (37 ± 1) °C for (24 ± 3) h<br>➢ Xylose Lysine Desoxycholate agar, incubate at (37 ± 1) °C for (24 ± 3) h<br>➢ other selective medi(um)(a), incubate for specified time at the specified temperature |
| 4 | **Continue selective enrichment** | Incubate RVS, MKTTn and MSRV medium (see day 3) another 24 (± 3) hours at the relevant temperatures |
| 5 | **Second isolation after 24 h** | Inoculate from RVS, MKTTn,suspect MSRV plates and Other medi(um)(a) (see day 4)<br>➢ phenol red/brilliant green agar<br>➢ Xylose Lysine Desoxycholate agar<br>➢ other selective medi(um)(a) |
| 5 | **Biochemical confirmation** | Inoculate the media from first isolation media (day 4) for biochemical identification and incubate 24 (± 3)h at the specified temperature |
| 6 | **Biochemical confirmation** | Inoculate the media from second isolation media (day 5) for biochemical identification and incubate 24 (± 3)h at the specified temperature |

# Annex 8. Test report

**BACTERIOLOGICAL COLLABORATIVE STUDY VII (2003)**
**ON THE DETECTION OF *SALMONELLA* spp.**
**organised by CRL-*Salmonella***

| | |
|---|---|
| Laboratory code | |
| Laboratory name | |
| Address | |
| Country | |
| | |
| Date of arrival of the parcels | …… - ……. - 2003 |
| Start time of storage at - 20°C | Date:……. Time:…… |
| Parcels damaged? | **YES** **NO** |
| Starting date testing | …… - ……. - 2003 |

| | |
|---|---|
| Is your laboratory accredited or certified for the determination of *Salmonella*. If yes, according to which system ? If no, are you planning to be accredited or certified in the near future ? | |

| **PRE-ENRICHMENT – Buffered Peptone Water (BPW) (I)** |
|---|

| **Medium information BPW** |
|---|
| What did you use to prepare the BPW? |
| Individual ingredients |
| Dehydrated medium |
| Ready-to-use medium |

| **In case of dehydrated or ready-to-use medium , give information on the manufacturer of BPW** | |
|---|---|
| Name | |
| Code number | |
| Batch number | |
| Expire date | |

| **Specific data of composition of BPW medium. What is the concentration of the following compounds in 1000 ml water:** | |
|---|---|
| Enzymatic digest of casein | |
| Sodium chloride | |
| Disodium hydrogen phosphate dodecahydrate | |
| Potassium dihydrogen phosphate | |

| **Preparation of BPW** | |
|---|---|
| Date of preparation | …… - ……. - 2003 |
| pH after preparation | …….., measured at …… °C |
| pH at the day of use | …….., measured at …… °C |
| Did you perform quality control of BPW? | Yes<br>No |

| PRE-ENRICHMENT – Buffered Peptone Water (BPW) (II) |
|---|

| Prewarming time and temperature of the BPW | |
|---|---|
| At the start | Date: …… - ……. - 2003<br>time: ……... h ……..…… min<br>temperature incubator: ……..…… °C |
| At the end | Date: …… - ……. - 2003<br>time: …… h …… min<br>temperature incubator: ……...…… °C |

| Incubation time and temperature for dissolving the capsules | |
|---|---|
| At the start | Date: …… - ……. - 2003<br>time: ……... h ……..…… min<br>temperature incubator: ……..…… °C |
| At the end | time: …… h …… min<br>temperature incubator: ……...…… °C |

| Incubation time and temperature for pre-enrichment (after adding the faeces) | |
|---|---|
| At the start | Date: …… - ……. - 2003<br>time: …….. h ……..…… min<br>temperature incubator: ……..…… °C |
| At the end | Date: …… - ……. - 2003<br>time: …….. h ……..…… min<br>temperature incubator: ……..…… °C |

| **SELECTIVE ENRICHMENT - Rappaport Vassiliadis Soya medium (RVS) (I)** |
|---|

| **Medium information RVS** |
|---|
| What did you use to prepare the RVS? |
| Individual ingredients |
| Dehydrated medium |
| Ready-to-use medium |

| **In case of dehydrated or ready-to-use medium , give information on the manufacturer of RVS** | |
|---|---|
| Name | |
| Code number | |
| Batch number | |
| Expire date | |

| **Specific data of composition of RVS medium. What is the concentration of the following compounds in 1000 ml water:** | |
|---|---|
| Soya Peptone | |
| Sodium chloride | |
| Potassium dihydrogen phosphate | |
| Dipotassium hydrogen phosphate | |
| Magnesium chloride anhydrous | |
| Magnesium chloride.$6H_2O$ | |
| Malachite green | |

| **Preparation of RVS** | |
|---|---|
| Date of preparation | …… - ……. - 2003 |
| pH after preparation | …….., measured at …… °C |
| pH at the day of use | …….., measured at …… °C |
| Did you perform quality control of RVS? | Yes<br>No |

| **SELECTIVE ENRICHMENT - Rappaport Vassiliadis Soya medium (RVS) (II)** |
|---|

| **Incubation time and temperature for selective enrichment** | |
|---|---|
| At the start of the first period | Date: …… - ……. - 2003<br>time: …….. h ……..…… min<br>temperature incubator: ……..…… °C |
| At the end of the first period | Date: …… - ……. - 2003<br>time: …….. h ……..…… min<br>temperature incubator: ……..…… °C |
| At the start of the second period | Date: …… - ……. - 2003<br>time: …….. h ……..…… min<br>temperature incubator: ……..…… °C |
| At the end of the second period | Date: …… - ……. - 2003<br>time: …….. h ……..…… min<br>temperature incubator: ……..…… °C |

| **SELECTIVE ENRICHMENT - Muller Kauffmann Tetra Thionate + novobiocin (MKTTn) (I)** |
|---|

| **Medium information MKTTn** |
|---|
| What did you use to prepare the MKTTn? |
| Individual ingredients |
| Dehydrated medium |
| Ready-to-use medium |

| **In case of dehydrated or ready-to-use medium , give information on the manufacturer of MKTTn** | |
|---|---|
| Name | |
| Code number | |
| Batch number | |
| Expire date | |

| **Specific data of composition of MKTTn medium. What is the concentration of the following compounds in 1000 ml water:** | |
|---|---|
| Enzymatic digest of meat extract | |
| Enzymatic digest of casein | |
| Sodium chloride (NaCl) | |
| Calcium carbonate ($CaCO_3$) | |
| Sodium thiosulfate pentahydrate ($Na_2S_2O_3.5H_2O$) | |
| Ox bile for bacteriological use | |
| Brilliant green | |

| **Preparation of MKTTn** | |
|---|---|
| Date of preparation | …… - ……. - 2003 |
| pH after preparation | …….., measured at …… °C |
| pH at the day of use | …….., measured at …… °C |
| Did you perform quality control of MKTTn? | Yes<br>No |

| **SELECTIVE ENRICHMENT - Muller Kauffmann Tetra Thionate + novobiocin (MKTTn) (II)** |
|---|

| **Incubation time and temperature for selective enrichment** | |
|---|---|
| At the start of the first period | Date: …… - ……. - 2003<br>time: …….. h ……..…… min<br>temperature incubator: ……..…… °C |
| At the end of the first period | Date: …… - ……. - 2003<br>time: …….. h ……..…… min<br>temperature incubator: ……..…… °C |
| At the start of the second period | Date: …… - ……. - 2003<br>time: …….. h ……..…… min<br>temperature incubator: ……..…… °C |
| At the end of the second period | Date: …… - ……. - 2003<br>time: …….. h ……..…… min<br>temperature incubator: ……..…… °C |

| **SELECTIVE ENRICHMENT - Modified Semi solid Rappaport Vassiliadis medium (MSRV) (I)** |
|---|

| **Medium information MSRV** |
|---|
| What did you use to prepare the MSRV? |
| Individual ingredients |
| Dehydrated medium |
| Ready-to-use medium |

| **In case of dehydrated or ready-to-use medium , give information on the manufacturer of MSRV** | |
|---|---|
| Name | |
| Code number | |
| Batch number | |
| Expire date | |

| **Specific data of composition of MSRV medium. What is the concentration of the following compounds in 1000 ml water:** | |
|---|---|
| | |
| | |
| | |
| | |
| | |
| | |
| | |

| **Preparation of MSRV** | |
|---|---|
| Date of preparation | …… - ……. - 2003 |
| pH after preparation | …….., measured at …… °C |
| pH at the day of use | …….., measured at …… °C |
| Did you perform quality control of MSRV? | Yes<br>No |

| **SELECTIVE ENRICHMENT - Modified Semi solid Rappaport Vassiliadis medium (MSRV) (II)** |
|---|

| **Incubation time and temperature for selective enrichment** | |
|---|---|
| At the start of the first period | Date: …… - ……. - 2003<br>time: …….. h ……..…… min<br>temperature incubator: ……..…… °C |
| At the end of the first period | Date: …… - ……. - 2003<br>time: …….. h ……..…… min<br>temperature incubator: ……..…… °C |
| At the start of the second period | Date: …… - ……. - 2003<br>time: …….. h ……..…… min<br>temperature incubator: ……..…… °C |
| At the end of the second period | Date: …… - ……. - 2003<br>time: …….. h ……..…… min<br>temperature incubator: ……..…… °C |

| **SELECTIVE ENRICHMENT - Selective medium, routinely used in your laboratory (I)** |
|---|

| **If you use more selective media, please write these on an annex.** | |
|---|---|
| Medium: | |

| **Medium information** |
|---|
| What did you use to prepare the medium? |
| Individual ingredients |
| Dehydrated medium |
| Ready-to-use medium |

| **In case of dehydrated or ready-to-use medium , give information on the manufacturer of the medium** | |
|---|---|
| Name | |
| Code number | |
| Batch number | |
| Expire date | |

| **Specific data of composition of the medium. What is the concentration of the compounds in 1000 ml water:** | |
|---|---|
| | |
| | |
| | |
| | |
| | |
| | |

| **Preparation of the medium** | |
|---|---|
| Date of preparation | …… - ……. - 2003 |
| pH after preparation | …….., measured at …… °C |
| pH at the day of use | …….., measured at …… °C |
| Did you perform quality control of the medium? | Yes<br>No |

| **SELECTIVE ENRICHMENT - Selective medium, routinely used in your laboratory (II)** |
|---|

| **Further details concerning the medium** | |
|---|---|
| Volume of the medium per jar/tube in ml | |
| Inoculation volume of BPW | |
| Prescribed incubation temperature in $^{o}$C | |

| **Incubation time and temperature for selective enrichment** | |
|---|---|
| At the start of the first period | Date: …… - ……. - 2003<br>time: …….. h ……..…… min<br>temperature incubator: ……..…… °C |
| At the end of the first period | Date: …… - ……. - 2003<br>time: …….. h ……..…… min<br>temperature incubator: ……..…… °C |
| At the start of the second period | Date: …… - ……. - 2003<br>time: …….. h ……..…… min<br>temperature incubator: ……..…… °C |
| At the end of the second period | Date: …… - ……. - 2003<br>time: …….. h ……..…… min<br>temperature incubator: ……..…… °C |

| **FIRST AND SECOND ISOLATION - Phenol red/brilliant green agar (BGA) (I)** |
|---|

| **Medium information BGA** |
|---|
| What did you use to prepare the BGA ? |
| Individual ingredients |
| Dehydrated medium |
| Ready-to-use medium |

| **In case of dehydrated or ready-to-use medium , give information on the manufacturer of BGA** | |
|---|---|
| Name | |
| Code number | |
| Batch number | |
| Expire date | |

| **Specific data of composition of BGA medium. What is the concentration of the following compounds in 1000 ml water:** | |
|---|---|
| | |
| | |
| | |
| | |
| | |
| | |
| | |

| **Preparation of BGA** | |
|---|---|
| Date of preparation | …… - ……. - 2003 |
| pH after preparation | …….., measured at …… °C |
| pH at the day of use | …….., measured at …… °C |
| Did you perform quality control of BGA ? | Yes<br>No |

**FIRST AND SECOND ISOLATION - Phenol red/brilliant green agar (BGA) (II)**

| **Size of petri dishes** | | | |
|---|---|---|---|
| Size of petri dishes used | 90 mm | 100 mm | 140 mm |

| **Incubation time and temperature for isolation** | |
|---|---|
| At the start of the first period | Date: …… - ……. - 2003<br>time: …….. h ……..…… min<br>temperature incubator: ……..…… °C |
| At the end of the first period | Date: …… - ……. - 2003<br>time: …….. h ……..…… min<br>temperature incubator: ……..…… °C |
| At the start of the second period | Date: …… - ……. - 2003<br>time: …….. h ……..…… min<br>temperature incubator: ……..…… °C |
| At the end of the second period | Date: …… - ……. - 2003<br>time: …….. h ……..…… min<br>temperature incubator: ……..…… °C |

| **FIRST AND SECOND ISOLATION - Xylose Lysine Desoxycholate medium (XLD) (I)** |
|---|

| **Medium information XLD** |
|---|
| What did you use to prepare the XLD ? |
| Individual ingredients |
| Dehydrated medium |
| Ready-to-use medium |

| **In case of dehydrated or ready-to-use medium , give information on the manufacturer of XLD** | |
|---|---|
| Name | |
| Code number | |
| Batch number | |
| Expire date | |

| **Specific data of composition of XLD medium. What is the concentration of the following compounds in 1000 ml water:** | |
|---|---|
| | |
| | |
| | |
| | |
| | |
| | |
| | |

| **Preparation of XLD** | |
|---|---|
| Date of preparation | …… - ……. - 2003 |
| pH after preparation | …….., measured at …… °C |
| pH at the day of use | …….., measured at …… °C |
| Did you perform quality control of XLD ? | Yes<br>No |

**FIRST AND SECOND ISOLATION - Xylose Lysine Desoxycholate medium (XLD) (II)**

| **Size of petri dishes** | | | |
|---|---|---|---|
| Size of petri dishes used | 90 mm | 100 mm | 140 mm |

| **Incubation time and temperature for isolation** | |
|---|---|
| At the start of the first period | Date: …… - ……. - 2003<br>time: …….. h ……..…… min<br>temperature incubator: ……..…… °C |
| At the end of the first period | Date: …… - ……. - 2003<br>time: …….. h ……..…… min<br>temperature incubator: ……..…… °C |
| At the start of the second period | Date: …… - ……. - 2003<br>time: …….. h ……..…… min<br>temperature incubator: ……..…… °C |
| At the end of the second period | Date: …… - ……. - 2003<br>time: …….. h ……..…… min<br>temperature incubator: ……..…… °C |

| **FIRST AND SECOND ISOLATION - Isolation medium routinely used in your lab. (I)** |
|---|

| **If you use more selective media, please write these on an annex.** | |
|---|---|
| Name of the medium | |
| Prescribed incubation temperature in $^{o}$C | |

| **Medium information of your own medium** |
|---|
| What did you use to prepare your own medium ? |
| Individual ingredients |
| Dehydrated medium |
| Ready-to-use medium |

| **In case of dehydrated or ready-to-use medium , give information on the manufacturer of your own medium** | |
|---|---|
| Name | |
| Code number | |
| Batch number | |
| Expire date | |

| **Specific data of composition of your own medium. What is the concentration of the following compounds in 1000 ml water:** | |
|---|---|
| | |
| | |
| | |
| | |
| | |
| | |
| | |

**FIRST AND SECOND ISOLATION - Isolation medium routinely used in your lab. (II)**

| **Preparation of your own medium** | |
|---|---|
| Date of preparation | …… - ……. - 2003 |
| pH after preparation | …….., measured at …… °C |
| pH at the day of use | …….., measured at …… °C |
| Did you perform quality control ? | Yes<br>No |

| **Size of petri dishes** | | | |
|---|---|---|---|
| Size of petri dishes used | 90 mm | 100 mm | 140 mm |

| **Incubation time and temperature for isolation** | |
|---|---|
| At the start of the first period | Date: …… - ……. - 2003<br>time: …….. h ……..…… min<br>temperature incubator: ……..…… °C |
| At the end of the first period | Date: …… - ……. - 2003<br>time: …….. h ……..…… min<br>temperature incubator: ……..…… °C |
| At the start of the second period | Date: …… - ……. - 2003<br>time: …….. h ……..…… min<br>temperature incubator: ……..…… °C |
| At the end of the second period | Date: …… - ……. - 2003<br>time: …….. h ……..…… min<br>temperature incubator: ……..…… °C |

| **CONFIRMATION – Nutrient agar (I)** |
|---|

| **Did you streak the colonies on Nutrient agar before starting confirmation?** |
|---|
| yes no<br>If yes give further information on nutrient agar below |

| **Medium information Nutrient agar** |
|---|
| What did you use to prepare the nutrient agar ? |
| Individual ingredients |
| Dehydrated medium |
| Ready-to-use medium |

| **In case of dehydrated or ready-to-use medium , give information on the manufacturer of the nutrient agar** | |
|---|---|
| Name | |
| Code number | |
| Batch number | |
| Expire date | |

| **Specific data of composition of nutrient agar medium. What is the concentration of the following compounds in 1000 ml water:** | |
|---|---|
| | |
| | |
| | |
| | |
| | |
| | |
| | |

| **CONFIRMATION – Nutrient agar (II)** |
|---|

| **Preparation of the nutrient agar** | |
|---|---|
| Date of preparation | …… - ……. - 2003 |
| pH after preparation | …….., measured at …… °C |
| pH at the day of use | …….., measured at …… °C |
| Did you perform quality control of nutrient agar ? | yes no |

| **Size of petri dishes** | | | |
|---|---|---|---|
| Size of petri dishes used | 90 mm | 100 mm | 140 mm |

| **BIOCHEMICAL CONFIRMATION** |
|---|

| **Manufacturer of the TSI agar** | |
|---|---|
| Name | |
| Code number | |
| Batch number | |
| Expire date | |
| pH of the broth:…….. | Measured at……$^{o}$C<br>Date …… - ……. 2003 |

| **Manufacturer of the urea agar** | |
|---|---|
| Name | |
| Code number | |
| Batch number | |
| Expire date | |
| pH of the broth:…….. | Measured at……$^{o}$C<br>Date …… - ……. 2003 |

| **Manufacturer of the l-Lysine decarboxylation medium** | |
|---|---|
| Name | |
| Code number | |
| Batch number | |
| Expire date | |
| pH of the broth:…….. | Measured at……$^{o}$C<br>Date …… - ……. 2003 |

| **Manufacturer of other confirmation tests - ……** | |
|---|---|
| Name | |
| Code number | |
| Batch number | |
| Expire date | |
| pH of the broth:…….. | Measured at……$^{o}$C<br>Date …… - ……. 2003 |

| **DETECTION BY PCR** |
|---|

| **General questions** | |
|---|---|
| Is the PCR used commercially available | • Yes<br>• No |
| If yes, name of PCR, manufacturer and batch used in the study: | |
| Is the PCR validated | • Yes<br>• No |
| How much samples did you test for *Salmonella* using this PCR in 2002 ? | |
| At what moment did you start with the extraction/detection? | before or after incubation of BPW |
| Volume of pre-enrichment used for extraction | |
| Volume of DNA-sample obtained from extraction | |
| Volume of DNA-sample added to PCR-mixture | |

| **Composition of PCR-mixture** | | |
|---|---|---|
| Compound | Volume per sample | Manufacturer and batch of specific compound |
| | | |
| | | |
| | | |
| | | |

| | |
|---|---|
| Name of thermocycler | |
| Write down the cycles | |
| What kind of detection system is used | |

Table 1: Results of isolation using RVS (dish numbers 1-25)

| Sample no. | RVS 24 hours | | | | | | RVS 48 hours | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | BGA | | XLD | | Third medium | | BGA | | XLD | | Third medium | |
| | Col [a] | Sal [b] | Col | Sal | Col | Sal | Col | Sal | Col | Sal | Col | Sal |
| 1 | | | | | | | | | | | | |
| 2 | | | | | | | | | | | | |
| 3 | | | | | | | | | | | | |
| 4 | | | | | | | | | | | | |
| 5 | | | | | | | | | | | | |
| 6 | | | | | | | | | | | | |
| 7 | | | | | | | | | | | | |
| 8 | | | | | | | | | | | | |
| 9 | | | | | | | | | | | | |
| 10 | | | | | | | | | | | | |
| 11 | | | | | | | | | | | | |
| 12 | | | | | | | | | | | | |
| 13 | | | | | | | | | | | | |
| 14 | | | | | | | | | | | | |
| 15 | | | | | | | | | | | | |
| 16 | | | | | | | | | | | | |
| 17 | | | | | | | | | | | | |
| 18 | | | | | | | | | | | | |
| 19 | | | | | | | | | | | | |
| 20 | | | | | | | | | | | | |
| 21 | | | | | | | | | | | | |
| 22 | | | | | | | | | | | | |
| 23 | | | | | | | | | | | | |
| 24 | | | | | | | | | | | | |
| 25 | | | | | | | | | | | | |

Col [a] = number of colonies used for confirmation
Sal [b] = number of colonies confirmed as *Salmonella*

Table 1 (continued): Results of isolation using RVS (dish numbers N1-N20)

| sample no. | RVS 24 hours | | | | | | RVS 48 hours | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | BGA | | XLD | | Third medium | | BGA | | XLD | | Third medium | |
| | Col [a] | Sal [b] | Col | Sal | Col | Sal | Col | Sal | Col | Sal | Col | Sal |
| N1 | | | | | | | | | | | | |
| N2 | | | | | | | | | | | | |
| N3 | | | | | | | | | | | | |
| N4 | | | | | | | | | | | | |
| N5 | | | | | | | | | | | | |
| N6 | | | | | | | | | | | | |
| N7 | | | | | | | | | | | | |
| N8 | | | | | | | | | | | | |
| N9 | | | | | | | | | | | | |
| N10 | | | | | | | | | | | | |
| N11 | | | | | | | | | | | | |
| N12 | | | | | | | | | | | | |
| N13 | | | | | | | | | | | | |
| N14 | | | | | | | | | | | | |
| N15 | | | | | | | | | | | | |
| N16 | | | | | | | | | | | | |
| N17 | | | | | | | | | | | | |
| N18 | | | | | | | | | | | | |
| N19 | | | | | | | | | | | | |
| N20 | | | | | | | | | | | | |

Col [a] = number of colonies used for confirmation
Sal [b] = number of colonies confirmed as *Salmonella*

Table 1 (continued): Results of isolation using RVS (dish numbers C1-C12)

| Sample no. | RVS 24 hours | | | | | | RVS 48 hours | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | BGA | | XLD | | Third medium | | BGA | | XLD | | Third medium | |
| | Col [a] | Sal [b] | Col | Sal | Col | Sal | Col | Sal | Col | Sal | Col | Sal |
| C1 | | | | | | | | | | | | |
| C2 | | | | | | | | | | | | |
| C3 | | | | | | | | | | | | |
| C4 | | | | | | | | | | | | |
| C5 | | | | | | | | | | | | |
| C6 | | | | | | | | | | | | |
| C7 | | | | | | | | | | | | |
| C8 | | | | | | | | | | | | |
| C9 | | | | | | | | | | | | |
| C10 | | | | | | | | | | | | |
| C11 | | | | | | | | | | | | |
| C12 | | | | | | | | | | | | |

Col [a] = number of colonies used for confirmation
Sal [b] = number of colonies confirmed as *Salmonella*

Table 2: Results of isolation using MKTTn (dish numbers 1-25)

| sample no. | MKTTn 24 hours | | | | | | MKTTn 48 hours | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | BGA | | XLD | | Third medium | | BGA | | XLD | | Third medium | |
| | Col [a] | Sal [b] | Col | Sal | Col | Sal | Col | Sal | Col | Sal | Col | Sal |
| 1 | | | | | | | | | | | | |
| 2 | | | | | | | | | | | | |
| 3 | | | | | | | | | | | | |
| 4 | | | | | | | | | | | | |
| 5 | | | | | | | | | | | | |
| 6 | | | | | | | | | | | | |
| 7 | | | | | | | | | | | | |
| 8 | | | | | | | | | | | | |
| 9 | | | | | | | | | | | | |
| 10 | | | | | | | | | | | | |
| 11 | | | | | | | | | | | | |
| 12 | | | | | | | | | | | | |
| 13 | | | | | | | | | | | | |
| 14 | | | | | | | | | | | | |
| 15 | | | | | | | | | | | | |
| 16 | | | | | | | | | | | | |
| 17 | | | | | | | | | | | | |
| 18 | | | | | | | | | | | | |
| 19 | | | | | | | | | | | | |
| 20 | | | | | | | | | | | | |
| 21 | | | | | | | | | | | | |
| 22 | | | | | | | | | | | | |
| 23 | | | | | | | | | | | | |
| 24 | | | | | | | | | | | | |
| 25 | | | | | | | | | | | | |

Col [a] = number of colonies used for confirmation
Sal [b] = number of colonies confirmed as *Salmonella*

Table 2 (continued): Results of isolation using MKTTn (dish numbers N1-N20)

| sample no. | MKTTn 24 hours | | | | | | MKTTn 48 hours | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | BGA | | XLD | | third medium | | BGA | | XLD | | third medium | |
| | Col [a] | Sal [b] | Col | Sal | Col | Sal | Col | Sal | Col | Sal | Col | Sal |
| N1 | | | | | | | | | | | | |
| N2 | | | | | | | | | | | | |
| N3 | | | | | | | | | | | | |
| N4 | | | | | | | | | | | | |
| N5 | | | | | | | | | | | | |
| N6 | | | | | | | | | | | | |
| N7 | | | | | | | | | | | | |
| N8 | | | | | | | | | | | | |
| N9 | | | | | | | | | | | | |
| N10 | | | | | | | | | | | | |
| N11 | | | | | | | | | | | | |
| N12 | | | | | | | | | | | | |
| N13 | | | | | | | | | | | | |
| N14 | | | | | | | | | | | | |
| N15 | | | | | | | | | | | | |
| N16 | | | | | | | | | | | | |
| N17 | | | | | | | | | | | | |
| N18 | | | | | | | | | | | | |
| N19 | | | | | | | | | | | | |
| N20 | | | | | | | | | | | | |

Col [a] = number of colonies used for confirmation
Sal [b] = number of colonies confirmed as *Salmonella*

Table 2 (continued): Results of isolation using MKTTn (dish numbers C1-C12)

| | MKTTn 24 hours | | | | | | MKTTn 48 hours | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| sample no. | BGA | | XLD | | Third medium | | BGA | | XLD | | Third medium | |
| | Col [a] | Sal [b] | Col | Sal | Col | Sal | Col | Sal | Col | Sal | Col | Sal |
| C1 | | | | | | | | | | | | |
| C2 | | | | | | | | | | | | |
| C3 | | | | | | | | | | | | |
| C4 | | | | | | | | | | | | |
| C5 | | | | | | | | | | | | |
| C6 | | | | | | | | | | | | |
| C7 | | | | | | | | | | | | |
| C8 | | | | | | | | | | | | |
| C9 | | | | | | | | | | | | |
| C10 | | | | | | | | | | | | |
| C11 | | | | | | | | | | | | |
| C12 | | | | | | | | | | | | |

Col [a] = number of colonies used for confirmation
Sal [b] = number of colonies confirmed as *Salmonella*

Table 3: Results of isolation using MSRV (dish numbers 1-25)

| sample no. | MSRV 24 hours | | | | | | MSRV 48 hours | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | BGA | | XLD | | Third medium | | BGA | | XLD | | Third medium | |
| | Col [a] | Sal [b] | Col | Sal | Col | Sal | Col | Sal | Col | Sal | Col | Sal |
| 1 | | | | | | | | | | | | |
| 2 | | | | | | | | | | | | |
| 3 | | | | | | | | | | | | |
| 4 | | | | | | | | | | | | |
| 5 | | | | | | | | | | | | |
| 6 | | | | | | | | | | | | |
| 7 | | | | | | | | | | | | |
| 8 | | | | | | | | | | | | |
| 9 | | | | | | | | | | | | |
| 10 | | | | | | | | | | | | |
| 11 | | | | | | | | | | | | |
| 12 | | | | | | | | | | | | |
| 13 | | | | | | | | | | | | |
| 14 | | | | | | | | | | | | |
| 15 | | | | | | | | | | | | |
| 16 | | | | | | | | | | | | |
| 17 | | | | | | | | | | | | |
| 18 | | | | | | | | | | | | |
| 19 | | | | | | | | | | | | |
| 20 | | | | | | | | | | | | |
| 21 | | | | | | | | | | | | |
| 22 | | | | | | | | | | | | |
| 23 | | | | | | | | | | | | |
| 24 | | | | | | | | | | | | |
| 25 | | | | | | | | | | | | |

Col [a] = number of colonies used for confirmation
Sal [b] = number of colonies confirmed as *Salmonella*

Table 3 (continued): Results of isolation using MSRV (dish numbers N1-N20)

| sample no. | MSRV 24 hours | | | | | | MSRV 48 hours | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | BGA | | XLD | | Third medium | | BGA | | XLD | | Third medium | |
| | Col [a] | Sal [b] | Col | Sal | Col | Sal | Col | Sal | Col | Sal | Col | Sal |
| N1 | | | | | | | | | | | | |
| N2 | | | | | | | | | | | | |
| N3 | | | | | | | | | | | | |
| N4 | | | | | | | | | | | | |
| N5 | | | | | | | | | | | | |
| N6 | | | | | | | | | | | | |
| N7 | | | | | | | | | | | | |
| N8 | | | | | | | | | | | | |
| N9 | | | | | | | | | | | | |
| N10 | | | | | | | | | | | | |
| N11 | | | | | | | | | | | | |
| N12 | | | | | | | | | | | | |
| N13 | | | | | | | | | | | | |
| N14 | | | | | | | | | | | | |
| N15 | | | | | | | | | | | | |
| N16 | | | | | | | | | | | | |
| N17 | | | | | | | | | | | | |
| N18 | | | | | | | | | | | | |
| N19 | | | | | | | | | | | | |
| N20 | | | | | | | | | | | | |

Col [a] = number of colonies used for confirmation
Sal [b] = number of colonies confirmed as *Salmonella*

Table 3 (continued): Results of isolation using MSRV (dish numbers C1-C12)

| sample no. | MSRV 24 hours | | | | | | MSRV 48 hours | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | BGA | | XLD | | Third medium | | BGA | | XLD | | Third medium | |
| | Col [a] | Sal [b] | Col | Sal | Col | Sal | Col | Sal | Col | Sal | Col | Sal |
| C1 | | | | | | | | | | | | |
| C2 | | | | | | | | | | | | |
| C3 | | | | | | | | | | | | |
| C4 | | | | | | | | | | | | |
| C5 | | | | | | | | | | | | |
| C6 | | | | | | | | | | | | |
| C7 | | | | | | | | | | | | |
| C8 | | | | | | | | | | | | |
| C9 | | | | | | | | | | | | |
| C10 | | | | | | | | | | | | |
| C11 | | | | | | | | | | | | |
| C12 | | | | | | | | | | | | |

Col [a] = number of colonies used for confirmation
Sal [b] = number of colonies confirmed as *Salmonella*

Table 4: Results of isolation using own enrichment (dish numbers 1-25)

| | Own enrichment 24 hours | | | | | | Own enrichment 48 hours | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| sample no. | BGA | | XLD | | Third medium | | BGA | | XLD | | Third medium | |
| | Col [a] | Sal [b] | Col | Sal | Col | Sal | Col | Sal | Col | Sal | Col | Sal |
| 1 | | | | | | | | | | | | |
| 2 | | | | | | | | | | | | |
| 3 | | | | | | | | | | | | |
| 4 | | | | | | | | | | | | |
| 5 | | | | | | | | | | | | |
| 6 | | | | | | | | | | | | |
| 7 | | | | | | | | | | | | |
| 8 | | | | | | | | | | | | |
| 9 | | | | | | | | | | | | |
| 10 | | | | | | | | | | | | |
| 11 | | | | | | | | | | | | |
| 12 | | | | | | | | | | | | |
| 13 | | | | | | | | | | | | |
| 14 | | | | | | | | | | | | |
| 15 | | | | | | | | | | | | |
| 16 | | | | | | | | | | | | |
| 17 | | | | | | | | | | | | |
| 18 | | | | | | | | | | | | |
| 19 | | | | | | | | | | | | |
| 20 | | | | | | | | | | | | |
| 21 | | | | | | | | | | | | |
| 22 | | | | | | | | | | | | |
| 23 | | | | | | | | | | | | |
| 24 | | | | | | | | | | | | |
| 25 | | | | | | | | | | | | |

Col [a] = number of colonies used for confirmation
Sal [b] = number of colonies confirmed as *Salmonella*

Table 4 (continued): Results of isolation using own enrichment (dish numbers N1-N20)

| sample no. | Own enrichment 24 hours | | | | | | Own enrichment 48 hours | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | BGA | | XLD | | Third medium | | BGA | | XLD | | Third medium | |
| | Col [a] | Sal [b] | Col | Sal | Col | Sal | Col | Sal | Col | Sal | Col | Sal |
| N1 | | | | | | | | | | | | |
| N2 | | | | | | | | | | | | |
| N3 | | | | | | | | | | | | |
| N4 | | | | | | | | | | | | |
| N5 | | | | | | | | | | | | |
| N6 | | | | | | | | | | | | |
| N7 | | | | | | | | | | | | |
| N8 | | | | | | | | | | | | |
| N9 | | | | | | | | | | | | |
| N10 | | | | | | | | | | | | |
| N11 | | | | | | | | | | | | |
| N12 | | | | | | | | | | | | |
| N13 | | | | | | | | | | | | |
| N14 | | | | | | | | | | | | |
| N15 | | | | | | | | | | | | |
| N16 | | | | | | | | | | | | |
| N17 | | | | | | | | | | | | |
| N18 | | | | | | | | | | | | |
| N19 | | | | | | | | | | | | |
| N20 | | | | | | | | | | | | |

Col [a] = number of colonies used for confirmation
Sal [b] = number of colonies confirmed as *Salmonella*

Table 4 (continued): Results of isolation using own enrichment (dish numbers C1-C12)

| | Own enrichment 24 hours | | | | | | Own enrichment 48 hours | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| sample no. | BGA | | XLD | | Third medium | | BGA | | XLD | | Third medium | |
| | Col [a] | Sal [b] | Col | Sal | Col | Sal | Col | Sal | Col | Sal | Col | Sal |
| C1 | | | | | | | | | | | | |
| C2 | | | | | | | | | | | | |
| C3 | | | | | | | | | | | | |
| C4 | | | | | | | | | | | | |
| C5 | | | | | | | | | | | | |
| C6 | | | | | | | | | | | | |
| C7 | | | | | | | | | | | | |
| C8 | | | | | | | | | | | | |
| C9 | | | | | | | | | | | | |
| C10 | | | | | | | | | | | | |
| C11 | | | | | | | | | | | | |
| C12 | | | | | | | | | | | | |

Col [a] = number of colonies used for confirmation
Sal [b] = number of colonies confirmed as *Salmonella*

Table 5: Results of detection using PCR (dish numbers 1-25)

| sample no. | PCR + or - | | | | |
|---|---|---|---|---|---|
| | | no. | | no. | |
| 1 | | N1 | | C1 | |
| 2 | | N2 | | C2 | |
| 3 | | N3 | | C3 | |
| 4 | | N4 | | C4 | |
| 5 | | N5 | | C5 | |
| 6 | | N6 | | C6 | |
| 7 | | N7 | | C7 | |
| 8 | | N8 | | C8 | |
| 9 | | N9 | | C9 | |
| 10 | | N10 | | C10 | |
| 11 | | N11 | | C11 | |
| 12 | | N12 | | C12 | |
| 13 | | N13 | | | |
| 14 | | N14 | | | |
| 15 | | N15 | | | |
| 16 | | N16 | | | |
| 17 | | N17 | | | |
| 18 | | N18 | | | |
| 19 | | N19 | | | |
| 20 | | N20 | | | |
| 21 | | | | | |
| 22 | | | | | |
| 23 | | | | | |
| 24 | | | | | |
| 25 | | | | | |

| Comment(s) on operational details that might influence the test results: |
| --- |
| |

| | |
| --- | --- |
| Name of person (s) carrying out the seventh bacteriological collaborative study (2003) | |
| Date and signature | |

| | |
| --- | --- |
| Name of person in charge | |
| Date and signature | |