



The EQUAL Scheme for Indicator Organisms

Water Microbiology

Isolation Methods Survey December 2004



At our last User Group Meeting (UGM) in June 2004, we were asked by several delegates why the Scheme does not report on the different methods used by participants and if it would be possible to produce separate medians for different methods. A full transcript of the questions and answers can be found in the report of the UGM which can be accessed on our website (www.hpaweqa.org.uk).

Despite our belief that method comparison is not the purpose of EQA, but because similar questions had been raised several times by participants, particularly in relation to isolation media for coliform bacteria/*E.coli*, we agreed at the meeting to undertake a survey of isolation methods, the results of which follow.

170 (63.02%) participants returned the questionnaire, before the closing date of October 4th 2004.

Table 1 shows the number of respondents for each parameter and the proportions of these using a membrane filtration (MF) method, a most probable number (MPN) method or both methods. The totals for MF and MPN **include** those laboratories which use both methods.

Graph 1 illustrates the percentage proportions from table 1.

Table 2 illustrates, by country, the total number of respondents and also the number of respondents per parameter using MF, MPN or both methods.

Tables 3 - 9 illustrate, for each parameter, the numbers of laboratories using different isolation methods and media.

Tables 10 - 13 illustrate, for the coliform, *E.coli*, enterococci and *Clostridium perfringens* parameters, the numbers of laboratories using particular media for MF and the corresponding numbers of laboratories and media used for MPN, by those laboratories that use both methods.

Tables 14 - 18 are the keys to abbreviations used for the media for each parameter in graphs 2 to 7 and tables 18 to 23.

Graphs 2 - 7 illustrate, for each parameter, the numbers of laboratories using different isolation methods and media.

Tables 18 - 23 illustrate, by country and by parameter, the numbers of laboratories using different methods and media.

We are grateful to those who took part in the survey and hope you find the results helpful.

It can be seen that the range of media in use is wide for all parameters, but in particular for coliform bacteria and *E. coli*. Although there could be sufficient data from the more favoured methods/media, the Scheme advisors do not believe that it would be appropriate to undertake separate analyses for some popular methods and not others. To do so would certainly be unfair on those participants employing less widely used methods. This in itself we feel, justifies our position not to analyse data sets from laboratories using specific methods separately.

In addition however, it must be remembered that an EQA distribution cannot separate any effects associated with the method from those associated with the laboratory. If there is an indication that one method gives higher or lower results than another method, this should be established unequivocally by carrying out an appropriate method comparison, not only between laboratories, but also within a single laboratory using sufficient numbers of positive split samples analysed by both methods. It must also be noted that any one method may not necessarily be suitable for all situations and sample types, so when methods are compared it should be with the types of samples that are, or will be, routinely analysed by the laboratory. A laboratory is responsible for evaluating the performance of any new method prior to adopting it for routine use. Thus, any laboratory not using a reference or national method should have generated the necessary data with which to assess the advantages and disadvantages of the method they use, and its performance relative to national or reference methods. In other words, if a laboratory's

newly introduced method demonstrates similar or improved recovery of organisms relative to a reference or national method, then the laboratory will possess the necessary data to support this and in the light of it, be able to assess their EQA results accordingly.

Guidance on undertaking comparison of methods studies is given in *The Microbiology of Drinking Water (2002) – Part 3* and ISO 17994:2004.

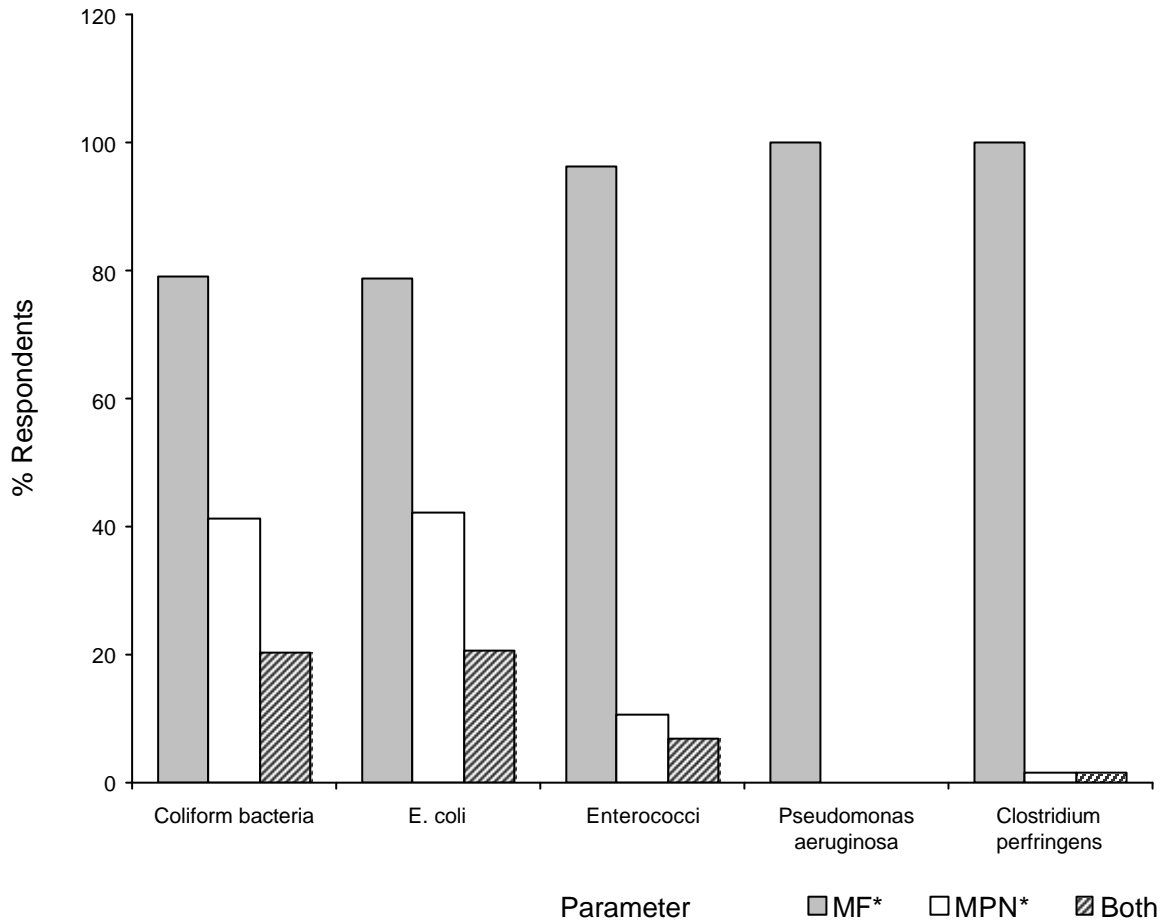
Table 1**Total number of respondents per parameter and the proportions using MF, MPN or both methods.**

| Parameter | Total number of respondents per parameter | Number of * respondents using MF (%) | Number of * respondents using MPN (%) | Number of respondents using both methods (%) |
|--------------------------------|---|--------------------------------------|---------------------------------------|--|
| Coliform bacteria | 167 | 132 (79.0) | 69 (41.3) | 34 (20.4) |
| <i>E.coli</i> | 169 | 133 (78.7) | 71 (42.0) | 35 (20.7) |
| Enterococci | 160 | 154 (96.3) | 17 (10.6) | 11 (6.9) |
| <i>Pseudomonas aeruginosa</i> | 145 | 145 (100) | 0 | 0 |
| <i>Clostridium perfringens</i> | 142 | 142 (100) | 2 (1.4) | 2 (1.4) |

* These totals **include** laboratories using both methods.

Graph 1.

Per parameter, percentages of respondents using a membrane filtration (MF) method, a most probable number (MPN) method or both methods.



* These percentages **include** laboratories using both methods

Table 2

By country, total number of respondents and number of respondents per parameter using MF, MPN or both methods

| Country | Total Number of Respondents | Number of Respondents per Parameter per Method | | | | | | | | | | | | | | |
|----------------------|-----------------------------|--|-----------|-----------|------------|-----------|-----------|-------------|-----------|-----------|----------------|-----|------|-----------------|----------|----------|
| | | Coliform bacteria | | | E.coli | | | Enterococci | | | Ps. aeruginosa | | | Cl. perfringens | | |
| | | MF | MPN | Both | MF | MPN | Both | MF | MPN | Both | MF | MPN | Both | MF | MPN | Both |
| Austria | 10 | 10 | 3 | 3 | 10 | 3 | 3 | 10 | | | 10 | | | 8 | | |
| Belgium | 2 | 2 | | | 2 | | | 2 | | | 2 | | | 2 | | |
| Cyprus | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | | 1 | | | 1 | | |
| Eire | 19 | 10 | 14 | 6 | 10 | 15 | 6 | 14 | 5 | 1 | 11 | | | 16 | | |
| England | 65 | 44 | 34 | 13 | 43 | 34 | 13 | 56 | 4 | 2 | 60 | | | 53 | 1 | 1 |
| Hong Kong | 1 | 1 | | | 1 | | | | | | | | | | | |
| Israel | 1 | 1 | | | 1 | | | 1 | | | | | | | | |
| Italy | 27 | 26 | 4 | 3 | 26 | 4 | 3 | 27 | 2 | 2 | 25 | | | 26 | | |
| N. Ireland | 6 | 4 | 2 | | 4 | 2 | | 6 | | | 2 | | | 5 | | |
| Portugal | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | | 1 | | |
| Scotland | 12 | 11 | 2 | 1 | 11 | 2 | 1 | 12 | | | 10 | | | 8 | | |
| Slovenia | 6 | 6 | 5 | 5 | 6 | 5 | 5 | 6 | 4 | 4 | 5 | | | 6 | | |
| S. Africa | 1 | | 1 | | | 1 | | 1 | | | 1 | | | 1 | | |
| Spain | 3 | 3 | | | 3 | 1 | 1 | 3 | 1 | 1 | 3 | | | 3 | | |
| Switzerland | 8 | 6 | | | 8 | | | 8 | | | 8 | | | 7 | | |
| United Arab Emirates | 1 | | 1 | | | 1 | | 1 | | | 1 | | | 1 | 1 | 1 |
| Wales | 6 | 6 | 1 | 1 | 6 | 1 | 1 | 5 | | | 5 | | | 4 | | |
| TOTAL | 170 | 132 | 69 | 34 | 133 | 71 | 35 | 154 | 17 | 11 | 145 | | | 142 | 2 | 2 |

Table 3
Coliform bacteria: numbers of laboratories using different isolation methods and media

| | Method | No. of Labs | Comment |
|-----|--|-------------|--|
| MF | Membrane lauryl sulphate broth (MLSB) | 56 | One intending to change to MLGA One additionally using MLGA One additionally using m-Endo agar LES |
| | Endo - type media | 26 | 1x Endo agar 21x m-Endo agar 3x m-Endo agar LES 1x m-Endo broth One of these additionally used Chromocult® coliform agar |
| | Lactose TTC + Tergitol 7 | 23 | One additionally used m-Endo agar |
| | Membrane lactose glucuronide agar (MLGA) | 9 | |
| | Membrane lauryl sulphate agar (MLSA) | 6 | |
| | Chromocult® Agar (Merck) | 4 | Two specified Chromocult® coliform agar Two did not specify which Chromocult® agar One used coli ID for surface waters |
| | C-EC Agar (Biolife-chromogenic and fluorogenic) | 2 | |
| | Chromogenic Coliform Agar (Biolife-chromogenic only) | 1 | |
| | m-FC agar | 1 | |
| | CHROMagar™ | 1 | |
| | Chromogenic medium (unspecified) | 1 | |
| | Violet Red Bile Lactose Agar | 1 | |
| | MacConkey (broth or agar not specified) | 1 | |
| MPN | Defined substrate (e.g. Colilert® 18) | 57 | One additionally used minerals modified glutamate |
| | Minerals modified glutamate | 5 | One intending to re-introduce Colilert® 18 in 2005 |
| | Lactose peptone broth + Andrade's indicator | 4 | |
| | Lauryl sulphate broth with MUG | 1 | |
| | Lauryl tryptose broth | 1 | |
| | MacConkey broth | 1 | |

Thirty four laboratories used both membrane filtration and most probable number methods for the coliform bacteria parameter (see table 10). Three of the thirty four said that they used MF only for heavily

contaminated samples and a defined substrate method (e.g. Colilert® 18) for potable and pool waters, where confirmation is required.

One said that MF was their back-up method, with a defined substrate method (e.g. Colilert® 18) the method of choice.

Another said they used MF for their EQA report, but a defined substrate method (e.g. Colilert® 18) for PWS samples (PWS wasn't expanded).

The remainder didn't specify.

Table 4
***E.coli*: numbers of laboratories using different isolation media**

| | Method | No. of Labs | Comment |
|--------------------------------|--|-------------|--|
| MF | Membrane lauryl sulphate broth (MLSB) | 51 | One intending to change to MLGA |
| | Lactose TTC + Tergitol 7 | 24 | One additionally using CHROMagar™ One additionally using m-FC agar |
| | Membrane lactose glucuronide agar (MLGA) | 12 | |
| | m-FC agar | 10 | One additionally using m-Endo agar LES Two additionally using Chromocult® coliform agar |
| | Tryptone Bile X-glucuronide (TBX) agar | 8 | |
| | EC X-Gluc agar | 5 | |
| | Membrane lauryl sulphate agar (MLSA) | 5 | |
| | Chromocult® agar | 4 | One specified Chromocult® coliform agar Three did not specify which Chromocult® agar |
| | m-FC broth | 2 | |
| | C-EC agar | 2 | |
| | Tryptone soy agar (TSA) and TBX Agar/C-EC agar | 1 | |
| | Chromogenic medium (unspecified) | 1 | |
| | Endo agar | 2 | |
| | E. coli Direct Agar MUG (OXOID) | 1 | |
| | TSA and TBX | 1 | |
| | TSA and Tryptone bile agar (TBA) | 1 | |
| | CHROMagar™ | 1 | |
| TSA and E.coli Direct Agar MUG | 1 | | |
| Fluorocult® | 1 | | |
| MPN | Defined substrate (e.g. Colilert® 18) | 58 | One additionally using minerals modified glutamate |
| | Minerals modified glutamate | 5 | One re-introducing Colilert® 18 in 2005 |
| | Lactose peptone broth + Andrade's indicator | 4 | |
| | Lauryl sulphate broth + MUG | 1 | |
| | Lauryl tryptose broth | 1 | |
| | MacConkey broth | 1 | |
| | Miniaturised method | 1 | |

Thirty five laboratories used both membrane filtration and most probable number methods for the *E.coli* parameter (see table 11).

As for the coliform parameter, it generally wasn't specified for which type of samples which method was used, but laboratories that commented on this for the coliform parameter made the same comments for the *E.coli* parameter.

Table 5
Enterococci: numbers of laboratories using different isolation media

| | Method | No. of Labs | Comment |
|-----|--|-------------|---|
| MF | m-Enterococcus agar (Slanetz and Bartley agar) | 145 | One had tried Enterolert™, but achieved low results |
| | KF-Streptococcus agar | 4 | |
| | Oxolinic acid aesculin azide agar | 2 | |
| | Bile aesculin azide agar | 1 | |
| | Kanamycin aesculin azide agar | 1 | |
| | Enterococcosel agar | 1 | |
| MPN | Defined substrate (e.g. Enterolert™) | 9 | |
| | Glucose azide broth | 5 | |
| | Chromocult® Enterococci Broth | 1 | |
| | Lactose peptone broth + Andrade's indicator | 1 | |
| | Miniaturised method | 1 | Surface and waste waters only |

Eleven laboratories used both membrane filtration and most probable number methods for the enterococci parameter (see table 12).

Table 6
***Pseudomonas aeruginosa*: numbers of laboratories using different isolation media**

| | Method | No. of Labs | Comment |
|----|--|-------------|--|
| MF | <i>Pseudomonas</i> agar + CN supplement | 103 | |
| | <i>Pseudomonas</i> agar + CFC supplement | 26 | One intending to change to CN supplement |
| | Cetrimide agar | 11 | |
| | Pseudosel agar | 1 | |
| | DIFCO <i>Pseudomonas</i> agar P | 1 | |
| | Endo agar | 1 | |
| | Membrane lauryl sulphate broth (MLSB) | 1 | |

Only membrane filtration methods were reported for *Pseudomonas aeruginosa*. No respondents examined for this parameter using a most probable number method.

Table 7

***Clostridium perfringens*: numbers of laboratories using different isolation media**

| | Method | No. of Labs | Comment |
|-----|--|--------------------|--|
| MF | Tryptose sulphite cycloserine (TSC) agar without egg yolk | 92 | Two included the fluorogenic substrate 4-Methylumbelliferyl phosphate (MUP). One included MUP and saccharose. One additionally used SPS agar One additionally used m-CP agar |
| | m-CP (membrane <i>Clostridium perfringens</i>) agar | 19 | |
| | TSC agar with egg yolk | 16 | |
| | OPSP (Oleandomycin polymyxin sulphadiazine perfringens) agar | 5 | |
| | SPS (sulphite polymyxin sulphadiazine) agar | 7 | |
| | SFP (Shahidi-Ferguson perfringens) agar | 1 | |
| | Reinforced clostridial medium | 1 | |
| | DRCM (Differential Reinforced Clostridial Medium) agar | 1 | |
| MPN | Differential Reinforced Clostridial Broth | 1 | |
| | Cooked meat medium | 1 | |

Two laboratories used both membrane filtration and most probable number methods for the *Clostridium perfringens* parameter (see table 13)

Table 8
Colony counts: numbers of laboratories using different isolation methods

| Method | No. of Labs | Comment |
|--------------------------|-------------|---|
| Pour Plate | 154 | Includes one laboratory that only uses this method at 22°C and MF's to R2A agar at 36°C. Includes two laboratories that didn't specify a medium. |
| Spread Plate | 3 | All use R2A agar |
| Membrane Filtration (MF) | 3 | Excludes laboratory that MF's onto R2A agar at 36°C only One uses YEA One uses PCA One uses R2A for chlorinated samples and nutrient agar for unchlorinated samples. |
| Spiral plating | 1 | Use YEA |
| IDEXX simplate | 1 | |

Table 9
Colony counts: numbers of laboratories using different isolation media

| Medium | No. of Labs | Comment |
|-----------------------------------|-------------|--|
| Yeast extract agar (YEA) | 124 | Includes 1 laboratory that additionally uses plate count agar. Includes 1 laboratory that uses this only at 22°C but MF's to R2A agar at 36°C. 122 use pour plate method. |
| Plate count agar (PCA) | 31 | 28 use pour plate method. One additionally uses Petrifilm. Includes two laboratories that didn't specify method. |
| R2A agar | 5 | Excludes 1 laboratory that MF's onto R2A agar at 36°C only, but uses yeast extract agar for pour plate at 22°C. Includes one lab that uses R2A agar only for chlorinated drinking water but uses nutrient agar for unchlorinated samples. |
| IDEXX simplate | 1 | |
| Tryptic Glucose Yeast agar (TGYA) | 1 | Use pour plate method |

Table 10

Coliform bacteria: of laboratories using both methods, the numbers using particular media for MF and the corresponding numbers and media used for MPN

| Membrane Filtration Method | | Most Probable Number Method | |
|--|--------------|---|--------------|
| Medium | No. of Labs. | Medium | No. of Labs. |
| Membrane lauryl sulphate broth | 16 | Defined substrate (e.g. Colilert® 18) Minerals modified glutamate | 13 3 |
| Endo-type | 7 | Defined substrate (e.g. Colilert® 18) Lactose peptone broth + Andrade's indicator Lauryl tryptose broth | 5 1 1 |
| Lactose TTC + Tergitol 7 | 6 | Lactose peptone broth + Andrade's indicator Defined substrate (e.g. Colilert® 18) MacConkey broth | 3 2 1 |
| Membrane lactose glucuronide agar | 2 | Lauryl sulphate broth + MUG Minerals modified glutamate | 1 1 |
| Membrane lauryl sulphate agar | 2 | Defined substrate (e.g. Colilert® 18) | 2 |
| MacConkey (broth or agar not specified) | 1 | Defined substrate (e.g. Colilert® 18) | 1 |

Table 11

E.coli: for laboratories using both methods, the numbers using particular media for MF and the corresponding numbers and media used for MPN

| Membrane Filtration Method | | Most Probable Number Method | |
|-----------------------------------|--------------|--|------------------|
| Medium | No. of Labs. | Medium | No. of Labs. |
| Membrane lauryl sulphate broth | 13 | Defined substrate (e.g. Colilert® 18) Minerals modified glutamate | 11 2 |
| Lactose TTC + Tergitol 7 | 7 | Lactose peptone broth + Andrade's indicator Defined substrate (e.g. Colilert® 18) Lauryl tryptose broth MacConkey broth | 3 2 1 1 |
| m-FC agar | 5 | Defined substrate (e.g. Colilert® 18) Lactose peptone broth + Andrade's indicator | 4 1 |
| Tryptone bile X-glucuronide agar | 3 | Defined substrate (e.g. Colilert® 18) Minerals modified glutamate | 2 1 |
| Membrane lactose glucuronide agar | 2 | Minerals modified glutamate Lauryl sulphate broth + MUG | 1 1 |
| m-FC broth | 2 | Defined substrate (e.g. Colilert® 18) | 2 |
| Fluorocult® | 1 | Defined substrate (e.g. Colilert® 18) | 1 |
| Membrane lauryl sulphate agar | 1 | Defined substrate (e.g. Colilert® 18) | 1 |
| Chromocult® agar | 1 | Miniaturised MPN method | 1 |

Table 12

Enterococci: of laboratories using both methods, the numbers using particular media for MF and the corresponding numbers and media used for MPN

| Membrane Filtration Method | | Most Probable Number Method | |
|---|--------------|---|--------------|
| Medium | No. of Labs. | Medium | No. of Labs. |
| m-Enterococcus agar (Slanetz and Bartley Agar) | 9 | Glucose azide broth | 4 |
| | | Defined substrate method (e.g. Enterolert™) | 4 |
| | | Chromocult® Enterococci broth | 1 |
| Bile aesculin azide agar | 1 | Lactose peptone broth + Andrade's indicator | 1 |
| Oxolinic acid aesculin azide agar | 1 | Miniaturised MPN method (for surface and waste waters) | 1 |

Table 13

***Clostridium perfringens*: MF medium and corresponding MPN media used by laboratories using both methods**

| Membrane Filtration Method | | Most Probable Number Method | |
|--|--------------|---|--------------|
| Medium | No. of Labs. | Medium | No. of Labs. |
| Tryptose sulphite cycloserine agar without egg yolk | 2 | Cooked meat medium | 1 |
| | | Differential Reinforced Clostridial Broth | 1 |

Table 14**Coliform bacteria/*E. coli*: key to media abbreviations used in graphs 2 and 3 and tables 18 and 19**

| | | |
|---------|--|---|
| MF | CCA | Chromogenic Coliform Agar (Biolife, chromogenic only) |
| | Ccult | Chromocult® Agar (Merck) |
| | C-EC | C-EC agar (Biolife, chromogenic and fluorogenic) |
| | CHROM | CHROMagar™ |
| | ECMUG | <i>E. coli</i> Direct Agar MUG (Oxoid) |
| | ECX | EC X-Gluc agar |
| | Endo | Endo agar |
| | Endo-t | Endo-type media, including Endo agar, m-Endo agar, m-Endo agar LES and m-Endo broth |
| | Fcult | Fluorocult® (exact medium not specified e.g. VRB, ECD) |
| | LTTC | Lactose TTC plus tergitol 7 |
| | Mac | MacConkey (broth or agar not specified) |
| | m-FC (a) | m-FC agar |
| | m-FC (b) | m-FC broth |
| | MLGA | Membrane lactose glucuronide agar |
| | MLSA | Membrane lauryl sulphate agar |
| | MLSB | Membrane lauryl sulphate broth |
| | TBX | Tryptone bile X-glucuronide agar |
| | TSA/B | Tryptone soy agar and tryptone bile agar |
| | TSA/M | Tryptone soy agar and <i>E. coli</i> Direct Agar MUG |
| | TSA/X | Tryptone soy agar and TBX agar |
| TSA/X/C | Tryptone soy agar and TBX Agar/C-EC agar | |
| Unsp | Chromogenic medium (unspecified) | |
| VRBLA | Violet red bile lactose agar | |
| MPN | Def. S | Defined substrate (e.g. Colilert® 18) |
| | LPBA | Lactose peptone broth with Andrade's indicator |
| | LSB+M | Lauryl sulphate broth with MUG |
| | LTB | Lauryl tryptose broth |
| | Mac.b | MacConkey broth |
| | Min | Miniaturised MPN method |
| | MMG | Minerals modified glutatmate |

Table 15**Enterococci: key to media abbreviations used in graph 4 and table 20**

| | | |
|-----|---------|--|
| MF | BAA | Bile aesculin azide agar |
| | Ent'c'l | Enterococcosel agar |
| | KAA | Kanamycin aesculin azide agar |
| | KF | KF-Streptococcus agar |
| | m-Ent | m-Enterococcus agar (Slanetz and Bartley agar) |
| | OAA | Oxolinic acid aesculin azide agar |
| MPN | CEB | Chromocult® Enterococci Broth |
| | D. sub | Defined substrate (e.g. Enterolert™) |
| | GAB | Glucose azide broth |
| | LPBA | Lactose peptone broth with Andrade's indicator |
| | Min | Miniaturised MPN method |

Table 16***Pseudomonas aeruginosa*: key to media abbreviations used in graph 5 and table 21**

| | |
|----------------------|--|
| Cet | Cetrimide agar |
| CFC | <i>Pseudomonas</i> agar + CF (cetrimide, fucidin and cephalosporin) supplement |
| CN | <i>Pseudomonas</i> agar + CN (cetrimide and sodium nalidixate) supplement |
| Endo | Endo agar |
| MLSB | Membrane lauryl sulphate broth |
| <i>Pseudomonas</i> P | DIFCO <i>Pseudomonas</i> agar P |
| Pseudosel | Pseudosel agar |

Table 17***Clostridium perfringens*: key to media abbreviations used in graph 6 and table 22**

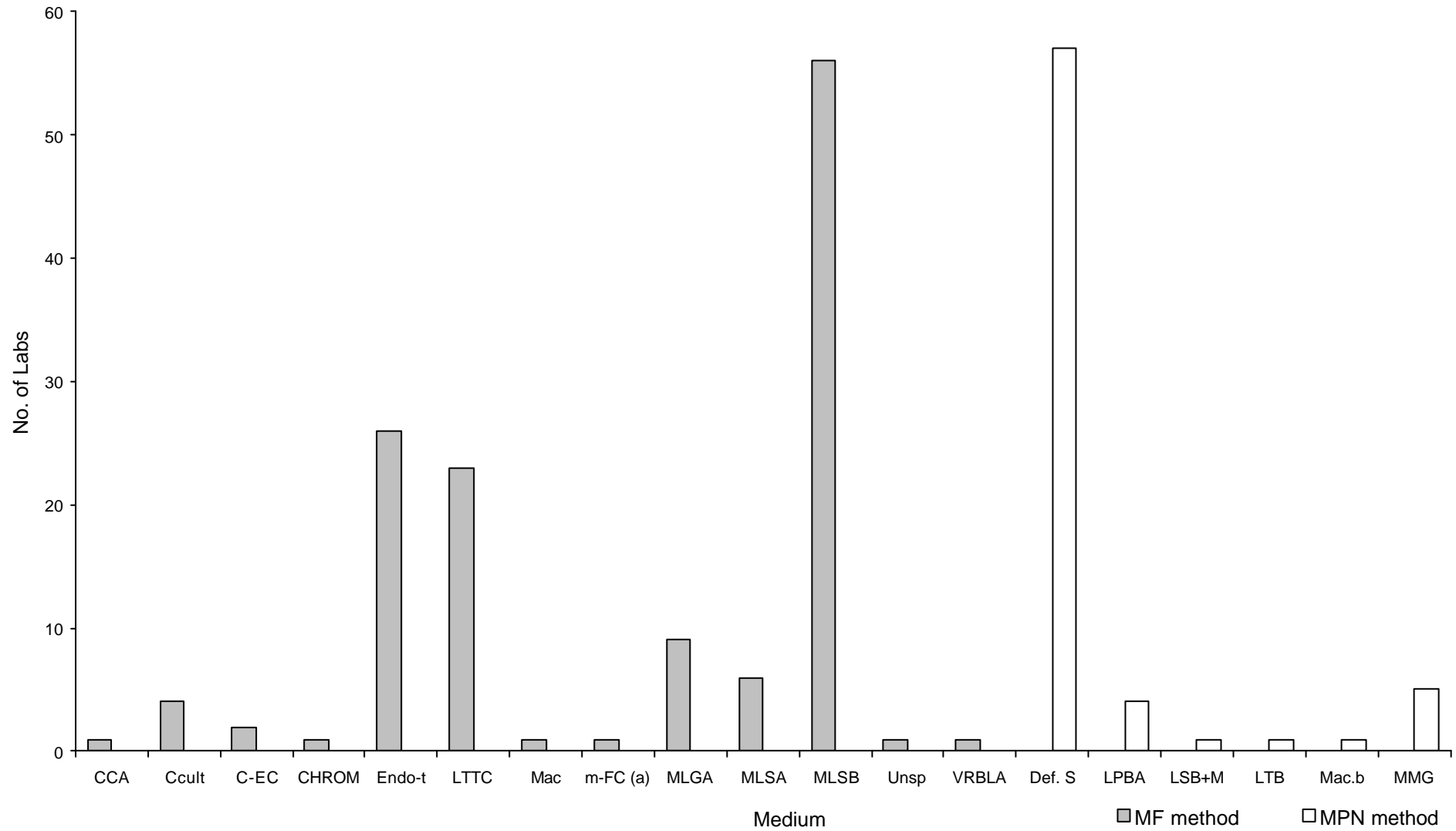
| | | |
|-----|-----------|--|
| MF | DRCM (a) | DRCM (differential reinforced clostridial medium) agar |
| | m-CP | Membrane <i>Clostridium perfringens</i> agar |
| | OPSP | Oleandomycin polymyxin sulphadiazine perfringens agar |
| | RCM | Reinforced clostridial medium |
| | SFP | Shahidi - Ferguson perfringens agar |
| | SPS | Sulphite polymyxin sulphadiazine agar |
| | TSC - egg | Tryptose sulphite cycloserine agar without egg yolk |
| | TSC + egg | Tryptose sulphite cycloserine agar with egg yolk |
| MPN | CMM | Cooked meat medium |
| | DRCM | Differential reinforced clostridial medium |

Table 18**Colony counts: key to media abbreviations used in graph 7 and table 23**

| | |
|-------|----------------------------|
| YEA | Yeast extract agar |
| PCA | Plate count agar |
| R2A | R2A agar |
| TGYA | Tryptic glucose yeast agar |
| IDEXX | IDEXX simplate |

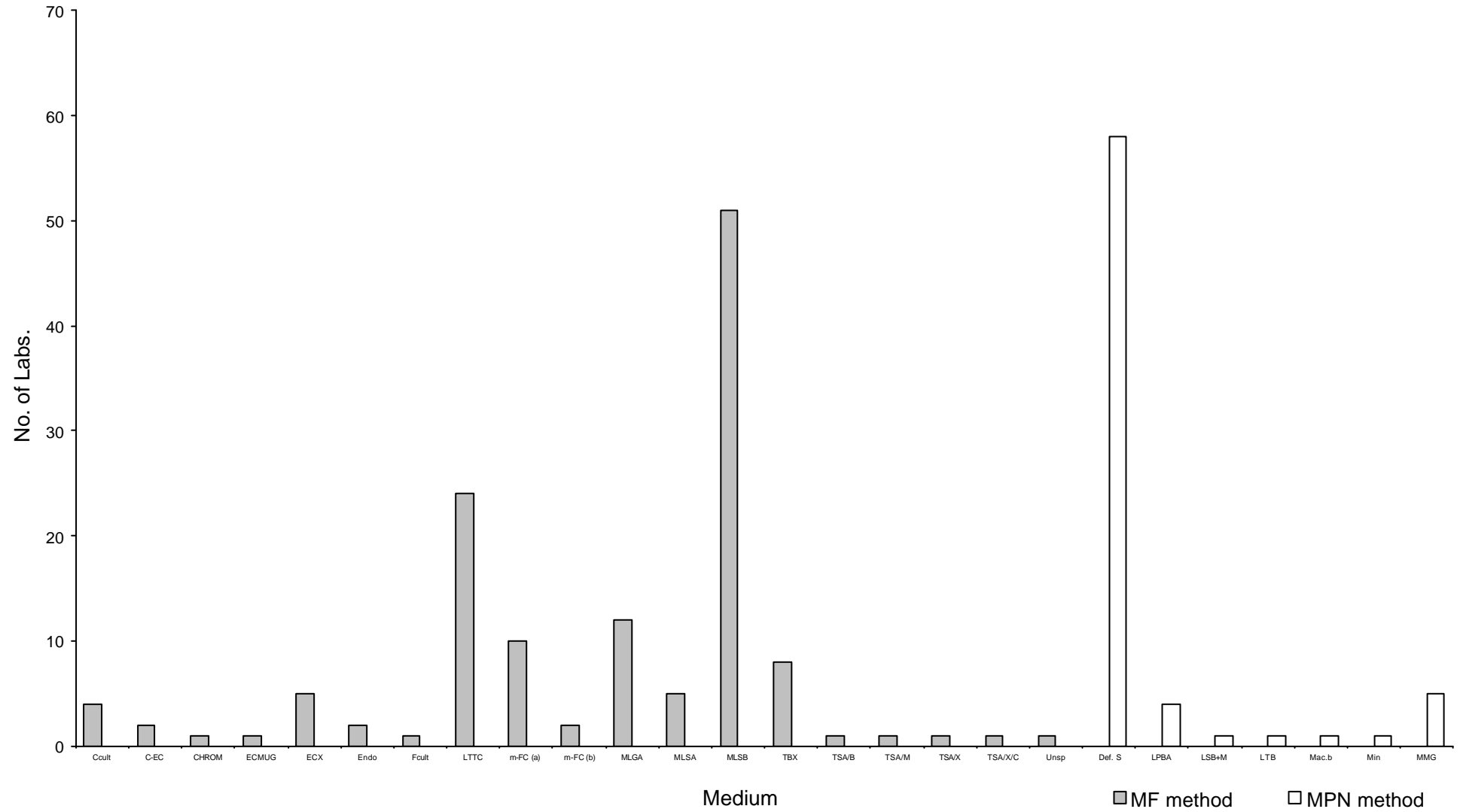
Graph 2

Coliform bacteria: numbers of laboratories using different isolation methods and media



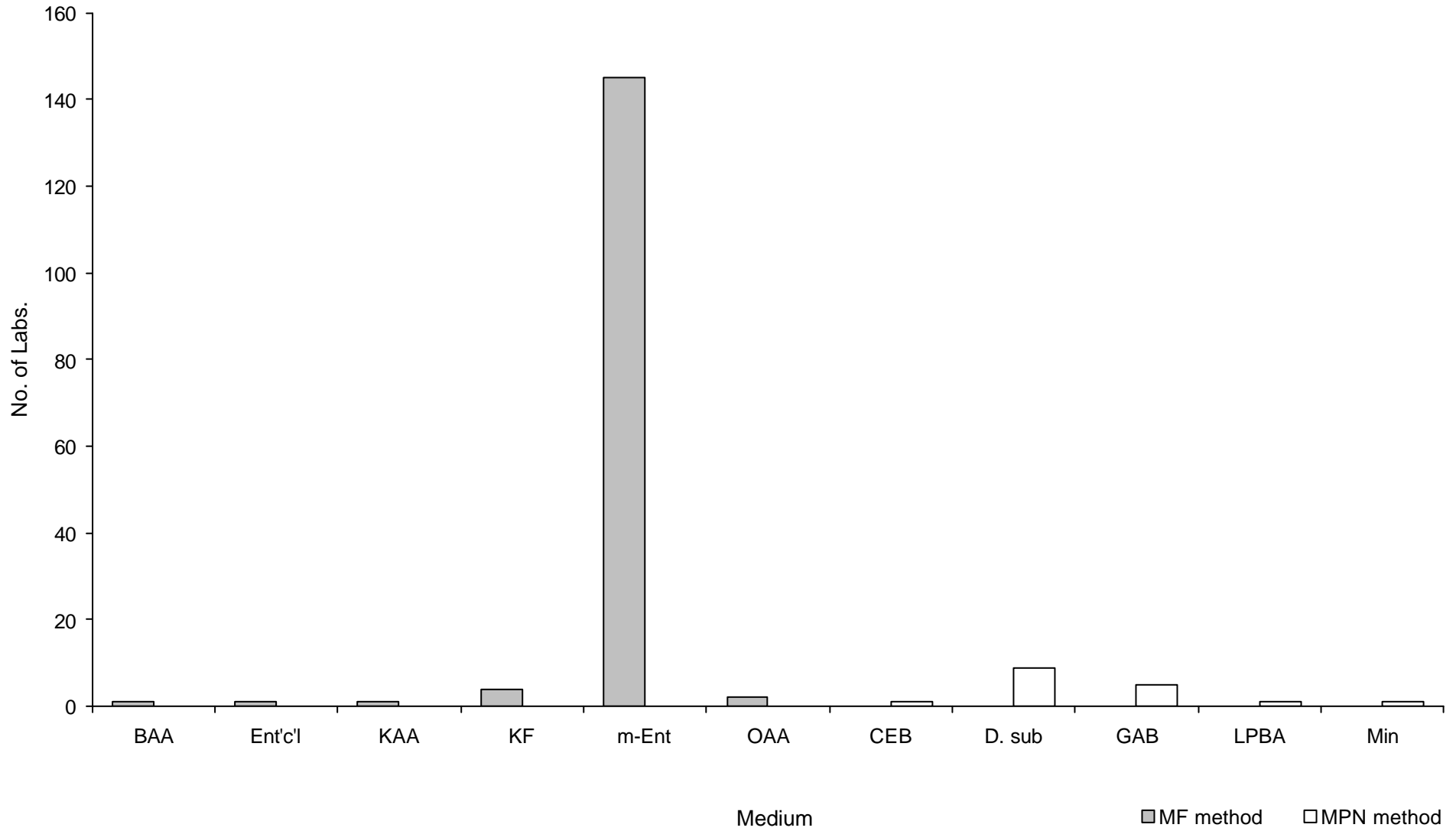
Graph 3

***E. coli*: numbers of laboratories using different isolation methods and media**



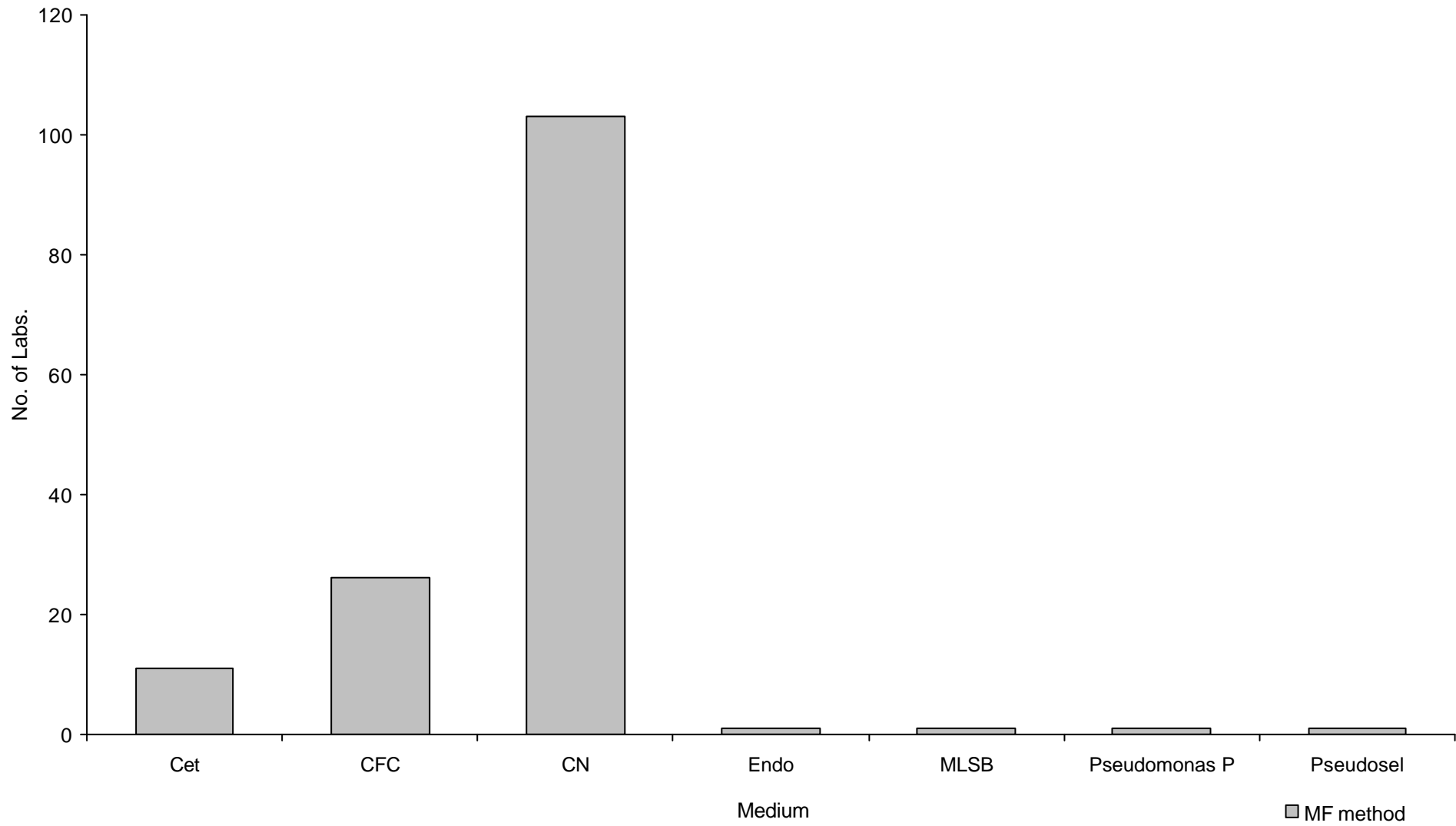
Graph 4

Enterococci: numbers of laboratories using different isolation methods and media

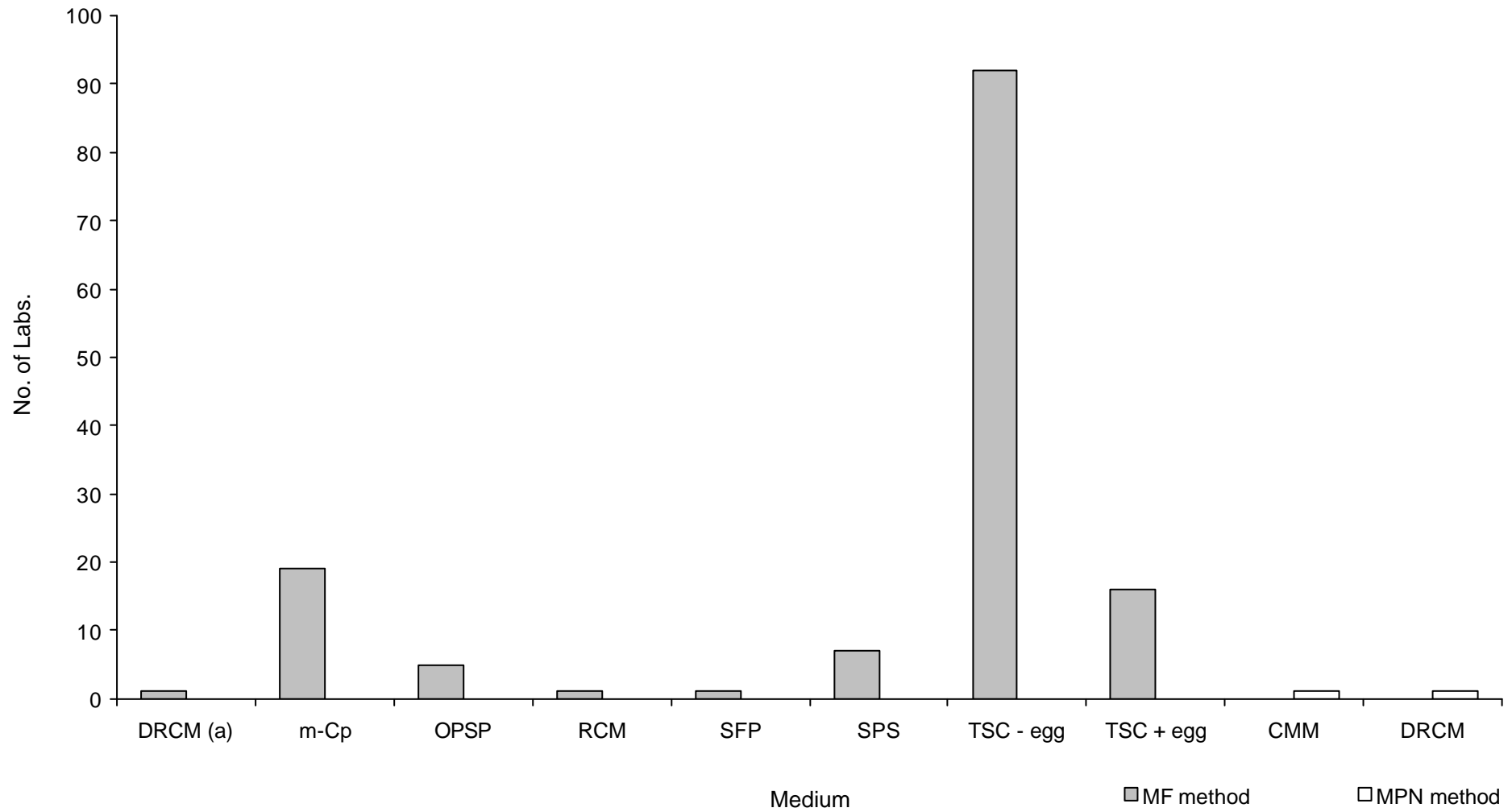


Graph 5

***Pseudomonas aeruginosa*: numbers of laboratories using different isolation media**



Graph 6
***Clostridium perfringens* : numbers of laboratories using different isolation methods and media**



Graph 7

Colony counts: numbers of laboratories using different isolation methods and media

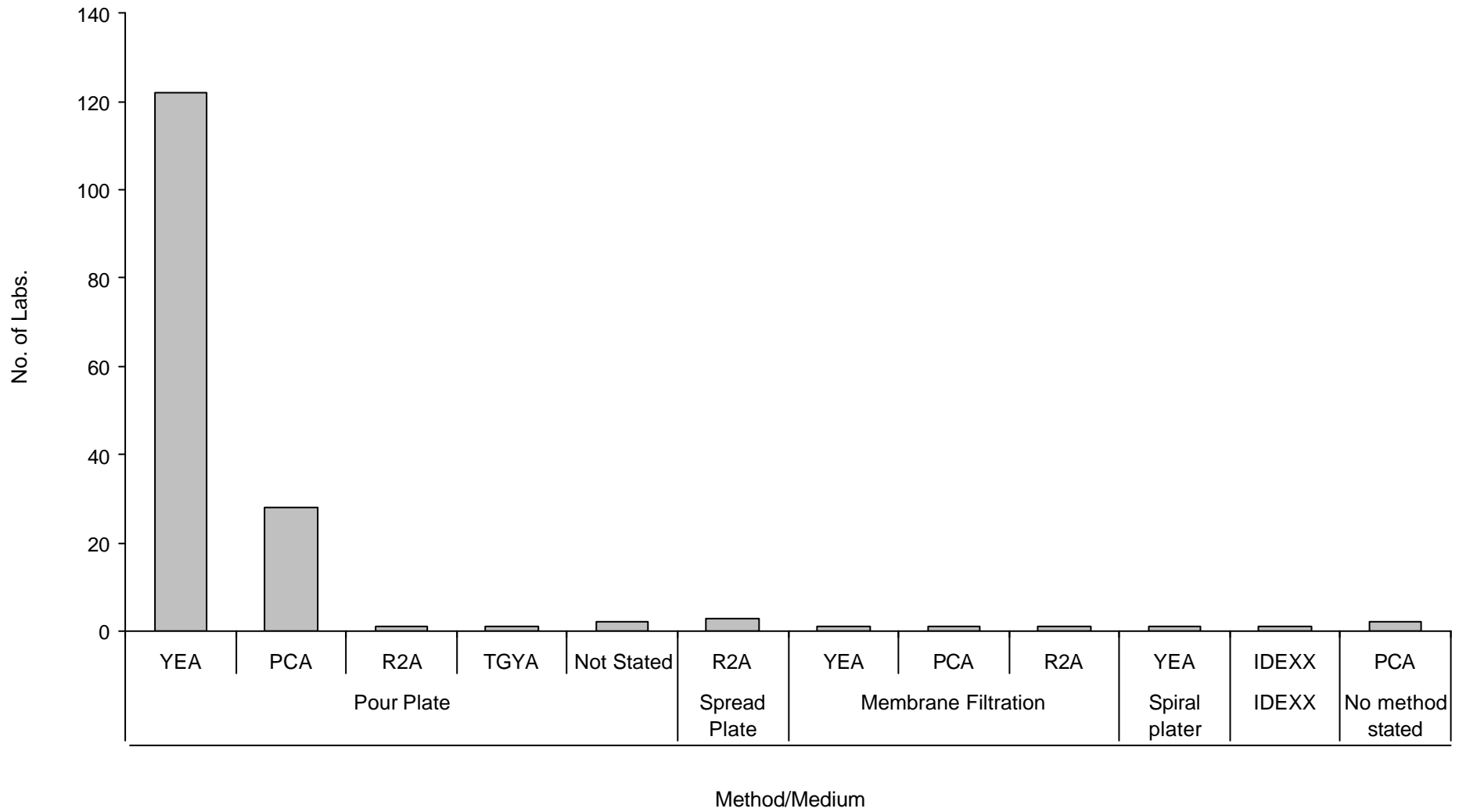


Table 18
Coliform bacteria: by country, numbers of laboratories using different methods and media

| Country | Number of Respondents per Method per Medium | | | | | | | | | | | | | | | | | | |
|----------------------|---|----------|----------|----------|-----------|-----------|----------|----------|----------|----------|-----------|----------|----------|-----------|----------|----------|----------|----------|----------|
| | MF | | | | | | | | | | | | | MPN | | | | | |
| | CCA | Ccult | C-EC | CHROM | Endo-t | LTTC | Mac | m-FC (a) | MLGA | MLSA | MLSB | Unsp | VRBLA | Def. S | LPBA | LSB+M | LTB | Mac. b | MMG |
| Austria | | | | | 2 | 6 | 1 | | 1 | | | | | 2 | | 1 | | | |
| Belgium | | | | | | 1 | | 1 | | | | | | | | | | | |
| Cyprus | | | | | 1 | | | | | | | | | 1 | | | | | |
| Eire | | | | | 3 | 2 | | | | 1 | 4 | | | 13 | | | 1 | | |
| England | | | | | | 1 | | | 6 | 3 | 34 | | | 31 | | | | | 3 |
| Hong Kong | | | | | | | | | | | 1 | | | | | | | | |
| Israel | | | | | 1 | | | | | | | | | | | | | | |
| Italy | | | 1 | | 17 | 7 | | | | | | 1 | | 4 | | | | | |
| N. Ireland | | | | | | | | | | 1 | 3 | | | 2 | | | | | |
| Portugal | | | | | | | | | | 1 | | | | 1 | | | | | |
| Scotland | | 1 | | 1 | | | | | 2 | | 7 | | | 1 | | | | | 1 |
| Slovenia | | | | | 1 | 5 | | | | | | | | | 4 | | | 1 | |
| S. Africa | | | | | | | | | | | | | | 1 | | | | | |
| Spain | | 2 | | | 1 | | | | | | | | | | | | | | |
| Switzerland | 1 | 1 | 1 | | | 1 | | | | | 1 | | 1 | | | | | | |
| United Arab Emirates | | | | | | | | | | | | | | 1 | | | | | |
| Wales | | | | | | | | | | | 6 | | | | | | | | 1 |
| TOTAL | 1 | 4 | 2 | 1 | 26 | 23 | 1 | 1 | 9 | 6 | 56 | 1 | 1 | 57 | 4 | 1 | 1 | 1 | 5 |

Table 19
***E. coli*: by country, numbers of laboratories using different methods and media**

| Country | Number of Respondents per Method per Medium | | | | | | | | | | | | | | | | | | | | | | | | | |
|----------------------|---|----------|----------|----------|----------|----------|----------|-----------|-----------|----------|-----------|----------|-----------|----------|----------|----------|----------|----------|----------|-----------|----------|----------|----------|----------|----------|----------|
| | MF | | | | | | | | | | | | | | | | | | | MPN | | | | | | |
| | Ccult | C-EC | CHROM | ECMUG | ECX | Endo | Fcult | LTTC | m-FC(a) | m-FC(b) | MLGA | MLSA | MLSB | TBX | TSA/B | TSA/M | TSA/X | TSA/X/C | Unsp | Def. S | LPBA | LSB+M | LTB | Mac. b | Min | MMG |
| Austria | | | | | | 2 | 1 | 6 | | 1 | | | | | | | | | | 2 | | 1 | | | | |
| Belgium | | | | | | | | 1 | 1 | | | | | | | | | | | | | | | | | |
| Cyprus | | | | | | | | | 1 | | | | | | | | | | | 1 | | | | | | |
| Eire | | | | | | | | 3 | | 2 | | 1 | 2 | 2 | | | | | | 14 | | | 1 | | | |
| England | | | | | | | | 1 | 1 | | 6 | 2 | 33 | | | | | | | 31 | | | | | | 3 |
| Hong Kong | | | | | | | | | | | | | 1 | | | | | | | | | | | | | |
| Israel | | | | | | | | | | 1 | | | | | | | | | | | | | | | | |
| Italy | | 2 | | | 5 | | | 8 | 6 | | 2 | | | 1 | 1 | | | | 1 | 4 | | | | | | |
| N. Ireland | | | | | | | | | | | | 1 | 3 | | | | | | | 2 | | | | | | |
| Portugal | | | | | | | | | | | | 1 | | | | | | | | 1 | | | | | | |
| Scotland | 1 | | 1 | | | | | | | | 2 | | 6 | 1 | | | | | | 1 | | | | | | 1 |
| Slovenia | | | | | | | | 5 | 1 | | | | | | | | | | | | 4 | | | | | |
| S. Africa | | | | | | | | | | | | | | | | | | | | 1 | | | | | | |
| Spain | 3 | | | | | | | | | | | | | | | | | | | | | | | | 1 | |
| Switzerland | | | | 1 | | | | | | | | | | 4 | | 1 | 1 | 1 | | | | | 1 | | | |
| United Arab Emirates | | | | | | | | | | | | | | | | | | | | 1 | | | | | | |
| Wales | | | | | | | | | | | | | 6 | | | | | | | | | | | | | 1 |
| TOTAL | 4 | 2 | 1 | 1 | 5 | 2 | 1 | 24 | 10 | 2 | 12 | 5 | 51 | 8 | 1 | 1 | 1 | 1 | 1 | 58 | 4 | 1 | 1 | 1 | 1 | 5 |

Table 20
Enterococci: by country, numbers of laboratories using different methods and media

| Country | Number of Respondents per Method per Medium | | | | | | | | | | |
|----------------------|---|----------|----------|----------|------------|----------|----------|----------|----------|----------|----------|
| | MF | | | | | | MPN | | | | |
| | BAA | Ent'c'l | KAA | KF | m-Ent | OAA | CEB | D. sub | GAB | LPBA | Min |
| Austria | | 1 | | | 9 | | | | | | |
| Belgium | | | | | 2 | | | | | | |
| Cyprus | | | | | 1 | | | | | | |
| Eire | | | | 2 | 12 | | | 4 | 1 | | |
| England | | | | | 56 | | | 2 | 2 | | |
| Hong Kong | | | | | | | | | | | |
| Israel | | | | | 1 | | | | | | |
| Italy | | | | 1 | 26 | | | 2 | | | |
| N. Ireland | | | | | 6 | | | | | | |
| Portugal | | | | | 1 | | | 1 | | | |
| Scotland | | | | 1 | 11 | | | | | | |
| Slovenia | 1 | | | | 5 | | 1 | | 2 | 1 | |
| S. Africa | | | | | 1 | | | | | | |
| Spain | | | 1 | | | 2 | | | | | 1 |
| Switzerland | | | | | 8 | | | | | | |
| United Arab Emirates | | | | | 1 | | | | | | |
| Wales | | | | | 5 | | | | | | |
| TOTAL | 1 | 1 | 1 | 4 | 145 | 2 | 1 | 9 | 5 | 1 | 1 |

Table 21

***Pseudomonas aeruginosa*: by country, numbers of laboratories using different media**

| Country | Number of Respondents per Medium | | | | | | |
|----------------------|----------------------------------|-----------|------------|----------|----------|---------------|-----------|
| | Cet | CFC | CN | Endo | MLSB | Pseudomonas P | Pseudosel |
| Austria | 2 | | 6 | 1 | | | 1 |
| Belgium | | | 2 | | | | |
| Cyprus | | | 1 | | | | |
| Eire | | 4 | 6 | | | 1 | |
| England | | 17 | 43 | | | | |
| Hong Kong | | | | | | | |
| Israel | | | | | | | |
| Italy | 8 | 1 | 16 | | | | |
| N. Ireland | | 1 | 1 | | | | |
| Portugal | | | 1 | | | | |
| Scotland | | 1 | 8 | | 1 | | |
| Slovenia | | 1 | 4 | | | | |
| S. Africa | | | 1 | | | | |
| Spain | | | 2 | | | | |
| Switzerland | 1 | | 7 | | | | |
| United Arab Emirates | | | 1 | | | | |
| Wales | | 1 | 4 | | | | |
| TOTAL | 11 | 26 | 103 | 1 | 1 | 1 | 1 |

Table 22***Clostridium perfringens*: by country, numbers of laboratories using different methods and media**

| Country | Number of Respondents per Method per Medium | | | | | | | | | |
|----------------------|---|-----------|----------|----------|----------|----------|-----------|-----------|----------|----------|
| | MF | | | | | | | | MPN | |
| | DRCM (a) | m-CP | OPSP | RCM | SFP | SPS | TSC- | TSC+ | CMM | DRCM |
| Austria | 1 | 2 | | | | | 5 | | | |
| Belgium | | | | | | | 2 | | | |
| Cyprus | | | | | | | 1 | | | |
| Eire | | 5 | | | | | 7 | 4 | | |
| England | | 1 | 3 | | 1 | | 39 | 9 | | 1 |
| Hong Kong | | | | | | | | | | |
| Israel | | | | | | | | | | |
| Italy | | 8 | | | | 7 | 10 | 1 | | |
| N. Ireland | | | | | | | 5 | | | |
| Portugal | | | | | | | 1 | | | |
| Scotland | | | | | | | 7 | 1 | | |
| Slovenia | | 2 | 1 | | | | 3 | | | |
| S. Africa | | | | 1 | | | | | | |
| Spain | | | | | | | 3 | | | |
| Switzerland | | 1 | | | | | 6 | | | |
| United Arab Emirates | | | | | | | 1 | | 1 | |
| Wales | | | 1 | | | | 2 | 1 | | |
| TOTAL | 1 | 19 | 5 | 1 | 1 | 7 | 92 | 16 | 1 | 1 |

Table 23**Colony counts: by country, numbers of laboratories using different methods and media**

| Country | Number of Respondents per Method per Medium | | | | | | | | | | | | |
|----------------------|---|-----------|----------|----------|------------|--------------|---------------------|----------|----------|---------------|----------|------------------|--|
| | Pour Plate | | | | | Spread Plate | Membrane Filtration | | | Spiral Plater | IDEXX | No Method stated | |
| | YEA | PCA | R2A | TGYA | Not Stated | R2A | YEA | PCA | R2A | YEA | IDEXX | PCA | |
| Austria | 8 | 1 | | | | | 1 | | | | | | |
| Belgium | 2 | | | | | | | | | | | | |
| Cyprus | 1 | | | | | | | | | | | | |
| Eire | 12 | 3 | | | 1 | | | | 1 | | 1 | | |
| England | 60 | 3 | | | | 1 | | | | 1 | | | |
| Hong Kong | 1 | | | | | | | | | | | | |
| Israel | | | 1 | | | | | | | | | | |
| Italy | 13 | 10 | | | 1 | | | 1 | | | | 2 | |
| N. Ireland | 5 | | | | | | | | | | | | |
| Portugal | 1 | | | | | | | | | | | | |
| Scotland | 8 | 1 | | | | | | | | | | | |
| Slovenia | 4 | 1 | | 1 | | | | | | | | | |
| S. Africa | 1 | | | | | | | | | | | | |
| Spain | 1 | | | | | 2 | | | | | | | |
| Switzerland | | 8 | | | | | | | | | | | |
| United Arab Emirates | | 1 | | | | | | | | | | | |
| Wales | 5 | | | | | | | | | | | | |
| TOTAL | 122 | 28 | 1 | 1 | 2 | 3 | 1 | 1 | 1 | 1 | 1 | 2 | |