



# The EQUAL Scheme for Indicator Organisms

Water Microbiology

Isolation Methods Survey  
March 2007



Health Protection Agency Water EQA, EQUAL Scheme for Indicator Organisms, Newcastle Laboratory,  
Institute of Pathology, General Hospital, Westgate Road, Newcastle upon Tyne, NE4 6BE  
Tel + 44 (0)191 272 4585 Fax +44 (0)191 273 7292 e-mail [weqa@hpa.org.uk](mailto:weqa@hpa.org.uk) [www.hpaweqa.org.uk](http://www.hpaweqa.org.uk)

R/PA/CS/8

In February of this year we undertook the second of our occasional 'mini' Isolation Methods Surveys, to monitor underlying shifts and trends in methods used for the isolation of indicator organisms.

Participants were asked to indicate their most frequently used methods by ticking only one box of four available for each parameter. Three boxes represented the top three methods, as identified by the comprehensive Isolation Methods Survey undertaken in August 2004 and the fourth box represented all other methods.

It was never our intention to link participants' EQA results to methods. So the fact that in some laboratories, the most frequently used method may be a reflection of the most frequently examined sample type, but not necessarily the method used for all of their EQA samples, does not invalidate the survey.

We were prompted to undertake these surveys by and for the benefit of participating laboratories. Of 250 laboratory code numbers to whom questionnaires were despatched 129 (52%) responded. This response rate is lower than in the previous 2004 and 2005 surveys, when 63% responded.

Charts 1 – 6 illustrate, for each parameter, the percentage of respondents in each of the four method categories.

Tables 1 – 6 compare, for each parameter, the percentages of respondents in the four method categories in surveys undertaken in 2004, 2005 and 2007.

The respondents haven't been from the same laboratories in all three surveys. However, of the 129 respondents in this survey, 70% had also responded in 2005 and 54% had been respondents in all three surveys.

There don't seem to have been any major shifts in the methods used.

For coliform bacteria and *E. coli*, as in 2005, the same two methods continue to vie with one another for the top spot. These are, of course, membrane filtration (MF) onto membrane lauryl sulphate broth (mlsb) and a most probable number (MPN) defined substrate method (e.g. Colilert® 18). The percentages of respondents using each of the two methods are almost identical and virtually unchanged from 2005. There has been a slight increase in the percentage of laboratories using MF onto Lactose TTC + Tergitol 7 since 2004 whilst the marked decrease between 2004 and 2005 in the percentage of respondents using 'other' methods has levelled out.

For each of the other parameters, the same method as in 2004 and 2005 convincingly retains the top spot.

For enterococci, this is MF onto m-Enterococcus (Slanetz and Bartley) agar. It is notable that MPN defined substrate methodology is used much less for enterococci than for the coliform parameters.

For *Pseudomonas aeruginosa*, use of CN supplement in Pseudomonas agar has increased with a concomitant decrease in use of CFC supplement. There were no longer any respondents using methods in the 'other' category for this parameter.

Addition of egg yolk to Tryptone Sulphite Cycloserine (TSC) agar, for *Clostridium perfringens* isolation, appears to be steadily losing support in favour of TSC without egg yolk. Popularity of m-CP agar has remained the same as in 2005, whilst in the 'other' methods category, numbers continue to fall slightly.

For colony counts, there were no major changes and 79% of respondents favour the pour plate method with yeast extract agar.

We included one additional question in the survey this year, to try to determine the extent to which laboratories are using molecular methods in water microbiology. Of 129 respondents, 114 answered this question and of these 2 were using PCR with Legionella, but not with Indicator Organisms.

We are grateful to all those who participated in the surveys and hope you find these results helpful.

Sheila Eaton  
May 2007

Chart 1 : Coliform bacteria

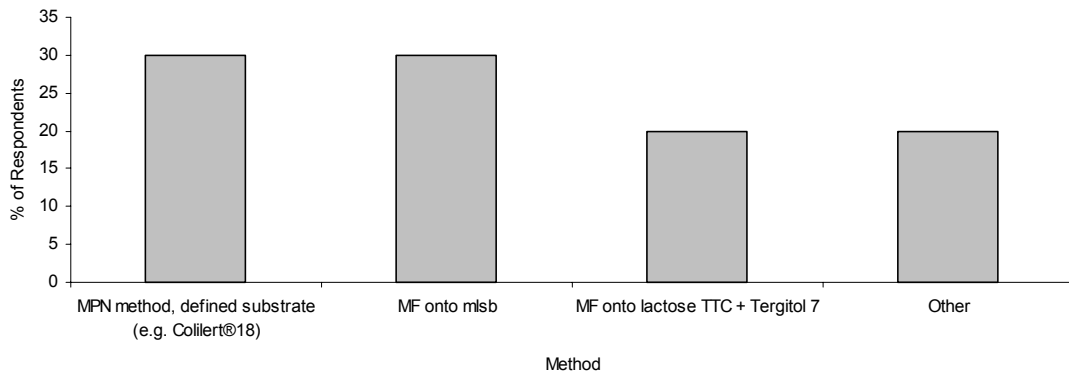


Chart 2 : *E. coli*

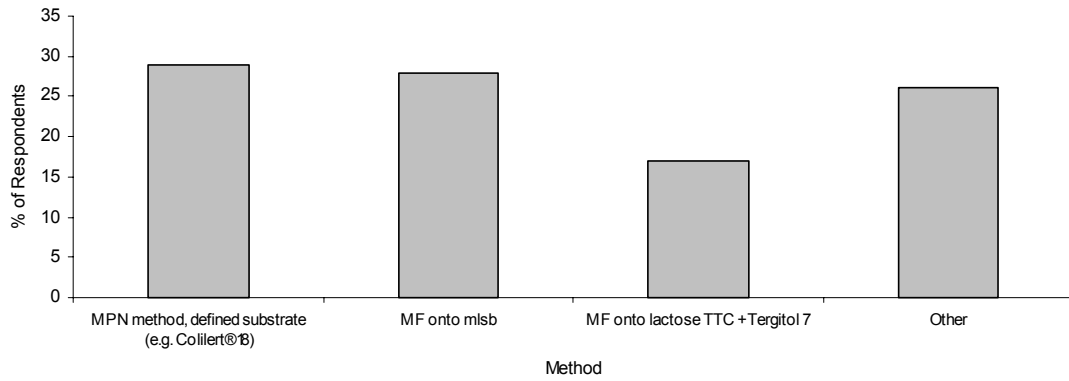


Chart 3 : Enterococci

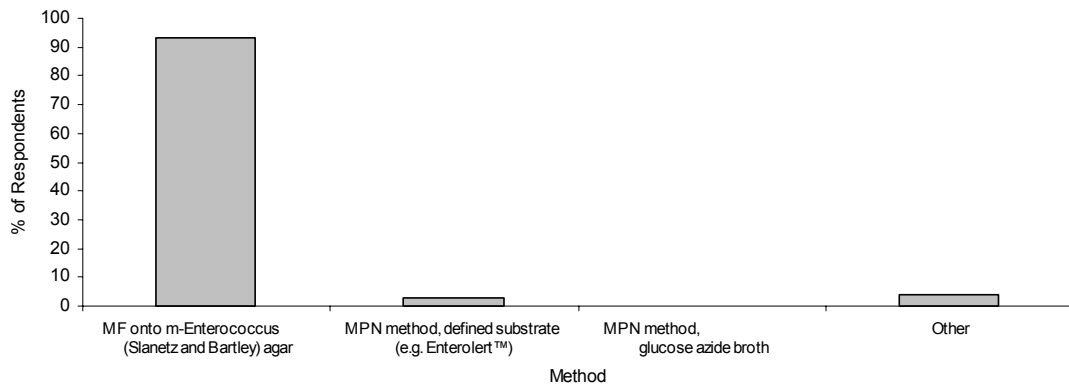


Chart 4 : *Pseudomonas aeruginosa*

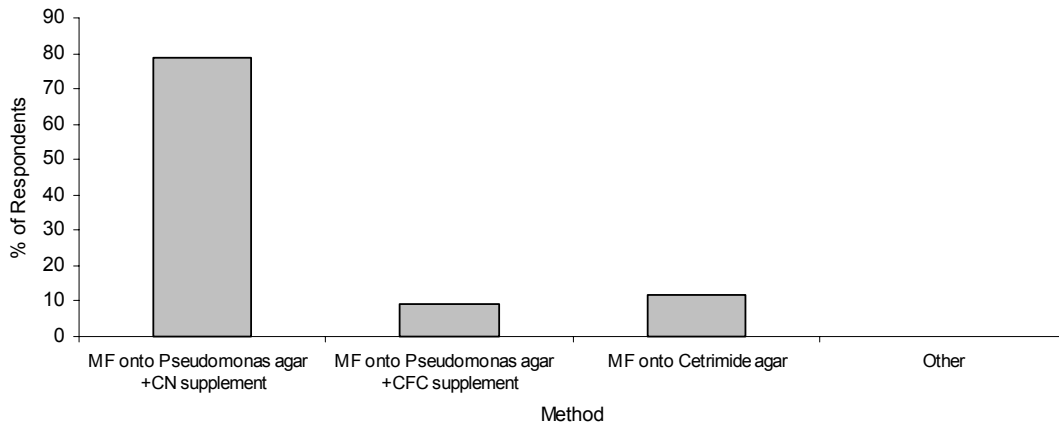


Chart 5 : *Clostridium perfringens*

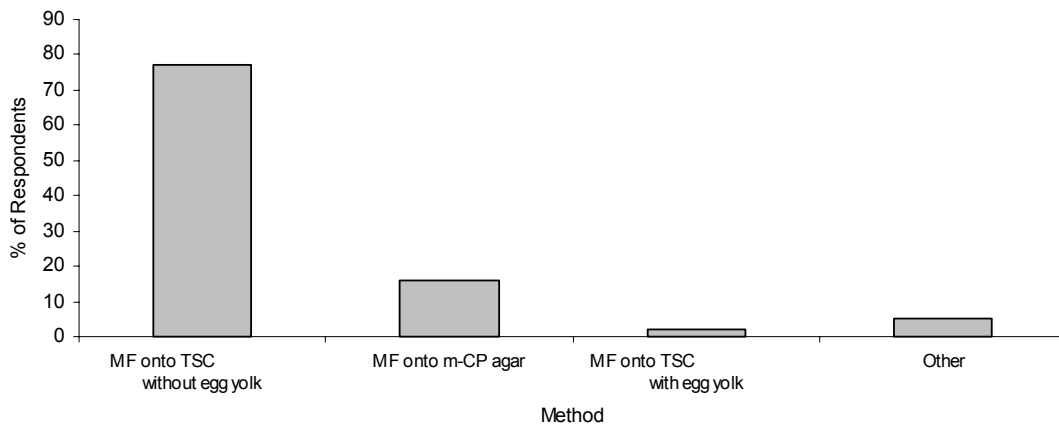


Chart 6 : Colony counts

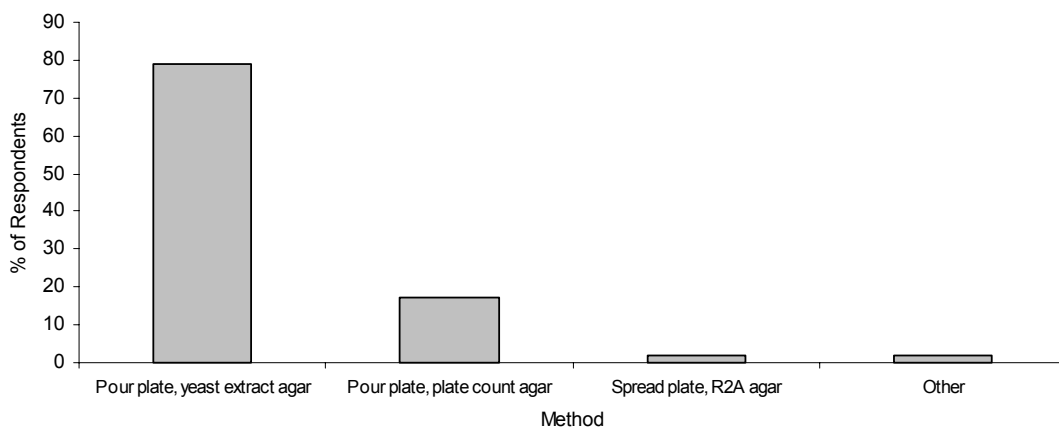


Chart 7: Using Molecular Methods

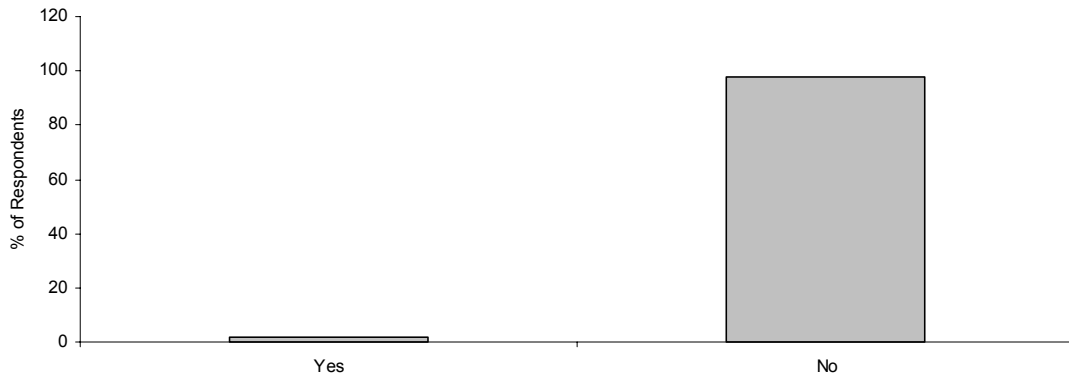


Table 1 : Coliform bacteria

Method	% survey respondents using method		
	2004	2005	2007
MPN defined substrate (e.g. Colilert® 18)	34	29	30
MF onto mlsb	34	30	30
MF onto LTCC + Tergitol 7	14	19	20
Other	39	22	20
	2004	2005	2007
Total number of respondents	167	156	125

34 respondents in 2004 were using 2 methods and were included in totals for both.

Table 2 : *E. coli*

Method	% survey respondents using method		
	2004	2005	2007
MPN defined substrate (e.g. Colilert® 18)	34	28	29
MF onto mlsb	30	27	28
MF onto LTCC + Tergitol 7	14	20	17
Other	42	25	26
	2004	2005	2007
Total number of respondents	169	157	129

35 respondents in 2004 were using 2 methods and were included in totals for both.

Table 3 : Enterococci

Method	% survey respondents using method		
	2004	2005	2007
MF onto m-Enterococcus (Slanetz and Bartley) agar	91	94	93
MPN defined substrate (e.g. Enterolert®)	6	2	3
MPN glucose azide broth	3	0	0
Other	8	4	4
	2004	2005	2007
Total number of respondents	160	147	121

11 respondents in 2004 were using 2 methods and were included in totals for both.

Table 4 : *Pseudomonas aeruginosa*

Method	% survey respondents using method		
	2004	2005	2007
MF onto Pseudomonas agar +CN supplement	71	69	79
MF onto Pseudomonas agar +CFC supplement	18	15	9
MF onto Cetrimide agar	8	14	12
Other	3	2	0
	2004	2005	2007
Total number of respondents	145	134	107

Table 5 : *Clostridium perfringens*

Method	% survey respondents using method		
	2004	2005	2007
MF onto TSC agar without egg yolk	65	71	77
MF onto m-CP agar	13	16	16
MF onto TSC agar with egg yolk	11	6	2
Other	12	7	5
	2004	2005	2007
Total number of respondents	142	135	106

2 respondents in 2004 were using 2 methods and were included in totals for both.

Table 6 : Colony counts

Method	% survey respondents using method		
	2004	2005	2007
Pour plate, yeast extract agar	74	70	79
Pour plate, plate count agar	17	24	17
Spread plate, R2A agar	2	1	2
Other	7	5	2
	2004	2005	2007
Total number of respondents	164	152	125

Table 7 : Molecular Methods

	% survey respondents using molecular methods
Yes	2
No	98
Total number of respondents	114