Cefas

European Community Reference laboratory for monitoring bacteriological and viral contamination of bivalve molluscs

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Comparison of Two and Three Class plans for evaluating E-coli levels in Live Bivalve Molluscs

1. Introduction

The following report was compiled in response to the document (CODEX STAN 292-2008) presented to NRLs in 2009. In this report a comparison is made between the following plans for acceptance of E-coli levels in live bivalve molluscs:

2 Class Plan (2CP): n=1, c=0, m=0 M=230
3 Class plan (3CP): n=5, c=1, m=230, M=700

So under the two class plan no samples out of one are allowed to have E-coli levels of 230 or more, whereas under the three class plan a maximum of 1 sample out of five can fall between 230 and <700 and the other 4 must be <230.

When making the comparison of the plans the following questions will be addressed:

Q1: If a site had 99% compliance with the 2CP what is the expected % compliance with the 3CP? How does this vary as we change M?

Q2: If a site had 99% compliance with the 3CP, what would its compliance be with the 2CP.

Q3: what would we need to reduce m to in a 3CP to achieve the same 99% compliance as the 2CP?

Q4: what would we need to increase m to in a 2CP to achieve the same 99% compliance as the 3CP?

2. Methods

The comparison will be made using the theoretical properties of the 3 by 5 tube Most-Probable Number (MPN) method used for shell fish E-coli testing. This assumes E-coli are distributed randomly within the sample which has three 10 fold dilutions (1g, 0.1g 0.01g) and has 5 tubes at each dilution which test as positive or negative.

The MPNs (per 100g) for the various tube combinations are given in Appendix I. A few extra MPNs were added for tube combinations that are unlikely, but not very unlikely.

For a given true concentration (y) the probability of each possible tube combination is calculated and this tube combination mapped to it's MPN. It is assumed that where MPN's do not exist (due to a very unlikely tube combination) then the sample would be re-tested. The probability can then be calculated that a single sample has an MPN less than (Pm) and also that 5 out of 5 samples are less than a given m (P5m) and also the probability that if 5 out of 5 are not less than m then 4 out of 5 are less than m and 1 out of 5 between m and less than M (P5mM).

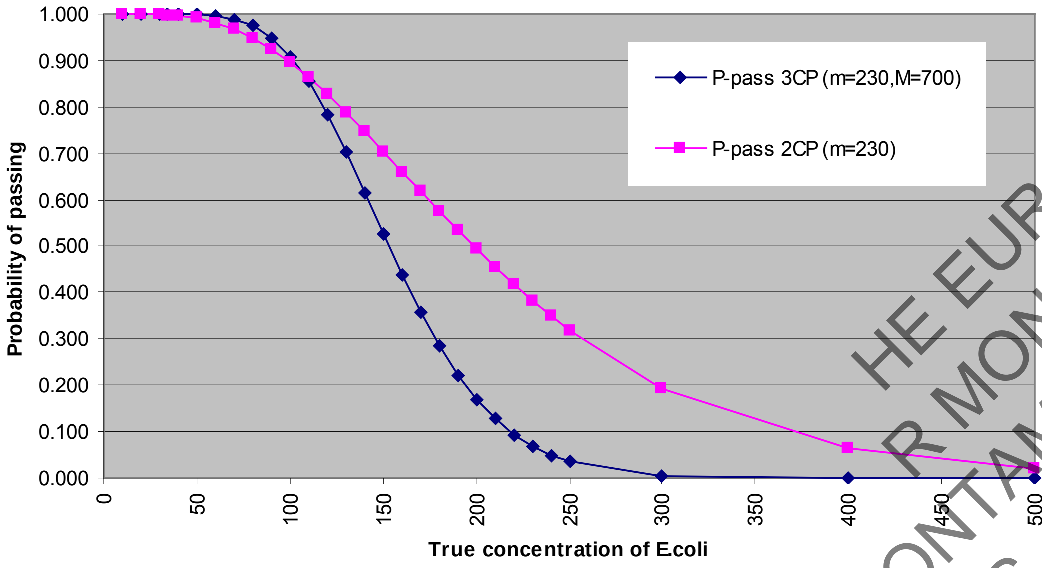
For the 2 plan scheme the probability of passing for a given true y is Pm(y)
For the 3 plan scheme it is P5m(y) + P5mM(y)

These probabilities are calculated for y=between 10 and 250. An Excel spreadsheet is used to perform the calculations.

3. Results

Figure1 shows the properties of the 2 CP(m=230) and 3CP (m=230, M=700).

Figure1: Comparison of different plans



Q1: If a site had 99% compliance with the 2CP what is the expected % compliance with the 3CP? How does this vary as we change M?

Compliance (i.e. probability of passing) is exactly 99% with the 2CP when the true mean is 50. At this true mean the compliance with the 3CP is 99.9%. So at this level almost all those who fail the2CP would pass the 3CP. As the true mean increase figure 1 shows that the probability of passing is higher for the 3CP until the mean reaches 100, after which it is higher for the 2CP.

Looking at when the true mean is 50 (99% compliance with 2CP) the % compliance with a 3CP would be as follows a M varies:

M	**Probability of passing**
700	99.9%
600	99.9%
500	99.9%
400	99.8%
300	98.8%
230	95.3%

The jump down from 98.8% to 95.3% is due to the relatively likely MPN of 5,0,0 = 230. So any 3CP that allows a pass if one result is 5,0,0 (230) will give an increase in the probability of passing from 95.3% to 98.8% at a true mean of 50.

Q2: If a site had 99% compliance with the 3CP, what would its compliance be with the 2CP.

Figure1 shows that exactly 99% compliance with the 3CP is achieved at a true concentration of 70. At this true concentration the compliance with the 2CP would be 96.7%. Clearly if a site has a true concentration of well below 70 then the 2CP compliance will be higher than 96.7% (after all – not all sites will have a true concentration that gives exactly 99% compliance).

Q3: what would we need to reduce m to in a 3CP to achieve the same 99% compliance as the 2CP?

Q4: what would we need to increase m to in a 2CP to achieve the same 99% compliance as the 3CP?

Figure2 shows two further plans that answer the above questions (figure 2b is the same as 2 but looks more closely at the top of the figure). They show that for a 3CP to give 99% compliance at a true mean of 50 (the level that gives 99% compliance with the 2CP) we would need to reduce m to 140 (M=700). This plan can be seen to then give much lower probabilities of passing as the true mean increases.

For a 2CP to give 99% compliance at a true mean of 70 (the level that gives 99% compliance with the 3CP) then m needs to be increased to 330. This plan, however, then gives much higher probabilities of passing as the true mean increases.

Figure2: Comparison of 4 different plans

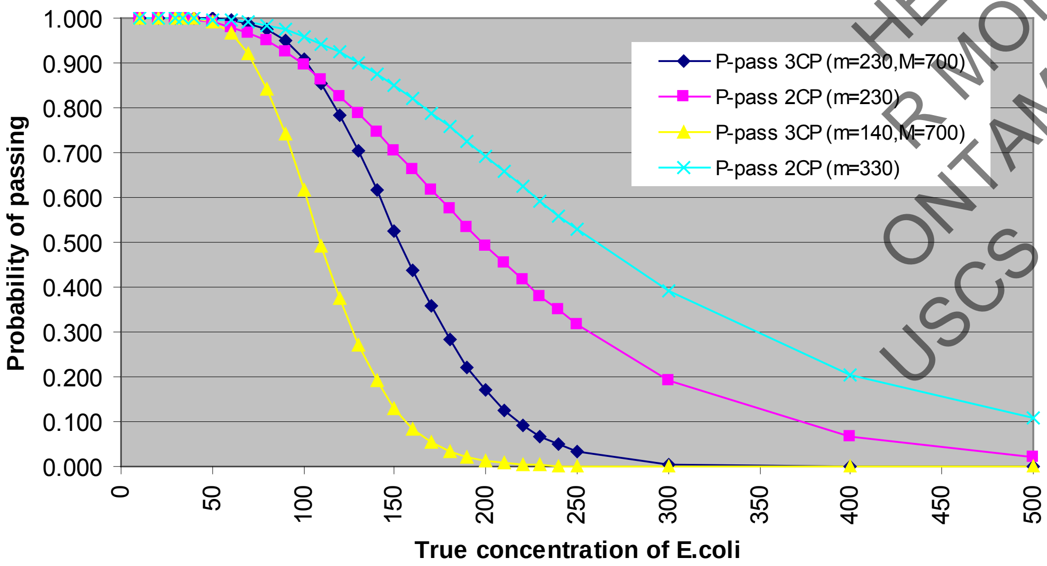
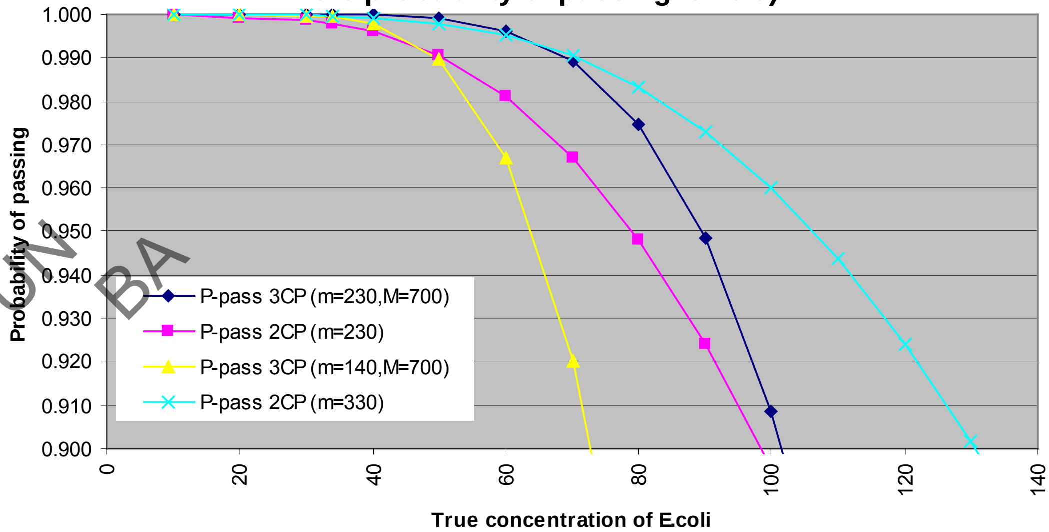


Figure 2b:Comparison of 4different plans (in the range where probability of passing is >0.9)



Appendix 2 gives the actual probabilities for the 4 plans.

4. Discussion

The 3CP (m=230,M=700) will lead to fewer sites failing who are at or very close to the level that gives 99% compliance to the 2CP. For example a site which is just within the 99% compliance for the 2CP will almost always pass the 3CP. However, a site which is much poorer (e.g. true level = 130) will eventually fail by both plans, but it will fail sooner by the 3CP (mean time 3 tests) than the 2CP (mean time 5 tests). Note that mean time to failure is calculated as 1/(1-prob of passing).

A 3CP with m=140 and M=700 is equivalent to the 2CP (m=230) at the concentration that gives 99% compliance, but then will give a greater chance of failing a site as the true concentration increases. This could be seen as an improvement on the 2CP depending on at which concentration you want ensure a high chance of failure.

A 2CP with m=330 is equivalent to the 3CP (m=230, M=700) at the concentration that gives 99% compliance by the 3CP, but then will give less chance of failing a site as the true concentration increases.

An ideal plan would be one that gives a probability of passing of 100% until a certain concentration that is deemed unacceptable is reached, and then will give 100% chance of failing. Clearly this is not possible, but the results do suggest that a 3CP could be designed that is more effective than a 2CP at doing this.

Appendix I: MPN tube combinations (CEFAS table)

Number of tubes giving positive reaction			MPN per 100g
5 of 1 g	**5 of 0,1 g**	**5 of 0,01 g**	
0	0	0	<20
0	1	0	20
0	2	0	40
1	0	0	20
1	0	1	40
1	1	0	40
1	1	1	60
2	0	0	60
2	0	1	70
2	1	0	70
2	1	1	90
2	2	0	90
2	3	0	120
3	0	0	80
3	0	1	110
3	1	0	110
3	1	1	140
3	2	0	140
3	2	1	170
3	3	0	170
4	0	0	130
4	0	1	170
4	1	0	170
4	1	1	210
4	1	2	260
4	2	0	220
4	2	1	260
4	3	0	270
4	3	1	330
4	4	0	340
5	0	0	230
5	0	1	310
5	0	2	430
5	1	0	330
5	1	1	460
5	1	2	630
5	2	0	490
5	2	1	700
5	2	2	940
5	3	0	790
5	3	1	1100
5	3	2	1400
5	3	3	1800
5	4	0	1300
5	4	1	1700
5	4	2	2200
5	4	3	2800
5	4	4	3500
5	5	0	2400
5	5	1	3500
5	5	2	5400
5	5	3	9200
5	5	4	16000
5	5	5	>18000

Additional MPN's used (all others are assumed to be retested)

Number of tubes giving positive reaction			MPN per 100g
5 of 1 g	**5 of 0,1 g**	**5 of 0,01 g**	
0	1	1	***60***
1	2	0	***50***
1	3	0	***80***
2	2	1	***120***
3	3	1	***200***
4	4	1	***380***
5	1	3	***850***
5	3	4	***2100***
5	4	5	***4600***

Appendix2: Probability of passing 4 different plans

True Mean	P-pass 3CP (m=230,M=700)	P-pass 2CP (m=230)	P-pass 3CP (m=140,M=700)	P-pass 2CP (m=330)
10	1.000	1.000	1.000	1.000
20	1.000	0.999	1.000	1.000
30	1.000	0.999	1.000	1.000
40	1.000	0.996	0.998	0.999
50	0.999	0.990	0.990	0.998
60	0.996	0.981	0.967	0.995
70	0.989	0.967	0.920	0.990
80	0.975	0.948	0.844	0.983
90	0.949	0.924	0.740	0.973
100	0.909	0.896	0.619	0.960
110	0.854	0.863	0.493	0.944
120	0.784	0.827	0.374	0.924
130	0.703	0.788	0.272	0.902
140	0.615	0.746	0.190	0.876
150	0.525	0.704	0.128	0.849
160	0.438	0.661	0.083	0.819
170	0.356	0.617	0.053	0.788
180	0.284	0.574	0.033	0.756
190	0.221	0.533	0.020	0.723
200	0.169	0.492	0.012	0.690
210	0.127	0.453	0.007	0.657
220	0.094	0.416	0.004	0.624
230	0.068	0.381	0.002	0.591
240	0.049	0.348	0.001	0.559
250	0.034	0.317	0.001	0.528
300	0.005	0.193	0.000	0.390
400	0.000	0.065	0.000	0.203
500	0.000	0.020	0.000	0.106