Cefas

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

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Report of the EU-RL regarding possible harmonisation of EU and Codex microbiological standards for live bivalve molluscs

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Issue

Codex Ailmentarius has recently published a trade standard for live and raw bivalve molluscs (CODEX STAN 292). This includes microbiological criteria for live bivalve molluscs (LBM) placed on the market and traded between countries. The *E. coli* element of these criteria is a critical consideration since it controls the general extent of faecal pollution permitted in traded products and thus the risk from faecally derived pathogens such as *Salmonella* spp.and enteric viruses. An *E. coli* criterion is not absolute protection against pathogen contamination but instead sets an upper risk threshold for general faecal pollution. *E. coli* (or the related faecal coliforms) standards are used internationally for LBMs and are key compliance criteria with important trade and public health impacts. Significantly the Codex *E. coli* criterion differs from the analogous EU criterion contained in EU food legislation. The EU-RL was requested by the EU Commission to consider the implications of this discrepancy and to make recommendations.

Approach

The EU-RL has undertaken a statistical comparison of the Codex and EU standards, has discussed with the NRL network of laboratories at the 8th and 9th annual Workshop, and has collaborated with 5 NRLs representing MS with classified production areas to consider the practical implications for monitoring. The findings are summarised in this report.

Statistical comparison

The Codex criterion is a three class plan (n=5, c=1, m=230 and M=700 *E. coli* MPN/100g) whereas the EU criteria (Regulation 2073/2005) is a two class plan (n=1, c=0, M=230 *E. coli* MPN /100g). Thus the Codex plan requires 5 samples to be taken, all must be less than 700 *E. coli* MPN/100g, and 1 sample is allowed to fall between 230 and 700 *E. coli* MPN/100 g. The EU plan requires 1 sample to be taken and it must be least than or equal to 230 *E. coli* MPN/100g. The statistical evaluation performed is detailed in Annex I. Essentially; a two class plan equivalent to the three class Codex standard would give a compliance range up to 330 *E. coli* MPN/100g in comparison to the EU range of up to 230 *E. coli* MPN/100g. However, it also showed that a 3

class plan was more likely to detect non-compliant samples particularly as contamination levels approached the regulatory limit.

Implications

The statistical comparison shows that the EU criterion is more stringent than the Codex criterion. However, the EU-RL considers that these differences are marginal and unlikely, in practice, to lead to differential health status with respect to pathogen contamination. The implications for trade are that products compliant with Codex standards could fail EU standards when tested during border inspections. This could lead to international trade disputes and potentially referral to World Trade Organisation. Conversely, EU exports are being required to satisfy a more stringent standard than required by Codex. From the public health perspective the Codex 3 class plan was more likely to detect non-compliant samples, particularly as contamination levels approached the regulatory limit.

Opinion of the EU-RL and NRLs

The scientific basis for 2 class and 3 class sampling plans for LBMs was discussed with NRLs at the 8th and 9th NRLs workshops. The consensus was that, given the known heterogeneous nature of *E. coli* contamination in LBMs, a 3 class plan for marketed products was scientifically preferable and would give better health protection. It was also noted that this was the approach adopted for the other microbiological criteria with EU Regulation (2073/2005). The workshop concluded that adoption of a 3 class plan for the *E. coli* microbiological criteria for LBMs in Regulation 2073 should be supported (see Resolution 1 of 8th Workshop[1], Resolution 3 of 9th Workshop[2]). The workshop further concluded that adoption of the Codex criteria was the most pragmatic approach and would also resolve any potential trade issues.

Consequential issues for production area monitoring

The EU microbiological criterion for marketed products set out in Regulation 2073/2005 is also the standard required of LBMs harvested from Class A designated production areas in Regulation (854/2004). Thus it is necessary to consider the consequential implications for harvesting area classification of adoption of a 3 class plan (such as the Codex criterion) for marketed products. An immediate practical implication is that simple adoption of the same criterion for production area monitoring would increase sampling requirements 5 fold. The scientific benefit of such a large increase in cost is debatable given that the objective of production area monitoring is to ensure harvested bivalves continue to be compliant with the criterion rather than to accurately measure the contamination status of individual marketed batches. An alternative less resource intensive approach would be to apply the 3 class plan criterion over time i.e. for class A areas no samples can exceed 700 *E. coli* MPN/100g and 80% of samples must be ≤230 *E. coli* MPN/100g. Following discussion at the 9th workshop 5 NRLs agreed to evaluate the

[1] http://www.crlcefas.org/InformationCentre/documents.asp?action=list&Section_ID=17
[2] http://www.crlcefas.org/InformationCentre/docs/Resolutions_of_the_9th_workshop_final.pdf

implications of possible adoption of the Codex 3 class plan values for production area monitoring. The summary findings are given in Annex II. Essentially the NRLs considered that adoption of the Codex standard for class A areas would have little practical impact on classifications awarded by Competent Authorities. The opinion of the NRLs was that adoption of the Codex values was realistic for production area monitoring, had scientific merit, and should therefore be supported. This view is also supported by the EU-RL.

Recommendation of NRLs and the EU-RL to the Commission and MS Competent Authorities

The recommendation is to harmonise EU and Codex end-product microbiological standards for LBMs by adoption of the Codex criteria (n=5, c=1, m=230 and M=700 *E. coli* MPN/100g) in EU Regulation 2073/2005. The Codex 3 class standard is scientifically preferable for detection of non-conforming batches and consistent with the approach adopted for other food commodities in this regulation.

A consequential recommendation is to amend EU Regulation 854/2004 to specify that LBMs harvested from class A designated production areas must comply either with the criteria specified in EU Regulation 2073/2005 (following amendment as above) or with a monitoring programme where during the review period no samples exceed 700 *E. coli* MPN/100g and 80% of samples are ≤230 *E. coli* MPN/100g.

EU-RL
April 2011

Annex I.

Comparison of 2 and 3 Class plans for evaluating E-coli levels in Live Bivalve Molluscs

1. Summary

Statistical equivalence in terms of public health risk of the Commission Regulation (EC) No. 2073/2004 and the CODEX STAN 292-2008 for live bivalve molluscs placed on the market.

The theoretical equivalence in terms of public health risks (as judged by the faecal indicator bacterium, *E. coli* levels in 100g shellfish flesh) of Commission Regulation (EC) No. 2073/2004 and the CODEX STAN 292-2008 were examined. For practicality in the evaluation the following parameters were considered:

- 99% compliance with a 2 class plan: n=1, c=0, m=0 M=230 (derived from Commission Regulation (EC) No. 2073/2004) - no sample from one > 230 *E. coli* MPN/100g.

- 99% compliance with a 3 class plan: n=5, c=1, m=230, M=700 (CODEX STAN 292-2008) – one sample out of five can fall between 230 and <700 and the other four must be <230.

In summary, it was established that a 3 class plan with n=5, c=1, m=140 and M=700 was equivalent to the 2 class plan (m=230) i.e. existing EU requirements. And, that a 2 class plan with m=330 was equivalent to the 3 class plan with n=5, c=1, m=230 and M=700. The evaluation also identified that clean sites (true mean 50 *E. coli* MPN per 100g) which show 99% (or very close to 99%) compliance with the 2 class plan would fail less often if assessed using the Codex 3 class plan. For example at a true mean of 50 the probability of passing the 2 class plan was 99% and 99.9% for the 3 class plan approach. However, a more contaminated site (e.g. true mean 130) would eventually fail by both plans, but it would fail sooner by the 3 class plan (mean time 3 tests) than the 2 class plan (mean time 5 tests). Thus pristine sites (mean < 50 *E. coli* MPN per 100g) would fail the existing EU requirements more often than if assessed by the Codex approach. However, importantly as the contamination status of a site increased (mean ≥130 *E. coli* MPN per 100g) the site would over time fail by both plans but if assessed by the 3 class plan approach this would occur more quickly. Consequently, it could be considered that for more contaminated (and perhaps more risky) Class A sites the application of the 3 class plan would result in a better level of public health protection.

2. Introduction

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?

3. 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).

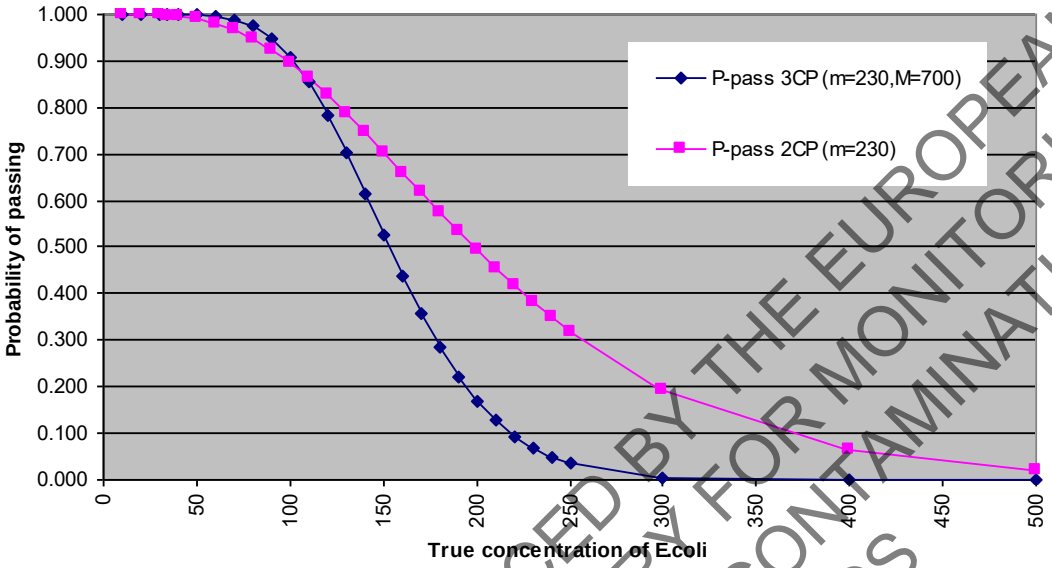
For the 2 plan scheme the probability of passing for a given true y is Pm(y)
For the 3 plan scheme it is P5m(y) + P5mM(y)

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

4. . 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

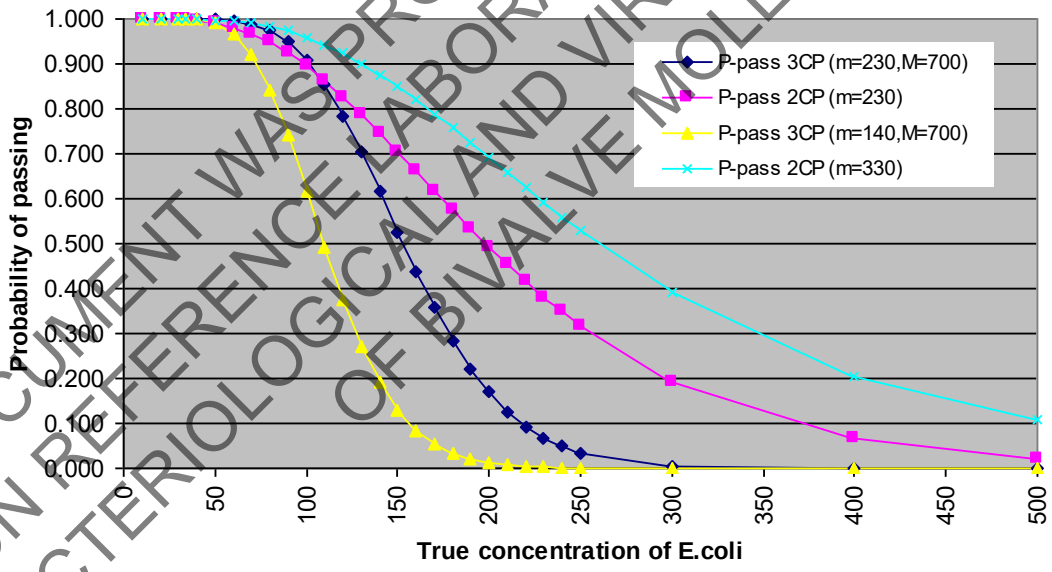
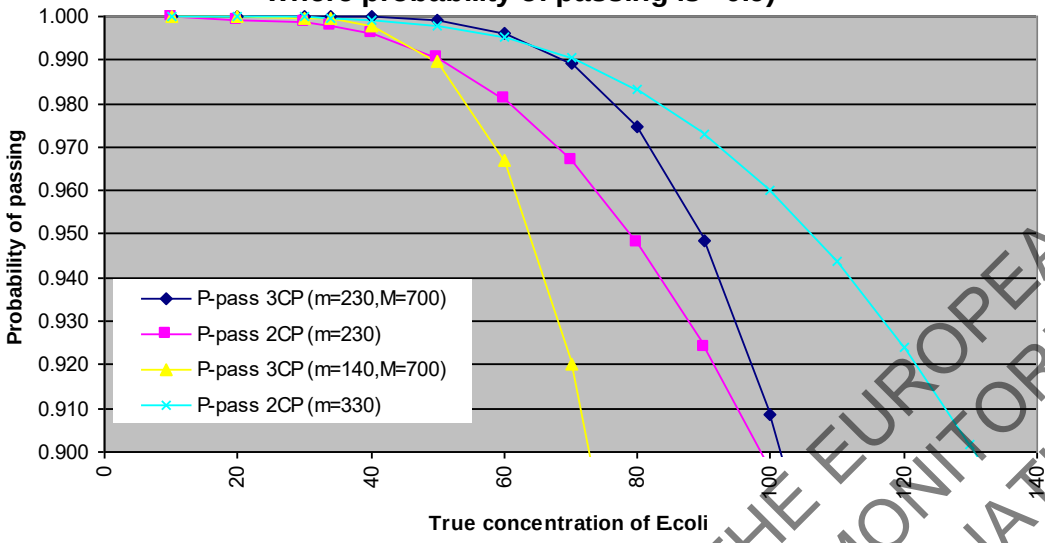


Figure 2b: Comparison of 4 different plans (in the range where probability of passing is >0.9)



Appendix 2 gives the actual probabilities for the 4 plans.

5. 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

Appendix 2: 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

Annex II- Summary of NRLs responses.

Analysis of class A datasets by NRLs
Following discussion at the 9th annual workshop NRLs were asked to challenge existing class A datasets with respect to the following scenarios:

1. To assess the impact of a 2 class plan with all samples <330 *E. coli* MPN per 100g.

2. To apply the Codex 3 class plan approach over time to monitoring data[1].

[1]applied over a minimum time period (for example 24 results available for 3 years or equivalent) 80% of sample results would be required to be ≤ 230 *E. coli* MPN/100g with up to 20% between >230 and an upper maximum of 700 *E. coli* MPN per 100g. The upper MPN limit of 700 is the upper 95% confidence limit of a 5 x 3 MPN test.

NRLs Denmark, France, Portugal, The Netherlands and U.K. responded to the request of the EU-RL.

Denmark
In Denmark production areas or line establishments were classified if more than 20 data points were available between 2006-2010 and available for 2009. Where less than twenty data points were available between 2006 and 2010 or no sampling had occurred in 2009 sites were not awarded a permanent classification and assigned U (outside permanent classification). These were not considered in the assessment.

Under existing EU legislation 16 sites (12 areas and 4 line establishments) were classified according to the above as class A. This represented 16% of the total bivalve production areas. Applying scenario 1 (m=330 *E. coli* MPN per 100g) to the dataset resulted in an increase to 19 sites (14 areas and 5 line establishments); a total of 19%. Application of the Codex guidance to time series data further increased the numbers of total production sites to 31% (19 production areas (30%); 12 line establishments (32%)).

France
The French NRL provided data for datasets comprising between 15 and 175 samples from 2007, 2008 and 2009 for 29 selected areas production areas. According to the absolute requirements of Commission Regulation (EC) No. 854/2004 by cross reference, 4 from 29 (≈14%) met the class A criterion. In France 10% exceedences of up to 1000 *E. coli* MPN per 100g are considered permissible (corresponding to the recommendations of the former EU expert working group on microbiological monitoring). Application of this tolerance for a practical management purposes increases the numbers of nominal class areas to 27 (93%). Applying scenario 2 further increased the percentage of compliant areas to 29 (100%). Scenario 1 (m=330 *E. coli* MPN per 100g) was applied to a smaller subset of data (n= 13) 4 of which met existent EU criterion (40%), and all of which met the requirements set of in the French order (10% tolerance up to 1000). All samples gave maximum results of ≤ 330 *E. coli* MPN per 100g. It was noted that NRL France favoured the implementation of scenario 2.

Portugal
NRL Portugal provided data for typical class A area from three years of monitoring (n=16). Using this approach one data point exceeded 330 *E. coli* MPN per 100g but was less than 700. Thus this area would have been classified as B under scenario 1 (all samples <330) and class A under scenario 2. The Portuguese NRL proposed support for scenario 2.

The Netherlands

In The Netherlands 12 production areas were considered according to both scenarios, between 128 and 216 sample results were evaluated. According to the data presented four areas produced results all < 230 *E. coli* MPN per 100g (25%), assessment by scenario 1, i.e. all samples ≤330, increased this by one (to 42%). Application of scenario 2 increased the number of compliant sites to 6 (50%). The site that failed under scenario 1 but passed when assessed by scenario 2 gave just one result between 230 and 700 (660) n = 196. This provides some support for the statistical observation that apparently cleaner sites would fulfil 2 class plan criteria less often than the Codex approach.

The United Kingdom

The UK provided information from a selected subset of less contaminated harvesting areas throughout the UK, based upon the presumption that none of the other areas in the UK would comply with either scenario. Furthermore only datasets that comprised at least 24 samples from a three year period were included, this was in accordance with EU-RL recommendations for a full classification (Anon 2010). Using this approach it was reported that 15 areas would comply with scenario 1, whereas 26 areas would be compliant with the recommendation contained within in scenario 2, 10 areas met the existing class A requirements.

Summary of NRL responses

NRLs interpreted the request from the EU-RL in slightly different ways and provided varying levels of detail thus direct comparisons of responses were not possible. However, application of both scenarios increased the proportion of compliant areas with in all cases slightly more areas identified as nominal Class A when the Codex 3 class plan was applied to times series data. Where preferences were specified NRLs supported the introduction of scenario 2 – harmonisation with Codex 3 class plan applied to times series data over specified time frames. These observations were supported by the data.