

APPENDIX E. LABORATORY QUALITY ASSURANCE TESTS

E.1 Laboratory Quality Assurance Tests

- E.1.1 In order that the results are admissible in a Court of Law, in the event of a prosecution by the DWI, the following laboratory quality assurance tests are considered necessary to ensure the validity of the results.
- E.1.2 Each approved laboratory shall have a formal system of internal quality control checks in accordance with the following procedures. Records and relevant charts will be kept and maintained such that not only a daily check is made but also the results will be trended to determine any long term changes which could affect the method.

E.2 PRINCIPLE

- E.2.1 The basic principle of the procedure is that every approved laboratory will carry out every day the laboratory is analysing regulatory samples an analysis of a spiked sample from the validation sampling rig. The sample shall be taken in accordance with the Regulations and the SOP. A full chain of custody is not required providing that the sampling is undertaken by approved *Cryptosporidium* laboratory staff and the validation sampling rig is in a secure location under the sole control of the *Cryptosporidium* laboratory.
- E.2.2 After the spiked sample has been taken from the validation sampling rig the sample will be transferred to the *Cryptosporidium* laboratory and signed in the appropriate analysis book(s). The sample will be treated as A N other sample and analysed alongside the other regulatory samples.
- E.2.3 The analysis will be undertaken by staff approved to undertake regulatory analysis. The rotation of staff is not a specific requirement but over a period of time all approved staff must be involved in the analysis of the daily validation sample. The results of the daily validation sample will be plotted on a chart with upper and lower limits to ensure appropriate action is taken if the result is outside the action limits. Any result outside the action limit must be investigated and the results of the investigation recorded.
- E.2.4 Each laboratory shall undertake to prepare, run and analyse a quality control sample on each day the laboratory is analysing regulatory samples. The quality control sample shall be valid until the next quality control sample result is available. Where a laboratory operates two separate shifts with separate staff operating on each shift then each shift will have to prepare, run and analyse a quality control sample on each day that the laboratory shift is analysing regulatory samples.

E.3 SECURE CONDITIONS FOR THE SAMPLE

- E.3.1 The sample taken for internal quality control shall be taken under 'secure' conditions. That is:

- (a) if the validation sampling rig is in an open access area then it must be in a locked cabinet with the key only available to approved *Cryptosporidium* laboratory staff; or
- (b) if the validation sampling rig is in a secure room with a unique key available only to approved *Cryptosporidium* laboratory staff, including the *Cryptosporidium* laboratory then a board mounted validation sampling rig may be used.

E.4 THE VALIDATION SAMPLING RIG

E.4.1 The validation sampling rig should conform to the following specification. A suitable sampling rig is available from Hydraulic Modelling Services Limited (HMS). The rig is available in two forms, either in a cabinet or mounted on a board.

E.4.2 Details of a suitable septum, needle and syringe are given below. Whilst these products have been found to be satisfactory for use with the validation rig the SOP does not endorse that they are the only suitable products available. The laboratory may use a product of equivalent specification.

E.4.2 Specification of the Validation Rig

E.4.2.1

E.4.3 Specification of a Suitable Septum

E.4.3.1 A suitable septum is supplied by:

Crawford Scientific
Holm Street
Strathaven
ML10 6NB

Tel No: 01357 – 522961

High pressure GC septum part **5183-4757-50** (11mm) 50 or 100 per pack.

Or a septum of equivalent specification.

E.4.3.2 It is recommended that the septum is changed each day of use prior to injection of the oocysts to minimize the possibility of loss of the oocysts.

E.4.4 Injection Syringe and Needle

E.4.4.1 A suitable syringe is supplied by:

BD (Becton, Dickinson and Company)
Plastipak Luer Fitting 10ml

Ref: 302188

A supplier is: 3S Healthcare, George House, Unit 6, Delta Park Industrial Estate, Millmarsh Lane, Enfield, EN3 7QJ

Tel: 0870 8734901

Or a syringe with an equivalent specification.

E.4.4.2 A suitable needle:

BD (Becton, Dickinson and Company)
Microlance 3, 19G x 2" (1.1mm x 50mm)
Ref: 301750

Supplied by: 3S Healthcare, George House, Unit 6, Delta Park Industrial Estate, Millmarsh Lane, Enfield, EN3 7QJ

Tel: 0870 8734901

Or a needle with an equivalent specification.

E.4.4.3 It is recommended that each syringe and needle is used once and safely disposed of in accordance with the laboratory's health and safety policy and practice.

E.5 SPIKING SUSPENSION

E.5.1 A spiking suspension may be made either

- (a) a flow cytometer may be used to prepare a suspension of 100 oocysts and subject to approved quality control procedures; or
- (b) *EasyseedTM*, which are test tubes containing one hundred *Cryptosporidium* oocysts in approximately 1 ml of saline solution; or
- (c) an approved commercial product containing 100 oocysts with certificated tolerances equivalent to existing approved products.

E.6 PERFORMANCE

E.6.1 Using the method outlined above experience has shown that recoveries of 40% or greater can be achieved. Similarly the recovery should not exceed 100% due to the inherent potential loss of oocysts that could occur during the analysis of the sample.

E.6.2 Experience will determine the recovery each laboratory will make. It is anticipated that there will be a variation in the initial stages. Once the staff have become familiar with the equipment and the analysis it is anticipated that recovery will become more consistent and this will be reflected in the

standard deviation. The Inspectorate will be monitoring the recoveries obtained by the laboratories but each will be looked at on an individual basis noting the percentage recovery, consistency of results and the standard deviation. In addition, the charts will be monitored to determine if they follow the guidance in Section E.9.4.

E.7 PROCEDURE

E.7.1 Procedure for use of Idexx Filta-Max® filter module in the Validation Sampling Rig

E.7.1.1 It is important that the same batch of filters is used both for the validation sample and the regulatory samples. The batch of filter housings must be detailed as part of the information recorded in the validation log.

E.7.1.2 Insert a filter housing into a filter module and ensure that it is tightened according to the manufacturers instructions to prevent leaking. The filter housing(s) is/are placed in the appropriate holder(s) on the spiking rig. Record the time, date, analyst and meter reading. The water is turned on up to a maximum pressure of 150kPa (1.5 bar) to wet and coat the filters for at least one hour. Check to see that the filter is not leaking if after one hour there is no leak then the spike can be prepared and injected.

E.7.1.3 Prepare EasySeed spike as per instructions. Draw each rinse stage (PBS and RO water) into a 10ml syringe ensuring no liquid remains in the needle. Record the details of batch numbers, date, time and analyst; these must be the same for both left and right filters. The time between preparing the spike and injecting into the sample line must be as short as possible.

E.7.1.4 Reduce the flow so that the pressure reads below 0.5 bar. Inject the spike into the sample line, holding the plunger down for 10-20 seconds. Care should be taken as there may be some back pressure, safety glasses or visor should be worn. As the plunger is released water will be drawn into the syringe. Fill and empty the syringe to rinse twice, without withdrawing the needle from the sample line. Remove the needle and reset the flow to give an appropriate volume filtered over 24 hrs. Record the time, date, analyst and meter readings.

E.7.2 Procedure for use of Pall Envirochek™ HV filter module in the Validation Sampling Rig

E.7.2.1 Insert a Pall Life Sciences Envirochek™ HV filter module into a stainless steel filter housing and ensure that it is tightened according to the manufacturers instructions to prevent leaking. The filter housing(s) is/are placed in the appropriate holder(s) on the spiking rig. Record the time, date, analyst and meter reading. The water is turned on up to a maximum pressure of 150kPa (1.5 bar) to wet and coat the filters for at least one hour. Check to see that the filter is not leaking if after one hour there is no leak then the spike can be prepared and injected.

- E.7.2.2 Prepare EasySeed spike as per instructions. Draw each rinse stage (PBS and RO water) into a 10ml syringe ensuring no liquid remains in the needle. Record the details of batch numbers, date, time and analyst; these must be the same for both left and right filters. The time between preparing the spike and injecting into the sample line must be as short as possible.
- E.7.2.3 Reduce the flow so that the pressure reads below 0.5 bar. Inject the spike into the sample line, holding the plunger down for 10-20 seconds. Care should be taken as there may be some back pressure, safety glasses or visor should be worn. As the plunger is released water will be drawn into the syringe. Fill and empty the syringe to rinse twice, without withdrawing the needle from the sample line. Remove the needle and reset the flow to give an appropriate volume filtered over 24 hrs. Record the time, date, analyst and meter readings.

E.8 RECORDS OF LABORATORY QUALITY ASSURANCE TESTS

- E.8.1 Records must be kept of all 'Laboratory Quality Assurance Tests' and these must be available for any audit undertaken by the Inspectorate. The records can be electronic (such as linked spreadsheets and graphs) or hard copy or both.
- E.8.2 A full record of each daily AQC sample shall be kept in a bound and sequentially numbered AQC analysis book. The records will provide information on the water sampled, the spike used (with an auditable trail to the supplier), details of reagents used. All records must be capable of being audited back to either the sample or the analyst.
- E.8.3 A full auditable record must be kept of the actions taken in response to any exceedance of the 'triggers' detailed in E.9.4.2. This record must be available for any inspection undertaken at the laboratory.

E.9 THE STATISTICS TO BE USED AND GUIDANCE ON THE CHARTS TO BE USED FOR PLOTTING RESULTS FROM DAILY *CRYPTOSPORIDIUM* QC SAMPLES

E.9.1 Introduction

- E.9.1.1 A large daily volume spiked sample is processed through an approved rig after injection of a known number of oocysts. Following analysis of the filter this provides an oocyst count from the filter. The method of analysis of the filter shall be the method used for analysing regulatory samples and the sample shall be treated as another regulatory sample. The spike shall be 100 oocysts with a known tolerance: with Easyseed of plus or minus one oocyst and with a flow cytometer of plus or minus two oocysts.
- E.9.1.2 The data from these QC samples will be recorded in an AQC analysis book, so that information on process details, names of analysts and any other relevant background facts are readily to hand. The results will be recorded and plotted on charts. See Section 8 above 'Records of Laboratory Assurance Tests'.

E.9.1.3 The aim of these QC charts is to give assurance that oocyst recovery performance is being maintained and to trigger investigations when performance appears to change.

E.9.1.4 Quality control charts were developed for operational control in manufacturing industry and have been used successfully in the water industry for demonstrating control of chemical testing, in particular using Shewhart charts. The concept has been extended to microbiological laboratories to demonstrate consistent microbial enumeration, using reference material.

E.9.1.5 In industry the measurement being controlled might be physical (e.g. life-time of a light bulb) and have a defined tolerance. A sample of the bulbs would be tested and if the average life time was seen to deteriorate and the measurements cross an 'action' line then the production process would be judged to be out of control and appropriate action taken. In water chemistry the ability of the laboratory to make accurate measurements is monitored using reference material. The regular tests would be expected to give consistent and repeatable results. A small amount of variation would be observed and tolerated. If QC results drift or become more erratic, with results on the control chart crossing the 'warning' or 'action' lines, then an investigation would be undertaken to pinpoint any problems in the analytical procedures (or possibly a problem with the reference material).

E.9.1.6 In microbiology there is usually much more variation than in chemistry in the results from sequential samples from reference material - principally because of random variation in the numbers of organisms present in each test portion and partly because of the difficulty in keeping stable reference material. Therefore 'control' charts have been approached in a less judgmental way and are often referred to as 'guidance' charts (*Microbiology of Drinking Water (2002) - Part 3 -Practices and procedures for laboratories, section 8.5.1*). An apparent problem won't necessarily reflect a true deterioration in laboratory performance, it may just be microbial behaviour or problems with the samples. However the chart does act as a screening tool for possible laboratory problems. The charts are designed with 'response' lines which, if crossed, trigger investigation without automatically labelling the laboratory as 'out of control' and thus discrediting all that batch of results.

E.9.1.7 In this *Cryptosporidium* QC scheme laboratories have an advantage over most microbiology QC in that the numbers of organisms spiked into the sample will be known to a much greater accuracy than can be achieved for bacterial spiking suspensions. There may be problems with maintaining consistent reference material for spiking the samples day after day, and with using water with consistent properties.

E.9.2. Aims of internal QC daily samples.

E.9.2.1 The results of the daily QC samples should be used as part of the laboratory's Quality Assurance programme. The results will be plotted on a suitable guidance chart. This will provide a screening tool for possible problems in recovering and enumerating oocysts. Furthermore, the charts and the

associated records will provide evidence to DWI that performance is either being maintained or that observed evidence of possible problems is being promptly investigated, with the results of that investigation properly recorded and any necessary action taken.

E.9.3 Constructing guidance charts

E.9.3.1 Plot the numbers of oocysts recovered sequentially. Once the rig is functioning satisfactorily and staff are accustomed to its use then this plot should settle down to show a small scatter around a steady average. It is customary to use a sequence of steady data (a minimum of 20 consecutive results) to calculate the average and standard deviation of the observations. These statistical parameters are then used to construct the warning and response lines against which future QC samples are compared.

E.9.3.2 The guidance chart will show the preliminary sequence of data. Horizontal lines of the mean of these preliminary data and lines at ± 2 x standard deviation (warning lines) and at ± 3 x standard deviation (response lines) are drawn on the chart.

E.9.3.3 The reason these lines are chosen is because, if the data are distributed approximately Normally (*i.e.* with a frequency distribution which would look bell-shaped and have this mean and standard deviation) then the warning lines should not be crossed, by chance, more than about 1 in 20 observations, and the response lines more than 1 in 370 observations. In reality microbiology counts are, at best, only approximately Normally distributed but this approach usually provides a working, objective solution.

E.9.3.4 Part of the management of the QC work will be to re-assess the values of the guidance lines at regular intervals and to check that they are set at suitable levels.

E.9.4 Plotting routine results

E.9.4.1 Once the levels have been set and the guidance chart is functional then the following procedures should be followed and logged:

E.9.4.2 Plot the new QC result on the guidance chart and note whether any of the following has occurred:

- (i) this result falls outside a response (action) line;
- (ii) two out of three successive results fall outside the same warning line;
- (iii) nine consecutive results fall on the same side of the mean line; and
- (iv) six consecutive counts show a trend which continuously rises or falls.

These represent four 'triggers' which have been tried and tested in microbiology laboratories and which are very unlikely to occur by chance unless the rate of

recovery of oocysts has changed (Microbiological Analysis of Food and Water: Guidance for Quality Assurance. Ed NF Lightfoot & EA Maier, pub Elsevier, 1998. page 170). By having four triggers there is a better chance of detecting problems with gradual as well as sudden onset. These triggers should initiate a pre-planned response. An explanation should be sought as to whether there is a change in laboratory performance or, for example, a change in the QC sample material. Note, however, that these events can happen, rarely, by chance.

E.9.5 Response to triggers

E.9.5.1 Pre-planned responses to 'trigger' events should be kept on file and be available for audit when required.

E.9.5.2 Responses to any of the four triggers will typically involve an appraisal of the whole analytical process including the preparation of the associated daily samples from which the data have been calculated. Apart from the assessment of the actual analytical and spiking procedures (including observation of the analysts' techniques), it is appropriate to check whether the changes may be associated with the introduction of a new batch of a reagent (e.g. IMS beads, IFA stain *etc.*).

E.9.6 Monthly review

E.9.6.1 Assess the lines on the guidance chart and check that they are fulfilling their required functions.

E.9.6.2 If the warning lines are never being crossed (and they should, by chance, be exceeded occasionally) then the estimate of the standard deviation is probably too high. This can happen when the chart is first set up because 20 observations is quite a small number for obtaining an accurate estimate. Re-calculate the mean and standard deviation using the last 50 results.

E.9.6.3 If the first three of the four triggers have been happening several times without explanation then consider whether the initial standard deviation was too low or the initial mean was too low or high. Re-set them using the last 50 results.

E.9.6.4 Once the QC procedures have settled down it should not be necessary to keep re-calculating the parameters. In theory the 2 x s.d. lines is likely to be crossed approximately one in 20 data points, but this will be a chance and not a regular process. Some months will have no crossings and others will have multiple crossings. Similarly the data points will be expected to be scattered above and below the mean line but some months may have a slight excess on one side and other months on the other side, by chance. Further guidance should be possible in the light of experience - when this scheme has been running in all laboratories for at least a year.

E.9.6.5 Changes in the values of the statistical parameters must be logged. Adjustments in the early months of setting up the guidance charts are justifiable but any changes after the charts have been used successfully for a few months

must be highlighted and discussed in the log. They may well indicate a shift in performance.

E.9.7 Summary of procedures

E.9.7.1 A summary of the procedures are detailed below:

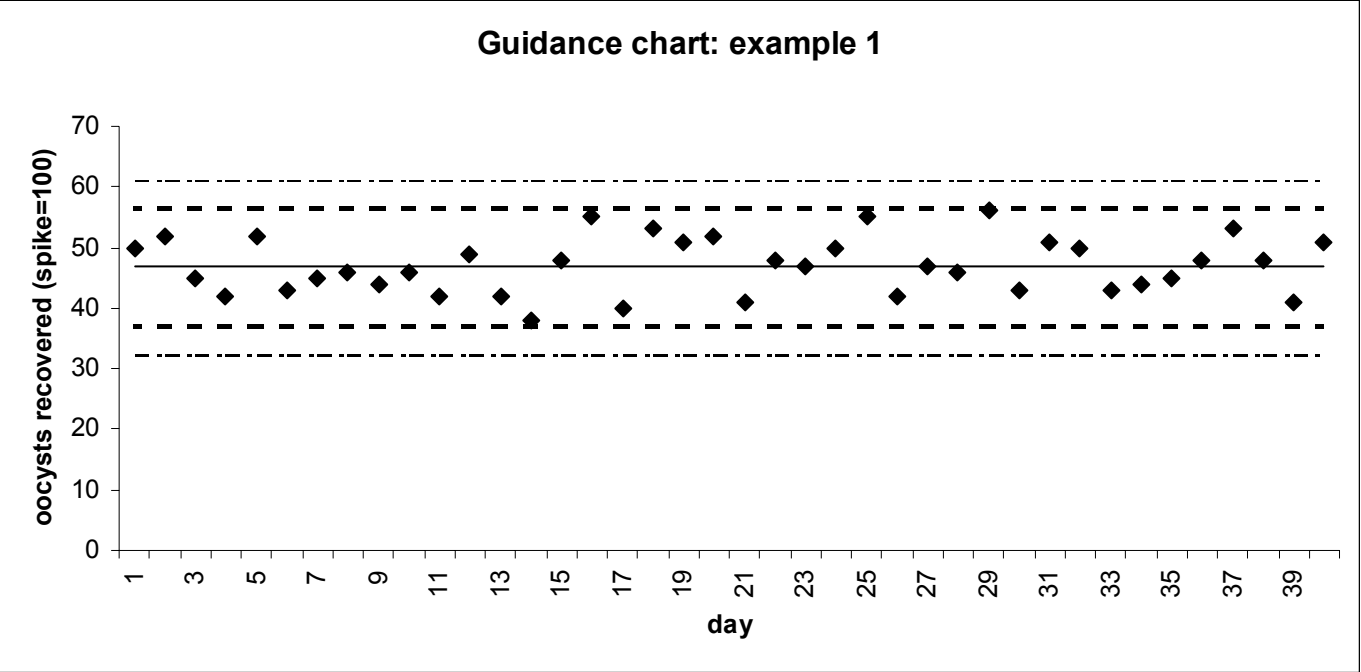
- (a) daily samples will be processed according to the DWI protocol, details recorded and result plotted;
- (b) when the routine is established a sequence of at least 20 results will be used to calculate mean and standard deviation and these will be used to plot response lines on the guidance chart;
- (c) each subsequent daily result will be plotted and checked against the set of four 'triggers';
- (d) the performance of the chart will be reviewed after every month (i.e. initially after 50 data points have accumulated and thereafter monthly) to assess, long-term, the appropriateness of the response lines; and
- (e) after the scheme has been running for about a year this document will be reviewed. Further worked examples will be provided using real data.

E.9.8 Examples of QC Charts for Information and Guidance

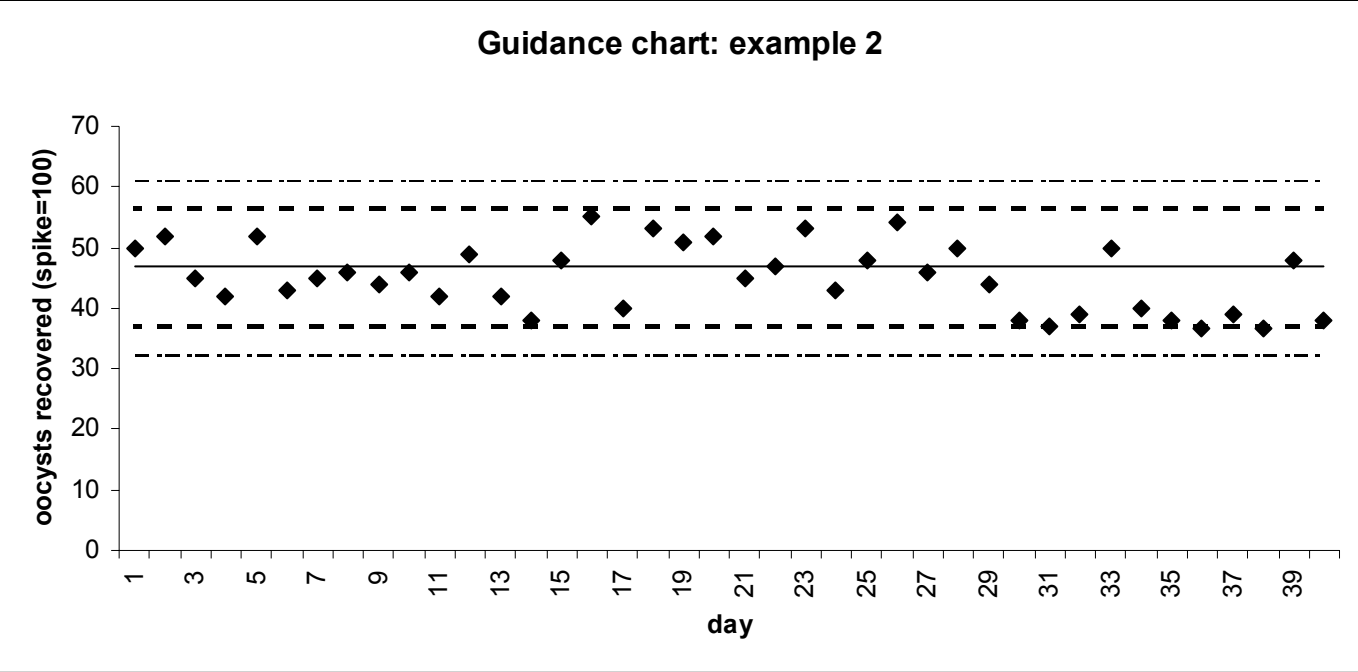
E.9.8.1 Example 1 shows a 40-day series. The first 20 observations were taken from real data and their mean (46.8) and standard deviation (4.83) were used to calculate warning and response lines. Because no further data were available for this example, the next 20 results were computer-generated to have the same parameters (*i.e.* mean and s.d.). Therefore this example illustrates a laboratory performing consistently; no possible problems are detected. Note that the upper warning line is reached once (on day 29) and this was a chance occurrence.

E.9.8.2 In Example 2 the same 20 observations as in example 1 were used to set up the chart. The next 10 results were computer-generated to have the same parameters but then the average recovery was reduced by 10%, from day 31 onwards. Visual inspection would have raised suspicion that the average recovery had in fact fallen. And by day 38 one of the 'trigger' rules had come into effect - two out of three successive results fall below the lower warning line. In real life this would lead to investigations, a record in the log book of this occurrence and a report on the outcome of the investigations and any action taken.

E.9.8.3 Guidance Chart Example 1



E.9.8.4 Guidance Chart Example 2



E.9.9 Introduction of New Approved Products into the Laboratory

E.9.9.1 Before any new approved product can be introduced in an approved laboratory for the sampling and analysis of regulatory samples, it must be shown to be at least as good as or better than recovering oocysts at limit concentration, than the existing approved method of analysis. [The existing approved method of analysis is that method which is posted on the DWI website, thus over time the method will change as improvements and alternatives are brought in.] The method whereby validation is to be achieved is set down below.

E.9.9.2 The revision of the SOP has introduced the concept of 'equivalent' in the testing of new approved products for the analysis of regulatory *Cryptosporidium* samples in approved laboratories. This is defined below:

Equivalent

The required performance of the trial method is that it should be at least equivalent (or better) than the standard method. 'Equivalent' will be interpreted as either 'significantly better' or as 'not significantly different', but the latter must be to a level of confidence acceptable to DWI. Thus, if the trial method is not finding significantly larger numbers of oocysts then the average difference between results from the two methods should be compatible with the null hypothesis of zero difference (with 95% probability). This statistical analysis will automatically lead to a statement about the likely range of the 'true average difference' between the methods, which is the average you would get from an infinite number of samples of the same types of water. This range is usually expressed as a 95% confidence interval and the lower end of this should rule out unacceptably worse recovery.

So that any new product has to be shown that it is at least 'equivalent' in performance or better than the existing approved product as defined above.

E.9.9.3 The definition of 'equivalent' will be used to introduce new approved products into the laboratory. A new product will be tested, as part of an analysis, against the existing approved method of analysis employed in the laboratory.

E.9.9.4 The laboratory validation sampling rig will be used to produce the samples for analysis in the laboratory to determine 'equivalence'. One line of the validation sampling rig would be the daily QC filter module (the result would be used as the QC sample, as well as, using the result as part of the validation of the new product) and the other line would be used to test the new product. Using the laboratory validation rig, the two filters are tested in parallel using the following procedure for checking the verification:

- (i) seed the two filter modules with oocysts, each at the limit concentration. Where a product other than a filter module is being tested then two filter modules of the same type should be seeded;

- (ii) connect the filters to the validation sampling rig which is on the laboratory standard water supply. The flow-meter connected downstream of each filter unit to measure the volume sampled is read prior to turning on the water supply to the validation sampling rig. The water is turned on, so that the pressure gauge reads 100-150 kPa (1-1.5 Bar), for at least one hour. [Providing the filter has run for at least one hour the time the oocysts are injected is not critical.] The pressure to the sampling lines is turned down to 50 kPa (0.5 Bar) to make injection of the oocysts easier. The oocysts are then injected into each filter line. After injection of the oocysts the syringe is flushed at least twice into the flow line. When this has been completed the pressure is increased to 100-150 kPa (1-1.5 Bar) to ensure that at least 1,000 litres of water will pass through the filter over a 24 hour period. The filter is run continuously for a 24 hour period at a flow rate not less than 40 litres per hour. At the end of the 24 hour period the filter is removed and the sample analysed in the laboratory along with the regulatory samples. That is it would be treated in a similar manner to any regulatory sample analysed in the laboratory and not in any special way;
- (iii) the validation sampling rig is run on twelve separate days and the filters run for a 24 hour period and the volume filtered is measured, which should be greater than 1,000 litres. Fresh filters are used for each 24 hour period.
- (iv) It is not required that a negative control is run with these filters.
- (v) The data generated must demonstrate that one approved product is equivalent or (better) than the other approved product. 'Equivalent' will be interpreted as either 'significantly better' or as 'not significantly different', but the latter must be to a level of confidence acceptable to DWI. Should the data fail to demonstrate equivalence then more replicates will be required for this to be achieved.

E.9.10 Statistics to be used to Validate a New Product in the Laboratory

E.9.10.1 The laboratory checking the *alternative* or modified approved method needs to establish equivalence between the two methods. The statistics for this together with two worked examples are given in Annex B of Part 3 of the SOP 'Validation Of New Methods Or Parts Of Methods For Sampling And Analysis'. It may also be apparent that there is a difference in recovery of oocysts between the water sources used by different laboratories for the validation. Providing the results are consistent with the QC sample for the supply and the method is 'in control' then the result is acceptable.

E.9.11 Documentation

E.9.11.1 Full documentation must be kept of each validation in a separate file. The completed file has to be available for audit purposes to show that any new product has been properly validated. All results, data and calculations

(including any rejected results) must be kept and copies put on the file. The written report of any validation should clearly show the setting down of the results, the calculation of the statistics and that the new approved product is 'equivalent' to the existing approved product being used in the laboratory. The format of the report must follow the format set down in Part 3 of the SOP 'Validation Of New Methods Or Parts Of Methods For Sampling And Analysis'.