

PROTOCOL FOR THE *crypts* CRYPTOSPORIDIUM PROFICIENCY TESTING SCHEME

1. Aim of the Scheme

The primary aim of the scheme is to promote quality in the measurement of *Cryptosporidium* oocysts in treated water supplies to satisfy the Water Supply (Water Quality) (Amendment) Regulations 1999, SI No. 1524. The scheme operates under Drinking Water Inspectorate (DWI) licence. *crypts* provides the DWI and laboratories with a means of independently assessing their performance over time for a range of analyte levels and methods. The scheme enables participants to demonstrate to regulatory bodies and customers, on an international basis, the validity of their results.

crypts also enables laboratories and regulatory bodies concerned with the analysis of *Cryptosporidium* to gain information on the efficacy of methods and assist in the development of new methods.

2. Organisation of *crypts*

The structure and organisation of *crypts* follows ISO/IEC Guide 43:1997 for the Development and Operation of Laboratory Proficiency Testing¹ and ILAC Guide G13 Guidelines for Requirements for the Competence of Providers of Proficiency Testing Schemes². The scheme combines the expertise of both the Proficiency Testing Team at LGC and the Scottish Parasite Diagnostic Laboratory (SPDL). Overall administration of the scheme, including data processing and reporting will be the responsibility of LGC. Biological and scientific expertise pertinent to the organism, including sample preparation, dispatch, and homogeneity testing is the responsibility of the SPDL. LGC documents all practices and procedures relevant to the scheme administration in appropriate quality documentation as part of compliance with ISO Guide 43 accreditation. SPDL documents the technical aspects of the scheme pertinent to the organism. Collaboration between LGC and SPDL ensures that the scheme is operated at the highest possible standard.

Contacts at LGC:

| | | |
|--|-----------------|---------------------|
| Scheme Manager: | Nick Boley: | +44 (0)20 8943 7311 |
| Secretariat: | Derek Woods: | +44 (0)20 8943 7494 |
| Administration: | Eileen Doherty: | +44 (0)20 8943 7425 |
| FAX: | | +44 (0)20 8943 0654 |
| E-Mail: | | ptgroup@lgc.co.uk |
| Contract Manager and Liaison with DWI | Jo Peet: | +44 (0)20 8943 8411 |
| FAX: | | +44 (0)20 8943 1050 |

E-Mail: jo.peet@lgc.co.uk

Contacts at SPDL:

| | | |
|--|-----------------|---|
| Senior Technical Advisor: | Huw Smith: | +44 (0)141 201 3028 |
| Technical Officer, dispatch and return of samples: | Brian Campbell: | +44 (0)141 201 3029 |
| Enquiries: | | +44 (0)141 201 3029 |
| FAX: | | +44 (0)141 558 8000 |
| E-mail | | h.v.smith@spdl.org.uk b.campbell@spdl.org.uk |

Overall direction of the scheme is overseen by a Steering Committee which is designated by DWI.

The Steering Committee is chaired by a senior representative of LGC, with LGC providing the Secretariat. Membership of the Committee will normally include representatives of the Scheme Operator, LGC and SPDL, a representative of DWI, and a minimum of two representatives of participating laboratories (one nominated by the UK Water Industry and one independent), together with any additional members considered necessary by DWI.

The terms of reference of the Steering Committee are:

- To assess the results obtained in the scheme.
- To consider the nature and timing of proficiency testing rounds and the scope and direction in which the scheme should develop.
- To provide specialist advice to DWI and scheme organisers on technical and other matters to assist in the smooth running of the scheme.
- To advise on the need for and nature of any revision of the scheme protocol.
- To advise DWI and scheme organisers on the criteria for satisfactory performance.
- To advise on the scope of User Group Meetings.
- To advise DWI on action to be taken in the event of persistent poor performance by a laboratory or analyst.
- To resolve any disputes between the participants and the scheme organiser.

The Steering Committee will meet when necessary to ensure progression of the scheme. Meetings will be at least quarterly for the initial year of operation and every six months thereafter.

User Group Meetings will be held annually for participants of the scheme.

The structure of the scheme is as follows:

2.1 Framework

Twelve rounds of test materials are distributed each year to participants according to a schedule issued in advance. DWI reserve the right to reduce or increase the frequency of sample rounds, subject to notice.

Participants are required to return their results within the specified deadline, usually within one working week. The results are subject to review and participants are notified of their performance within a further three working weeks of the closing date for return of results. Participant confidentiality is assured by identifying them by confidential identification numbers only. DWI are notified of the identity of participants and their identification numbers.

The structure of each round of the scheme is as follows:

- 1 Sourcing, preparation, bottling and labelling of test materials;
- 2 Homogeneity testing of filters and suspensions, and initial counting of slides;
- 3 Distribution of test materials;
- 4 Participants' analysis of test materials and reporting of results to scheme organiser;
- 5 Count back of returned slides;
- 6 Results entered and tabulated and reports prepared;
- 7 Individual reports sent to participants and full report sent to Steering Group members;
- 8 Requirements of next round identified;
- 9 Commencement of next round.

SPDL take the lead responsibility for parts 1, 2, 3, 5 of the structure and LGC for parts 4, 6, 7. Parts 8, 9 are the joint responsibility of LGC, SPDL and the Steering Committee.

2.2 Test Materials

It is intended that the test materials used in the scheme reflect the types of materials generally encountered by participant laboratories. These include *Cryptosporidium* oocysts of the species *parvum*, *baileyi* and *muris* as well as other interferents of similar and different size and antibody reactivity. Sample materials are purchased, prepared then assessed, in the case of filters and suspensions, for homogeneity according to the procedure described in Annex 2. Each slide is examined and counted prior to dispatch.

Each round consists of three test materials:

- i) a microscope slide
- ii) a suspension
- iii) a filter (Genera Filta-Max™)

2.2.1

Microscope slides are selected from the following:

- i) stained slides displaying only *Cryptosporidium* oocysts;
and
- ii) stained slides displaying typical interferents or oocyst-like bodies other than *Cryptosporidium*, with or without *Cryptosporidium* present.

2.2.2.

Suspensions contain any level of *Cryptosporidium* relevant to the assessment of laboratory performance, including but not limited to:

- i) a standard solution to be filtered containing 80-120 oocysts;
- ii) a standard solution to be filtered containing 80-120 oocysts and additional potential interferents; and
- iii) an unspiked suspension.

2.2.3

Filters contain any level of *Cryptosporidium* relevant to the assessment of laboratory performance, including but not limited to:

- i) a filter spiked with water containing oocysts at levels near the regulatory limit;
- ii) a filter spiked with typical interferents or oocyst-like bodies other than *Cryptosporidium*, with or without *Cryptosporidium* present; and
- iii) an unspiked filter after use in an uncontaminated water supply.

2.3 Establishing the Assigned Value

The assigned values are established as follows:

- i) stained microscope slides:
Each stained microscope slide is ascribed a unique identification number and *Cryptosporidium* oocysts are enumerated by a trained analyst at SPDL prior to each distribution. The enumerated slides are then dispatched to participants. Following enumeration by participants, slides are returned to SPDL within the designated time and re-enumerated and the results entered against the unique identification number. The assigned value for each slide is determined from the two counts: Where both counts are the same this shall be the assigned value, where there is a difference between these counts, the assigned value will be calculated as follows:
 - when the participant's result lies within the range of counts at SPDL, the difference between the result and the assigned value will be deemed to be zero and the result described as "NV" to indicate a non-variant result;
 - when the participant's result is above the range of counts at SPDL, the assigned value will be the higher of the two SPDL counts;
 - when the participant's result is below the range of counts at SPDL, the assigned value will be the lower of the two SPDL counts.
- ii) Suspensions: Suspensions are formulated to give a target concentration and checked to validate this. The validation consists of determining the mean of the replicate validation counts of the flow cytometry homogeneity data (see below), which is then ascribed the assigned value. Laboratory returns are compared to this value and results recorded as percentage recoveries of the mean replicate counts.
- iii) Filters: The mean of the replicate counts of the flow cytometry homogeneity data (see below) is ascribed the assigned value. Laboratory returns are compared to this value and results recorded as percentage recoveries of the mean replicate counts.

2.4 Homogeneity of Test Samples

The homogeneity of samples is assessed according to the procedure described in Annex 2.

2.5 Stability of Test Samples

The stability and viability of test samples and the *Cryptosporidium* oocysts contained therein are assessed according to the procedure described in Annex 3.

2.6 Instructions to Participants

Full instructions are supplied to participants regarding the handling of samples, the period in which to carry out the tests and for return of non-disposable materials to SPDL.

2.7 Analytes to be Determined

Participants are requested only to count the number of *Cryptosporidium* oocysts in samples (as defined in the Standard operating protocol for the monitoring of *Cryptosporidium* oocysts in treated water supplies to satisfy water supply (water quality) (amendment) regulations 1999, S. no 1524 – Part 4 Requirements for inter-laboratory proficiency schemes). See also Annex 1.

2.8 Methods of Analysis

Participants must use the method specified in Part 2 of the DWI Standard Operating Protocol 1999, SI No 1524.

2.9 Assessment of Performance

Laboratories, and counters within laboratories are assessed by comparison with the assigned values. For slides, the difference between the counter's result and the assigned value will be indicated in the report. For filters and suspensions, the number of oocysts reported will be calculated as a percentage recovery, which is compared with the mean replicate counts obtained by SPDL in the homogeneity assessment of the samples.

Criteria for satisfactory performance, based upon results in the scheme, will be developed by the Steering Committee and DWI.

2.10 Reporting of Results

Results should be sent to the Scheme Secretariat by post, e-mail and/or FAX, using a proforma supplied by the Scheme Secretariat.

Results will not be edited by LGC. LGC reserves the right not to include any results received after the deadline for any particular round.

2.11 Reporting of Results to Participants

The appropriate reports are sent to participants within 15 working days of the result deadline.

2.12 Confidentiality

Information concerning the performance of individual laboratories is confidential between the individual laboratory, DWI and the scheme organiser, except where the individual laboratory decides otherwise, and informs the Secretariat in writing. The participant laboratory and counters' codes are notified to the laboratory upon registration, and remain the same unless the participant requests that they be changed, in writing to the Secretariat. DWI will be informed by the scheme co-ordinator of all laboratories obtaining unsatisfactory performance, failure to report results within the deadline and failure to submit results for any round.

DWI will be informed of any laboratory's full withdrawal from the scheme or a component of the scheme.

2.13 Liability and Disputes

The scheme management accept no liability to participants. If a dispute should arise it will be dealt with on an individual basis and appropriate action taken by the scheme management to redress the situation. All disputes will be discussed fully by the Steering Committee.

2.14 Collusion and Falsification

The scheme Secretariat takes all necessary actions to prevent any collusion or falsification of results. Where any collusion or falsification by any participant is proven, the results for that participant for the sample(s) concerned will be expunged.

3. Subscriptions

The subscription rate is provided before the commencement of each series of 12 rounds and each participant notifies LGC of their basic monthly subscription for the next 12 rounds. Participants may make changes to their subscription no less than 2 weeks prior to the dispatch date of the round they wish to change.

Participants are invoiced on a monthly basis.

4. References

1. ISO/IEC Guide 43 Part 1 (1997) "Proficiency Testing by Interlaboratory Comparisons", ISO, Geneva, 1997.

2. ILAC Guide G13:2000: "Guidelines for the Requirements for the Competence of Providers of Proficiency Testing Schemes", ILAC, Sydney 2000.
3. Drinking Water Inspectorate: Water Supply (Water Quality) (Amendment) Regulations 1999, SI No 1524.
4. Campbell, A T, Robertson, L J and Smith, H V (1992). Viability of *Cryptosporidium parvum* oocysts: correlation of *in vitro* excystation with inclusion/exclusion of fluorogenic vital dyes. **Journal of Applied and Environmental Microbiology** **58**: 3488-3493.
5. Robertson, L J, Campbell, A T and Smith, H V (1993). *In vitro* excystation of *Cryptosporidium parvum*. **Parasitology** **106**: 13-29.
6. Standard operating protocol for the monitoring of *Cryptosporidium* oocysts in treated water supplies to satisfy water supply (water quality) (amendment) regulations 1999, S. no 1524 Part 4 - Requirements for the inter-laboratory proficiency testing scheme.

Annex 1

Definitions and Terminology

Proficiency Testing Scheme

The system for objectively checking laboratory results by means of an external agency (ie the *crypts* Secretariat). It includes comparison of a laboratory's results at intervals with those of other laboratories, the main objective being to assess the trueness of test results. Proficiency testing is therefore designed to assess the accuracy of a laboratory's results.

True Value

The actual concentration of the analyte in the matrix.

Assigned Value

The value to be used as the "true" value by the *crypts* Secretariat in the statistical treatment of results. It is a practical estimate of the true value of the analyte in the matrix.

Target Value for Standard Deviation

A numerical value for the standard deviation of a measurement's results, which has been designated as a goal for measurement quality.

Accuracy

The closeness of agreement between a test result and the accepted reference value.

Note: The term accuracy, when applied to a set of test results, describes a combination of random components and a common systematic error or bias component.

Trueness

The closeness of agreement between the average value obtained from a large series of test results and an accepted reference value.

Note: The measure of trueness is usually expressed in terms of bias.

Bias

The difference between the expectation of the test results and an accepted reference value.

Note: Bias is a systematic error as contrasted to a random error. There may be one or more systematic error components contributing to the bias. A larger systematic difference from the accepted value is reflected in a larger bias value.

Laboratory Bias

The difference between the expectation of the test results from a particular laboratory and an accepted reference value.

Bias of the Measurement Method

The difference between the expectation of test results obtained from all laboratories using that method and an accepted reference value.

Precision

The closeness of agreement between independent test results obtained under prescribed conditions.

Cryptosporidium Oocysts

All species (active or inactive) of the *Cryptosporidium* species within the size range 4-6µm (According to Standard operating protocol for the monitoring of *Cryptosporidium* oocysts in treated water supplies to satisfy water supply (water quality) (amendment) regulations 1999, S. no 1524 – Part 4 Requirements for inter-laboratory proficiency schemes).

CLBs

Cryptosporidial oocyst-like bodies (According to Standard operating protocol for the monitoring of *Cryptosporidium* oocysts in treated water supplies to satisfy water supply (water quality) (amendment) regulations 1999, SI. no 1524 – Part 2 – Laboratory and Analytical Procedures).

Annex 2

Assessing a Test Material for Homogeneity

Slides

Each slide sample is counted prior to distribution and following receipt from the participating analysts. Slides are not re-used. No assessment of homogeneity is necessary.

Validation of flow cytometry prepared suspensions for distribution and for spiking of Genera Test Filters

Target oocyst densities are set in the DWI Standard Operating Protocol for the Monitoring of *Cryptosporidium* oocysts in Treated Water Supplies to Satisfy Water Supply (Water Quality) (Amendment) Regulations 1999, SI No. 1524 and can be modified by the Steering Committee. Target oocyst densities fall between 80 and 120 oocysts per test suspension and test filter.

Oocyst suspensions used for distribution are enumerated by flow cytometry at the West of Scotland Water Authority (WoSWA) laboratory. A representative sub-sample (10%) is then enumerated following the addition of FITC-CmAb on membrane filters. Sub-sample slides are submitted to the SPDL for re-enumeration and confirmation of WoSWA counts and, if satisfactory ($\pm 5\%$), suspensions are accepted into the scheme. Results of individual enumerations of suspensions chosen at random together with the requisite number of suspensions requested are supplied to SPDL prior to each distribution.

Annex 3

Assessment of Viability and Stability of Samples

Assessment of viability and *in vitro* Excystation of stock

Determination of *Cryptosporidium parvum* oocyst viability and *in vitro* excystation is performed prior to each distribution. Oocysts supplied by Moredun Animal Health (MAH) are used and the batch number recorded. If the percentage viability and excystation is above 75% the oocyst stock can be used to prepare the matrices used for distribution in the scheme.

Both maximised excystation *in vitro* and inclusion / exclusion of vital dyes are previous SPDL developments funded by the UK Department of the Environment^{4,5}.

Stability and Trip controls

In order to generate meaningful results, it is necessary to determine whether the matrices supplied by SPDL exert a detrimental effect on the number of oocysts present. Three matrices are supplied for each distribution of *crypts* namely, test slides, suspensions, and Genera filters all with or without oocysts.

Currently, no matrix is reused, and stability monitoring is performed on Trip controls.

Trip controls consist of test materials, packaged according to IATA regulations and in the same manner as participant samples, which are used to assess any changes / deterioration attributable to the act of dispatching test materials to participating laboratories.

Trip controls are dispatched to LGC using the standard dispatching system, where they are stored for 1 – 3 days at room temperature, and then sent back to SPDL for analysis. The despatch and storage time at LGC corresponds to at least double the time period that participating laboratories are expected to examine the matrices despatched to them. Each Trip control contains 2 test slides, one Genera Filta-Max™ filter and ten suspensions. Analysis of the trip control test matrices following their return from LGC is undertaken at SPDL.