CRYPTS

Cryptosporidium Proficiency Testing Scheme

Scheme Description

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Record of issue status and modifications

ISSUE	ISSUE DATE	DETAILS	AUTHORISED BY
1	April 2019	First issue (based on previous CRYPTS Scheme Protocol issue 9)	T.Noblett
2	Dec 2019	Removed ‘Standards’ from page 1	A McCarthy
3	Sep 2020	Removed Fax number	A McCarthy
4	July 2021	Updated email address and UKAS logo	A Collins
5	Oct 2022	Added Giardia, change need for slides to be returned for countback and updated format for Portal	T.Noblett

Notes:
Where this document has been translated, the English version shall remain the definitive version.

Scheme Aims and Organisation

The primary aim of the CRYPTS scheme is to promote quality in the measurement and enumeration of *Cryptosporidium* oocysts in treated water supplies to satisfy current drinking water legislation. CRYPTS also enables laboratories to monitor their performance and compare it with that of their peers and provides information to participants on technical issues and methodologies relating to these analyses.

The CRYPTS scheme year operates from January to December. Further information about CRYPTS, including test material availability, round despatch dates and reporting deadlines, are available on the current CRYPTS application form and on the website www.lgcstandards.com.

The scheme is carried out in collaboration with Moredun Scientific. Customer service, sales and marketing, administration, data processing and reporting is carried out by LGC. Sample preparation, dispatch, quality control and counting of slides is carried out by Moredun Scientific

The operation of the scheme is supported by a Steering Board comprising representatives from LGC, Moredun Scientific, scheme participants, and experts in the field of *Cryptosporidium* analysis.

Scheme Framework

Test Materials

Details of test materials available are given in Appendix A. It is intended that the test materials used in the scheme reflect the types of materials generally encountered by participant laboratories. The materials may be blank or may contain *Cryptosporidium* oocysts (active or inactive) of various species including *parvum, baileyi, hominis* and *muris*. Typical interferents or oocyst-like bodies may also be present of various size and antibody reactivity.

For quality control purposes, slide samples are individually counted at Moredun prior to distribution to participants, and are not tested for homogeneity. Suspension samples are prepared using flow cytometry (at Scottish Water) and for quality control purposes, a number of sub-samples are enumerated on membrane filters to validate the batch against the target value and for homogeneity. The same suspension samples are used to spike the filters

Some aspects of PT schemes, such as test material production, homogeneity testing and stability assessment, can from time to time be subcontracted. When subcontracting occurs, it is placed with a competent subcontractor and LGC is responsible for this work. The planning of the scheme, the evaluation of performance and the authorisation of the final report will never be subcontracted.

Test Material Analysis

Participants are only required to count the number of *Cryptosporidium* oocysts in samples with a size range of 4-6um (as described in Paragraph 1.3. of the Standard Operating Protocol for the monitoring of Cryptosporidium oocysts in treated water

supplies). However, the current advice from DWI is that analysing laboratories should report the presence of all oocysts confirmed as *Cryptosporidium* spp, irrespective of size, as well as details of any Cryptosporidium like bodies present. Participants should therefore separately report the presence and number of cells according to the following definitions; Crypto 4-6um, Other Crypto, Crypto-like bodies (CLB's). Giardia may also be present in the sample and should be counted and reported as such.

For example, a slide containing 4 *Cryptosporidium parvum*, 5 *Cryptosporidium muris* and an excysted oocyst would be reported as shown below;

Crypto 4 6um = 4
Other Crypto = 5
Crypto-like bodies = 1
Giardia = 0

Methods
Participants should use the method that is in routine use within their laboratories. It is expected that participants use either of the methods specified below;

- The method specified in the Standard operating protocol for the monitoring of Cryptosporidium oocysts in treated water supplies to satisfy The Water Supply (water Quality) Regulations 2000 England and the Water Supply (Water Quality) Regulations 2001 Wales – part 2 Laboratory and Analytical Procedures
- Methods A & D from the Microbiology of Drinking Water Blue Book (2010) – Part 14

Assigned values and Performance Assessment

Microscope slides
Slides are given a unique identification number and *Cryptosporidium* oocysts on each slide are enumerated by a trained microbiologist at Moredun Scientific prior to each distribution (referred to as the initial count). Following despatch and enumeration by participants, the participant count is compared to the Moredun Scientific initial counts. Participants are assessed on the different between their count and the Moredun count. In the event of disagreement, participants are asked to retain their slides in the original packaging so they can be returned to Moredun if a further count is required.

For performance assessment, a difference of greater than 3 between the analyst count and the assigned value will be considered as significant. However, consideration will be given as to whether a smaller acceptable difference would be appropriate for slides with a particularly low oocyst count, or vice versa. Analyst performance should be monitored over time in order to assess whether an analyst is consistently over or under-counting, which may be indicative of a performance issue.

Suspension and Filter Samples
The assigned value for suspension and filter samples is based on the number of oocysts present in the suspensions (used to seed the filter samples) prepared by

flow cytometry, which is formulated to give a target concentration. Validation of suspensions consists of enumerating a representative portion of the samples prepared. For suspension samples, the mean of the replicate enumeration counts is then taken as the assigned value. For filter samples, the mean of the replicate enumeration counts multiplied by the number of suspensions used for seeding each filter is then taken as the assigned value.
For suspension and filter samples, the number of oocysts reported by the participant is indicated as the percentage recovery of the assigned value. A simple statistical analysis of the performance of all participants is provided, including mean, median, upper and lower quartiles and standard deviation.

For performance assessment, the participant results can be compared with the median and quartile figures for each round. An ongoing assessment can then be made as to whether results are consistently outside the lower or upper quartiles, or whether there is a consistent trend of results on one side of the median.

It is advisable that a laboratory sets a lower recovery limit based upon appraisal of their performance. This should be reviewed regularly and may be increased as appropriate to reflect any improving performance. Failure to recover any oocysts from a positive sample should always result in a full investigation.

Results and Reports
CRYPTS results are returned through our electronic reporting software, PORTAL, full instructions for which are provided by email. Relevant comments submitted by participants and Moredun Scientific will be included in the report.

Results are not edited by LGC, and LGC reserve the right not to include results that are not submitted in the correct format or received after the reporting deadline. Results will also not be included if collusion between participants is apparent.

Each round, participants are provided with an individual report detailing their own results for slides, suspensions and filters, and also a summary report displaying results and graphs for all participants in the scheme, as well as general comments and conclusions. In order to maintain confidentiality, individual analysts are referred to by ‘counter’ number.

Members of the Steering Board are provided with copies of each round report containing summary results for slides, filters and suspensions, in this report laboratories are identified by a laboratory number.

All reports are provided in electronic (pdf) versions and are normally emailed within 12 working days of the results deadline.

APPENDIX A - Description of abbreviations used

Assigned Value (AV)
The value to be used as the ‘true’ value in the statistical treatment of results. It is a practical estimate of the true value of the analyte in the matrix.The assigned value may be derived in the following ways:

- From the robust mean (RMean). This is the median of participant results after the removal of test results that are inappropriate for statistical evaluation, e.g. miscalculations, transpositions and other gross errors. Generally, the assigned value will be set using results from all methods, unless the measurement is considered method-dependant, in which case the assigned value will be set by method as illustrated in the report tables.
  For some analytes, where there is a recognised reference method for that type of measurement, this may be used as the assigned value for a particular analyte i.e. it would be applied to results obtained by any method.

  Traceability: Assigned values which are derived from the participant results, or a sub-set of the results are not traceable to an international measurement standard. The uncertainty of assigned values derived in this way is estimated from the participant results, according to ISO 13528.

- From a formulation value (Formulation). This denotes the use of an assigned value derived from sample preparation details, where known and exact quantities of analyte have been used to prepare the sample.

  Traceability: Assigned values calculated from the formulation of the test sample are traceable, via an unbroken metrological traceability chain, to an international measurement standard. The measurement uncertainty of the assigned value is calculated using the contributions from each calibration in the traceability chain.

- From a qualitative formulation (Qual Form). This applies to qualitative tests where the assigned value is simply based on the presence/absence of the analyte in the test material.

  Traceability: Assigned values calculated from the qualitative formulation of the test sample are traceable to a certified reference standard or a microbiological reference strain.

- From expert labs (Expert). The assigned value for the analyte is provided by an ‘expert’ laboratory.

  Traceability: Assigned values provided by an ‘expert’ laboratory may be traceable to an international measurement standard, according to the laboratory and the method used. The uncertainty of measurement for an assigned value produced in this way will be provided by the laboratory undertaking the analysis. Details of traceability and the associated uncertainty will be provided in the report for the scheme/round.

Range
This indicates the concentration range at which the analyte may be present in the test material.

SDPA
The SDPA represents the ‘standard deviation for proficiency assessment’ which is used to assess participant performance for the measurement of each analyte. This may be a fixed value (as stated), a percentage (%) of the assigned value or based on the robust standard deviation of the participant measurement results, either across all methods or by method depending on whether the measurement made is method dependent (see assigned value).

Units
This indicates the units used for the assessment of data and in which participants should report their results. For some analytes in some schemes participants may have a choice of which units to report their results, however, the units stipulated in this scheme description are the default units to which any results reported using allowable alternative results will be converted to.

DP
This indicates the number of decimal places to which participants should report their measurement results.

APPENDIX A

Samples PT-CY-01 & 02 **Microscope slides**
Supplied as: **Dynal or Genera slides**

Analyte	Method	AV	Range (cells)	SDPA	Reporting units	DP
Enumeration of *Cryptosporidium* oocysts (4-6um)	Microscopy	Expert	0 to 100	NA	Number/slide	0
Enumeration of other Cryptosporidium	Microscopy	Expert	0 to 100	NA	Number/slide	0
Enumeration of Crypto-like bodies (CLB's)	Microscopy	Expert	0 to 100	NA	Number/slide	0
Enumeration of Giardia	Microscopy	Expert	0 to 100	NA	Number/slide	0

Sample PT-CY-03 **Suspension**
Supplied as: **Liquid suspension (volume between 1 to 2ml)**

Analyte	Method	AV	Range (cells)	SDPA	Reporting units	DP
Enumeration of *Cryptosporidium* oocysts	ALL	Formulation	0 to 200	NA	Number/suspension	0

Note: Suspensions are intended for use to test the IMS procedure and subsequent steps only. They are not intended to use as spikes for filter units.

Samples PT-CY-04 & 05 **Filters**
Supplied as: **Filta Max (04) or Filta Max Xpress (05) filter units**

Analyte	Method	AV	Range (cells)	SDPA	Reporting units	DP
Enumeration of *Cryptosporidium* oocysts	ALL	Formulation	0 to 200	NA	Number/filter	0