

**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 4 - Requirements for the Inter-laboratory Proficiency Schemes**

**June 1999**

## CONTENTS

<b>1.</b>	<b>Introduction to the Standard Operating Protocol</b>	<b>1</b>
<b>2.</b>	<b>Approved schemes</b>	<b>2</b>
<b>3.</b>	<b>Organisation of schemes</b>	<b>3</b>
3.1	General organisation	3
3.2	Steering Board	3
3.3	Scheme Organiser responsibilities	4
<b>4.</b>	<b>Operation</b>	<b>5</b>
4.1	Outline	5
4.2	Frequency and timing	5
4.3	Test Material	5
4.4	Homogeneity and Stability	6
4.5	Methods of Analysis	6
4.6	Testing by participants	6
4.7	Transmission of results	6
4.8	Establishing the Assigned Value	6
4.9	Assessment of performance	7
4.10	Reporting of performance	7
4.11	Notification	7
4.12	Confidentiality	8
<b>5.</b>	<b>References</b>	<b>9</b>
	<b>Annex 1: Definitions and Terminology</b>	<b>10</b>
	<b>Annex 2: Assessing a Test Material for Homogeneity</b>	<b>12</b>
	<b>Annex 3: Stability and Viability Monitoring for Slides and Suspensions</b>	<b>13</b>
	<b>Annex 4: Statistical Analysis</b>	<b>15</b>

## **1. INTRODUCTION TO THE STANDARD OPERATING PROTOCOL**

- 1.1 This Standard Operating Protocol (SOP) provides guidance from the Drinking Water Inspectorate (DWI) by the Secretary of State on the sampling and analysis requirements associated with the Water Supply (Water Quality) (Amendment) Regulations 1999. (The Regulations)
- 1.2 This Standard Operating Protocol is published in four parts:
  - Part 1 Sampling and Transportation of Samples.
  - Part 2 Laboratory and Analytical Procedures.
  - Part 3 Validation of New methods or Parts of Methods for Sampling and Analysis.
  - Part 4 Requirements for the Inter-laboratory Proficiency Schemes.
- 1.3 Wherever the terms *Cryptosporidium* or *Cryptosporidium* oocysts, or oocysts are used in this Standard Operating Protocol, it refers to all species (active or inactive) of this organism within the size range 4-6µm, *Cryptosporidium* spp.
- 1.4 This part of the protocol provides guidance on the requirement for an interlaboratory proficiency scheme. It provides details of the organisation and the requirements of a scheme to be approved.

## 2. APPROVED SCHEMES

- 2.1 Laboratories must participate in an approved scheme for the external assessment of *Cryptosporidium* monitoring performance. Satisfactory performance in an approved scheme is not a precondition to being an approved laboratory. A laboratory may be granted approval in parallel to participation in a scheme.
- 2.2 DWI will consider schemes for approval as compliant with this Standard Operating Protocol following applications from Scheme Organisers. Such applications must be accompanied by full details of the Scheme Organiser, any subcontractors employed in the operation of the scheme, the methods of preparing and distributing samples, methods of collecting and analysing results including software used, proposed format and method of reporting to participants, and any other pertinent details which may be requested by DWI. (Note: these details will normally be embodied in a Scheme Protocol specific to the particular scheme).
- 2.3 The number of schemes in operation will be at the discretion of the DWI.
- 2.4 Approved laboratories, water companies or subsidiary companies engaged in the determination of *Cryptosporidium* in accordance with the Water Supply (Water Quality) (Amendment) Regulations 1999, are not eligible to act as Scheme Organisers. A laboratory as a scheme organiser may take part in validation trials in accordance with Part 3 of this protocol.
- 2.5 DWI will reserve the right to inspect at any time the continuing operation of an approved scheme and any results and data associated with an approved scheme . DWI will reserve the right to withdraw approval for operation of a scheme at any time.

### **3. ORGANISATION OF SCHEMES**

#### **3.1 General organisation**

- 3.1.1 The operation of a scheme will be the responsibility of a Scheme Organiser designated by, and under the direction of, DWI. A Steering Board will advise the Scheme Organiser and the DWI on technical matters pertaining to the scheme.
- 3.1.2 Schemes must operate in accordance with a Scheme Protocol prepared by the Scheme Organiser in consultation with the Scheme Steering Board. The Scheme Protocol must be consistent with the requirements of this Standard Operating Protocol (Part 4).
- 3.1.3 The Scheme Protocol must detail the arrangements of the scheme, including details of the Scheme Organiser; Steering Board, including any procedure for nomination of participant representatives; sample preparation and distribution methods; frequency of testing and timescale for reporting; result reporting methods, format and procedure; format of reports to participants; any arrangements for participant meetings and any other details required to permit consistent operation of the scheme.
- 3.1.4 The Scheme Protocol must be available to all participants in a scheme.

#### **3.2 Steering Board**

- 3.2.1 A Steering Board will be designated by the DWI. The Steering Board will normally include a representative of the Scheme Organiser, a representative of the DWI, and two representatives of participating laboratories (one nominated by the UK Water Industry and one independent), together with any additional members considered necessary by the DWI.
- 3.2.3 The Steering Board must, taking account of the aims of the scheme:
  - i) periodically assess the results obtained in the scheme
  - ii) advise the DWI on the nature and timing of proficiency testing rounds
  - iii) advise the DWI on the need for and nature of any revision of the Scheme Protocol;
  - iv) advise the DWI on the criteria for satisfactory performance
  - v) advise the Scheme Organiser on the nature of samples circulated for analysis and on the methods to be used for assigning reference values for test materials; and
  - vi) advise the DWI on action to be taken in the event of persistent poor performance by a laboratory or analyst.
- 3.2.4 The Steering Board should meet when necessary to ensure progression of the scheme. Meetings must be at least quarterly for the initial year of operation of the scheme and every six months thereafter.

### **3.3 Scheme Organiser responsibilities**

3.3.1 The Scheme Organiser is responsible for:

- i) preparation, stability testing and homogeneity testing of circulated test materials;
- ii) retention of records on source, age and viability of oocyst suspensions;
- iii) distribution of test materials and instructions to participants, including repeats for poor performers;
- iv) statistical analysis of the results and assessment of individual laboratory performance;
- v) distribution of reports of performance to the participants;
- vi) provision of summary reports for each testing round to the Steering Board and DWI;
- vii) arranging Steering Board meetings; and
- viii) determining whether there is a need for suitable feedback and helpline facilities for participants and, if there is, implementing and maintaining such facilities.

3.3.2 The Scheme Organiser must advise the Steering Board on any matters of operation affecting the interpretation of results, as well as any practical difficulties or opportunities arising.

## **4. OPERATION**

### **4.1 Outline**

4.1.1 For each round of the scheme, the Scheme Organiser will arrange for test samples to be distributed to participants in the scheme. Participants will carry out the analysis required and return the results to the Scheme Organiser. The Scheme Organiser will collate the results, perform appropriate analysis and interpretation, and notify the participants of their performance for each round.

### **4.2 Frequency and timing**

4.2.1 Test materials will be distributed monthly to participants according to a schedule issued in advance by the Scheme Organiser. The DWI shall reserve the right to reduce or increase the frequency of rounds, subject to notice. Participants will be required to return the results within the specified deadline, usually one week. The results will be subject to statistical analysis and participants will be notified of their performance within two weeks of the closing date for return of results.

### **4.3 Test Material**

4.3.1 Three test materials must be distributed for analysis in each round:

- i) a microscope slide
- ii) a filter (General Filta-Max™) for analysis; and
- iii) a suspension.

4.3.2 Microscope slides should be selected from the following:

- i) stained or unstained slides displaying only *Cryptosporidium* oocysts at a level near the regulatory limit; and
- ii) stained or unstained slides displaying typical interferents or oocyst-like bodies other than *Cryptosporidium*, with or without *Cryptosporidium* present.

4.3.3 Filters may 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 suspension after use in an uncontaminated water supply.

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

#### **4.4 Homogeneity and stability**

- 4.4.1 Test materials must be checked for homogeneity to ensure that the interpretation of laboratory results is not prejudiced by variations in oocyst concentration between test items (see Annex 2). The oocysts spikes for use in 4.3.3 and 4.3.4 must be prepared by flow cytometry. Slides prepared for 4.3.2 should be completed prior to dispatch.
- 4.4.2 The durability of the slides and the stability of the oocysts will be monitored in accordance with the procedures in Annex 3.
- 4.4.3 The viability of oocysts in suspension will be monitored in accordance with the requirements in Annex 3.

#### **4.5 Methods of Analysis**

- 4.5.1 Participants must use the method specified in Part 2 of this Standard Operating Protocol.

#### **4.6 Testing by participants**

- 4.6.1 The participating laboratory must receive a separate slide for each qualified analyst (i.e. qualified to read microscopy slides) and each analyst must provide a reading from a separate slide for at least 10 of the 12 distributed rounds per year.
- 4.6.2 Each laboratory must provide one result for each filter and suspension, with replicates as required by the Scheme Organiser.

#### **4.7 Transmission of results**

- 4.7.1 Participants must report the results for all analyses within a time specified by the Scheme Organiser. Each result must be accompanied by a report of the time and date of analysis and the name of, or unique identifier for, the analyst who carried out the oocyst count.
- 4.7.2 Participants must provide results and other data in the format and units requested by the Scheme Organiser. This may be in electronic form to reduce time, costs and transcription errors; if so, the format and software employed must be notified to DWI or its designated agent and written approval obtained for their use.

#### **4.8 Establishing the Assigned Value**

- 4.8.1 Reference values (“assigned values”) must be assigned as follows:
  - i) stained microscope slides. Since slides are pre-prepared and stable, the assigned value must be determined as the mean of ten counts by a suitably trained analyst in a reference laboratory chosen by the Scheme Organiser; and
  - ii) suspensions. Suspensions should be formulated to give a target concentration and checked. The target concentration then forms the assigned value. Where subsequent analysis by participants or otherwise

shows significant changes, consensus values (see below) may be substituted with prior agreement from the Steering Board; and

iii) filters. Consensus assignment is recommended (see 4.8.2):

4.8.2 Consensus assignment is based on the results returned in each round. Acceptable alternatives are:

- i) the mean of all results excluding statistical outliers; and
- ii) a robust mean of all results (see Annex 4)

#### **4.9 Assessment of performance**

4.9.1 Laboratories and individual analysts must be assessed on the difference between their results and the assigned values. The assessment must consider performance for single rounds and performance over time (at least four rounds).

4.9.2 Two performance indexes must be calculated for each laboratory and analyst. These indexes must show the performance of the laboratory or analyst relative to other participants in each individual round, and the performance of the laboratory or analyst relative to other participants over a period of four rounds. Annex 4 provides suitable indexes.

4.9.3 The assessment will show acceptable, marginal and unacceptable results based on criteria set by the Steering Board and approved in writing by the DWI. The criteria must take into account

- i) the observed distribution of the data within and between rounds;
- ii) the risks attendant on action or inaction at particular levels; and
- iii) the action levels set by the DWI.

A recommended approach in the first instance is given in Annex 4.

#### **4.10 Reporting of performance**

4.10.1 The Scheme Organiser must distribute a report to each participant which must include:

- i) the results reported by the participant;
- ii) the assigned values;
- iii) the relevant performance index(es); and
- iv) the performance assessment for the laboratory and for each analyst separately identified.

4.10.2 In addition, a summary report must be supplied to the DWI and to each Steering Board member.

#### **4.11 Notification**

4.11.1 The DWI shall be informed if any laboratories or analysts have unacceptable scores. Failure to submit results or to meet the specified deadline is also notifiable. Action to be taken following notification of poor performance must be decided by the DWI and notified to the participants in advance. It must

include, but may not be limited to, requiring the laboratories or analysts to measure repeat slides, filters or suspensions, outside of the normal rounds.

#### **4.12 Confidentiality**

4.12.1 Information concerning the performance of individual laboratories and individual analysts will be confidential between the individual laboratory, the Scheme Organiser and DWI. To preserve confidentiality participants will be identified by a unique identification code number. The participant's code is notified to the participant upon registration, and will remain the same unless the participant requests that it be changed, in writing to the Scheme Organiser. All results are the property of the DWI and may not be copied, distributed or published without express permission from the DWI. DWI reserves the right to publish results from the scheme at any time. However, to preserve confidentiality, any such publication will not identify laboratories or individuals by name.

## **5. REFERENCES**

1. The International Harmonised Protocol for the Proficiency Testing of (Chemical) Analytical Laboratories. M Thompson, R Wood, Journal of AOAC International, Vol 76, No 4, 1993
2. Proficiency testing by inter-laboratory comparisons. ISO/IEC, Guide 43, part 1, Development and operation of proficiency testing schemes, 1997

## **ANNEX 1: DEFINITIONS AND TERMINOLOGY**

The following definitions are taken from relevant international documents<sup>1</sup>. “Explanatory notes” are specific to Parts 3 and 4 of this Standard Operating Protocol. Additional informative notes may be found in the source documentation.

### **Accuracy**

The closeness of agreement between a test result and the accepted reference value. When applied to a set of test results, the term involves a combination of random components and a common systematic error or bias component.

### **Assigned Value**

The value to be used as the 'true' value by the Scheme Organiser in the statistical treatment of results and the best available estimate of the true value of the analyte in the matrix.

Explanatory note: “The analyte” will normally refer to the oocyst concentration.

### **Bias**

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

Explanatory note: In the present Standard Operating Protocol, the “expectation” is generally represented by the mean of a large set of test results.

### **Outliers**

Observations in a sample, so far separated in value from the remainder as to suggest that they may be from a different population, or the result of an error in measurement.

Explanatory note: Outlier testing is a statistical procedure intended to identify observations which differ significantly from other members of a sample (set of data) at a specified level of confidence.

### **Precision**

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

Explanatory note: “Prescribed conditions” may be, for example, within-batch, between-batch, reproducibility conditions or repeatability conditions.

### **Proficiency testing scheme**

Methods of checking laboratory testing performance by means of inter-laboratory tests.

Explanatory note: Proficiency testing typically includes comparison of a laboratory’s results at intervals with those of other laboratories, the main objective being to assess the comparability of laboratory results.

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<sup>1</sup> Unless otherwise stated, definitions are quoted from the International Harmonised Protocol for the Proficiency Testing of (Chemical) Analytical Laboratories, M Thompson, R Wood, Journal of AOAC International, Vol 76, No 4, 1993, and conform to ISO standards current at the time of publication.

**Standard deviation**

The square root of the variance. [ISO 3534-1:1993]

**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.

**True Value**

The actual concentration of the analyte in the matrix.

Explanatory note: "Analyte" refers to oocyst concentration. "Matrix" refers to the solution or other substrate in which the oocysts are presented for analysis.

**Trueness**

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

Explanatory note: Trueness is usually expressed in terms of bias.

**Variance**

A measure of dispersion which is the sum of the squared deviations of observations from their average divided by one less than the number of observations. [ISO 3534-1:1993]

Explanatory note: For  $n$  observations  $x_1, x_2, \dots, x_n$ , the variance  $s^2$  is given by

$$s^2 = \frac{1}{n-1} \sum_n (x_i - \bar{x})^2$$

## ANNEX 2: ASSESSING A TEST MATERIAL FOR HOMOGENEITY

### 1 PROCEDURE

1.1 The procedure for homogeneity testing will be as follows:

- a) prepare the test materials in a clean, stable and safe environment by appropriate methods;
- b) place the materials into the containers that will be used for dispatch to the participants;
- c) for each test material select  $3 \times \sqrt[3]{N}$  (or ten, whichever is greater) of the containers strictly at random, where N is the total number of containers;
- d) for solutions or suspensions:
  - i) homogenise separately the contents of each of the selected containers and from each take two test portions; and
  - ii) analyse the test portions in a random order under repeatability conditions as a single analytical series, by an appropriate method. The analytical method must be sufficiently precise to allow a satisfactory estimation of the sampling standard deviation ( $S_s$ );
- e) for microscope slides, determine the oocyst count twice for the set of slides, in random order each time;
- f) form estimates of the between-group variance  $S_s^2$  (“sampling variance”) and the within-group variance  $S_a^2$  (“analytical variance”) by one-way analysis of variance (ANOVA), without exclusion of outliers; and
- g) report values of mean  $\bar{x}$ , median  $\tilde{x}$ , standard deviations  $S_s$ ,  $S_a$ , number of test portions analysed  $3 \times \sqrt[3]{N}$  and the result of the ANOVA F-test, at the 95% confidence level.

### 2 INTERPRETATION

2.1 If  $\sigma$  is the target value for the standard deviation for the proficiency test, the value of  $S_s/\sigma$  should be less than 0.3 for sufficient homogeneity. If the calculated estimate of  $S_s^2$  is negative, then  $S_a$  should be used instead of  $S_s$ .

2.2 Where  $S_s/\sigma$  exceeds 0.3, due allowance must be made in forming an assessment of laboratory performance. For example, if a z-score is used, the target standard deviation  $\sigma$  should be replaced by  $\sigma' = \sqrt{\sigma^2 + S_s^2}$

If the allowance increases the limits of acceptable performance by >30%, the test material is not sufficiently homogenous and must not be distributed.

## **ANNEX 3: STABILITY AND VIABILITY MONITORING FOR SLIDES AND SUSPENSIONS**

### **1. VIABILITY**

- 1.1 Recovery of oocysts in seeded trials is dependent on the age and viability of control suspensions. It is accordingly important that data is available on the age and viability of suspensions used in proficiency testing.
- 1.2 Oocyst suspensions must be assessed for viability prior to each testing round. Viability checks must include excystation and dye exclusion. Results must be retained by the Scheme Organiser and made available to the Steering Board as required.

### **2. STABILITY MONITORING FOR EACH ROUND**

#### **2.1 Slides**

- 2.1.1 Immediately prior to each round, the Scheme Organiser must select ten or  $3 \times \sqrt[3]{N}$  (whichever is less) of the slides strictly at random, where N is the total number of slides circulated. The selected slides must be given a unique identifier, and examined by a competent analyst before circulation, using the methods prescribed in Part 2 of this Standard Operating Protocol. Participants will be required to return these slides following analysis. The same slides must be re-analysed by the same competent analyst within two working days of their return, and the differences between counts before and after circulation recorded. The mean and standard deviation of the differences found must be recorded and reported to the Steering Board.
- 2.1.2 Where a significant difference is observed, the Scheme Organiser must take the magnitude of the change into account when reporting participant performance.
- 2.1.3 The Scheme Organiser must also note any laboratory for which appreciable degradation has occurred and inform the laboratory concerned.

#### **2.2 Suspensions**

- 2.2.1 For each round, five portions of the reference suspension must be transported to a laboratory chosen by the Scheme Organiser at the same time as the test materials are circulated, retained under the specified storage conditions, and analysed using the methods prescribed in Part 2 of this Standard Operating Protocol or a method approved by the DWI for the purpose of stability checking. This analysis must be on or as close as practically possible to the closing date for return of samples. The results must be transmitted to the Scheme Organiser

- 2.2.2 The mean and standard deviation of the values found and the difference between the mean value observed and the assigned value must be recorded and reported to the Steering Board.
- 2.2.3 Where a significant difference is observed, the Scheme Organiser must take the magnitude of the change into account when reporting participant performance.

### **3. SLIDE AND SUSPENSION STABILITY OVER TIME**

#### **3.1 Preliminary investigations**

- 3.1.1 The Scheme Organiser must conduct stability trials on prepared slides and suspensions to establish the stability of each under the conditions of the scheme. Such trials must consist of a sequence of at least four, equally spaced duplicate determinations in the same laboratory on each of three slides or suspensions over a time not less than twice the expected usable life of each material. The results must be tested for evidence of significant instability.

#### **3.2 Long term monitoring**

- 3.2.1 Where the same test items are to be circulated over several rounds in a scheme, each must be allocated a unique identification and the value checked by a laboratory designated by the Scheme Organiser at intervals not longer than half the nominal lifetime as confirmed by the long term stability check.
- 3.2.2 Suspensions will not be re-used following a circulation to participants.

## ANNEX 4: STATISTICAL ANALYSIS

### 1 ASSIGNING VALUES

#### 1.1 Robust Mean

1.1.1 It is recommended that robust statistical methods are used in the statistical evaluation of the results returned by participants in the scheme. Robust statistics cope effectively with many outliers in a data set, without resorting to their removal from the data set.

1.1.2 The recommended robust mean used is simply the median, here denoted by  $\bar{x}$ . If the data is arranged in order of magnitude, there are equal numbers of observations smaller and greater than the median. For a symmetrical normal distribution the mean and median have the same value. The median is more robust and is virtually unaffected by extreme values. The median of  $n$  observations is found as follows:

$$n \text{ even } (2, 4, 6\dots): \bar{x} = \frac{(x_{\text{int}(n/2)} + x_{\text{int}(n/2)+1})}{2}; \quad n \text{ odd } (1, 3, 5\dots): \bar{x} = \frac{(x_{\text{int}(n/2)+1})}{2}$$

where  $\text{int}(n/2)$  denotes the closest integer equal to or less than  $n/2$ .

#### 1.2 Robust estimate of Standard Deviation

1.2.1 The recommended robust estimate of the standard deviation is the  $\text{MAD}_E$  value, calculated as follows.

i) Calculate the Median Absolute Distance (MAD) from the sample median.

$$\text{MAD} = \text{median}(|x_i - \bar{x}|_{i=1, 2, \dots, n})$$

ii) Calculate  $\text{MAD}_E$  from

$$\text{MAD}_E = 1.483 \text{ MAD}$$

#### Example

Data	5 · 6	5 · 4	5 · 5	5 · 4	5 · 6	5 · 3	5 · 2
Ordered Data	5 · 2	5 · 3	5 · 4	5 · 4	5 · 5	5 · 6	5 · 6

$$\text{Median } \bar{x} = \frac{x_{(7+1)}}{2} \Rightarrow \bar{x} = x_4 \Rightarrow \bar{x} = 5 \cdot 4$$

$ x_i - \bar{x} $	0.2	0.1	0.0	0.0	0.1	0.2	0.2
Ordered difference	0.0	0.0	0.1	0.1	0.2	0.2	0.2

Median absolute difference (MAD) = 0.1

$$\text{MAD}_E = 1.483 \times 0.1 = 0.1483$$

In the example, which has no gross outliers, the robust estimates are close to the sample mean of 5.429 and the standard deviation of 0.149.

## 2 PERFORMANCE INDEXES

### 2.1 Calculation of Z Scores

2.1.1 Z scores provide an indication of performance relative to a target value and target standard deviation. The Z score for an individual result,  $x_i$  is calculated from the equation:-

$$\text{Z score} = \frac{x_i - \text{assigned value}}{\text{established standard deviation}}$$

2.1.2 The assigned value and established standard deviation are set by the Scheme Organiser in consultation with the Steering Board; section 4.8 and 4.9 of Part 4: requirements for inter-laboratory proficiency schemes refers.

### 2.2 Calculation of RSZ scores

$$\text{RSZ score} = \frac{\sum_{i=1}^n \mathbf{Z}_i}{\sqrt{n}}$$

2.2.1 The Rescaled Sum of Z scores provides an indication of performance over time. The RSZ score for an individual laboratory will be calculated from the results of the last n rounds reported (n is typically 4 rounds). Rescaling by  $\sqrt{n}$  converts the Sum of Z scores to the same scale as that associated with Z scores.

2.2.2 The RSZ value provides an indication of persistent bias in analytical systems; for example, a low RSZ score indicates a consistent negative bias.

### 3. INTERPRETATION

#### 3.1 Criteria

3.1.1 Criteria for acceptability should be set by the scheme Steering Board and notified to the participants in advance. The criteria must identify results that are:

- a) unacceptably high; or
- b) high acceptable; or
- c) acceptable;
- d) low acceptable; or
- e) unacceptably low.

#### 3.2 Z- and RSZ-scores

3.2.1 Z- and RSZ-scores for normally distributed data lead to the following probabilities and interpretation when the risks attendant on erroneously high and low results are comparable.

<b>Score</b>	<b>Probability (%)</b>	<b>Typical Interpretation</b>
$Z \text{ or } RSZ > 3$	0.14	Unacceptably high
$2 < Z \text{ or } RSZ < 3$	2.16	High
$-2 < Z \text{ or } RSZ < 2$	95.4	Acceptable
$-3 < Z \text{ or } RSZ < -2$	2.16	Low
$Z \text{ or } RSZ < -3$	0.14	Unacceptably low