ENVIRONMENT AGENCY

| PROCEDURE FOR QUALITY ASSURANCE FOR RIVPACS COMPATIBLE MACRO-INVERTEBRATE SAMPLES ANALYSED TO THE TAXONOMIC LEVEL NEEDED FOR THE BMWP-SCORE SYSTEM | | BT 003 | 77 Pages |
|---|---|---|---|
| Type: PROCEDURE | Issue No: 1.0 | Issue Date: 3 August 1999 | Copy No: |



## CONTENTS:

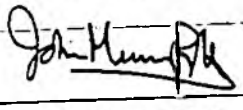
See page iii.

## DOCUMENT HISTORY:

| Issue date: | Issue No: | Summary of changes |
|---|---|---|
| 17 May 1996 | 0.1, Draft, issued to laboratories | |
| 14 June 1996 | 0.2, Draft (interim) | Definitions for parameters for AQC; Section 3.10, many smaller alterations |
| 21 October 1996 | 0.3, Draft, issued to laboratories | Revised AQC procedure: audit samples to be despatched in batches of 5, revised Table 3.1 and Appendix A, procedure for combining confidence limits added (Section 3.2.5) |
| 6 January 1998 | 0.4 ,Draft | Sections 3.3.5 and 3.3.6 split; Section 3.2.5 rewritten |
| 3 August 1999 | 1.0, First issue | As 0.4, with minor corrections, audit based on samples analysed to end of February |

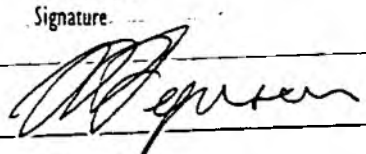
## ISSUE AUTHORITY:

Author's Signature



**John Murray-Bligh**
Ecologist
Thames Region

Authorization Signature



**Alastair Ferguson**
Manager, Environmental Monitoring
Head Office

Environment Agency
Rio House
Waterside Drive Aztec West
Almondsbury
BRISTOL BS12 4UD
Tel: 01454 62440
Fax: 01454 624409

ISBN 1 85705 195 5



**Dissemination Status**
Internal: Released to Regions
External: Released to public domain

**Statement of use**
The purpose of this document, which will be added to and updated, is to provide standardised procedures for the quality assurance of macro-invertebrate samples collected by procedures compatible with RIVPACS and analysed to the level needed for the BMWP-score system. This includes samples analysed for GQA.

This document was written for use within the Environment Agency by its own staff. The Environment Agency disclaims any responsibility for loss, damage or injury arising from the information contained in this document to anyone other than its own staff or its contractors whilst the are engaged in working for the Agency.

## CONTENTS

Page

LIST OF FIGURES ..... v

LIST OF TABLES ..... v

1 INTRODUCTION ..... 1.1
1.1 Purpose and scope ..... 1.1
1.2 Terms used in this document ..... 1.2
1.3 Using this document ..... 1.3

2 LITERATURE ..... 2.1
2.1 Internal Environment Agency sources ..... 2.1
2.2 External sources ..... 2.1

3 PROCEDURE ..... 3.1
3.1 Staff ..... 3.1
3.2 Common procedures for AQC and audit of sample analysis ..... 3.2
3.2.1 Modifications to primary sorting for AQC and auditing ..... 3.2
3.2.2 Storing samples ..... 3.2
3.2.3 Measures of quality ..... 3.3
3.2.4 The target quality ..... 3.4
3.2.5 Determining analytical quality ..... 3.4
3.2.6 Selecting samples for AQC or audit ..... 3.8
3.2.7 Likely causes of errors ..... 3.12
3.3 The AQC for sample analysis ..... 3.12
3.3.1 Staff covered by the AQC scheme ..... 3.13
3.3.2 Anonymity ..... 3.13
3.3.3 Quality of AQC inspection ..... 3.13
3.3.4 AQC inspection ..... 3.14
3.3.5 Running the AQC procedure: setting the AQC parameters ..... 3.15
3.3.6 Further information about the AQC procedure ..... 3.16
3.3.7 Running the AQC procedure: determining the state of the analysis ..... 3.18
3.3.8 Corrective action ..... 3.21
3.3.9 New or inexperienced analysts ..... 3.23
3.3.10 Analysing samples from a different Area or Region ..... 3.25
3.3.11 Maintaining the record of net gains ..... 3.26
3.4 Audit of sample analysis ..... 3.27
3.4.1 Analyst's identity ..... 3.28
3.4.2 The audit data sheet ..... 3.28
3.4.3 Despatching samples to the auditors ..... 3.30

3.4.4 The analysis undertaken by the auditors ..... 3.32
3.4.5 Audit results ..... 3.32
3.4.6 Corrective action ..... 3.33
3.4.7 Auditing samples analysed by another Environment Agency laboratory ..... 3.34
3.4.8 Auditing samples analysed by contractors ..... 3.35
3.5 Quality assurance for sample analysis to species ..... 3.37
3.6 Quality assurance for sample collection ..... 3.37
3.7 Quality assurance for sample traceability ..... 3.38
3.8 Quality assurance for data archiving and analysis ..... 3.38
3.9 Quality assurance of samples collected or analysed by other Environment Agency laboratories ..... 3.38
3.10 Quality assurance of samples collected or analysed by contractors ..... 3.39

4 EQUIPMENT SPECIFICATIONS ..... 4.1
4.1 Recording forms for the AQC and audit procedures ..... 4.1

5 GLOSSARY ..... 5.1

6 REFERENCES ..... 6.1

APPENDIX A CONFIDENCE LIMITS FOR THE MEAN OF A POISSON DISTRIBUTION ..... A.1

APPENDIX B SUMMARY OF QUALITY CONTROLLER'S DUTIES ..... B.1

APPENDIX C CHANGES TO THIS DOCUMENT ..... C.1

## LIST OF FIGURES

Page

3.1 Example of re-allocated AQC batches after scrapping and re-working primary data following an alarm ..... 3.11
3.2 Example of a completed cusum record form ..... 3.19
3.3 Recording misidentifications on audit data sheets ..... 3.29
3.4 Recording sorters' codes on audit data sheets ..... 3.30
3.5 Transport Emergency Card (TREM Card) for samples in fixative carried by road ..... 3.31
4.1 AQC sample record sheet ..... 4.2
4.2 AQC cusum record form ..... 4.3
4.3 Net gains record sheet ..... 4.4
4.4 Audit data sheet ..... 4.5

## LIST OF TABLES

Page

3.1 Values of R and D to be used for different values of $AQL_A$ ..... 3.16
3.2 Average run lengths for given values of $AQL_A$ ..... 3.17

# 1 INTRODUCTION

## 1.1 Purpose and scope

This document describes the quality assurance procedures for all macro-invertebrate samples collected in accordance with the standard methods for RIVPACS (See BT001 in this series) and analysed to the level required by the BMWP-score system. This includes samples for which the principal analysis will be to BMWP level but where some taxa have been identified further, either particular groups, or taxa whose further identity is readily apparent. This includes samples collected for General Quality Assessment (GQA, see BT002), as well as samples collected for other strategic and operational purposes. It does not encompass samples in which all or most of the taxa that can be identified to species have been, i.e. samples analysed to species (or mixed taxonomic level which does not always include difficult groups such as Chironomidae, Oligochaeta and Sphaeriidae). All staff must follow this procedure when analysing appropriate samples. This includes staff within the Environment Agency. Contractors working for the Agency may have their own analytical quality control (AQC) procedures instead of those described here, however their samples should be audited according to these procedures, so they will still need to follow the procedures in Section 3.2.1 and 3.2.2.

These procedures may be supplemented by additional procedures at the discretion of the individual laboratory or Region, so long as they do not interfere with these standard procedures.

The aim of quality assurance is to minimise and quantify errors. Full quality control and auditing would involve independent sampling, sorting and analysis. This is impractical because it would be too expensive and time-consuming. Instead, the formal systems are limited to laboratory analyses. Only brief guidance is included here on quality assurance for other sources of error, because they are covered more fully in BT001 and BT002.

Two parameters are used to measure quality: the number of gains (taxa that were present in the sample but are not recorded as being present) and number of losses (taxa that are recorded as being present but which were not present in the sample). Throughout the Environment Agency, the quality standard for the laboratory analysis of samples used to assess biological quality using the BMWP-score system is an average of no more than two gains (losses do not form part of this standard).

The audit provides an independent measure of the quality of the analysis of samples, and hence the quality of the data (the primary audit) and of the quality of AQC inspections (the AQC-audit). In the primary audit, the auditors results are compared with the primary data: in the AQC-audit they are compared with the AQC inspector's analyses. The audit is based on the re-analysis of a fixed number of samples collected in each calendar year. This provides estimates of the annual averages of the two parameters with an acceptable

degree of precision; however, these estimates will not be available until the end of the year, when the audit has been completed.

The analytical quality control (AQC) procedure provides each laboratory with a mechanism for ensuring that its data meets the required standard. It is a continuous process, providing rapid feedback when quality changes. It is based on a re-analysis, within the laboratory, of a percentage of the samples that it has analysed. The same parameters are measured as in the audit.

The standard AQC procedure should be applied to experienced staff analysing samples from their own Area. Inexperienced staff, staff new to the Area, or staff analysing samples from another Area will require more intensive checking, for which guidance is provided in this document. If the procedures have to be modified to suit local conditions, it is essential that the principles of the scheme and the anonymity of the AQC samples are maintained as far as possible.

The standard AQC procedure was introduced to all Environment Agency (then National Rivers Authority) laboratories in 1995 for the National GQA river quality survey. The audit was introduced in 1990 for the National River Quality Survey, and has continued in substantially the same form since then, although some modifications were introduced in 1995 to take account of the AQC procedure. Similar audits are undertaken by the Scottish Environment Protection Agency, The Department of the Environment in Northern Ireland, and the Government Laboratory in the Isle of Man. Both the AQC and audit procedures were refined further in 1996.

Much of the description of the AQC procedure in this document is based on van Dijk (1994b), modified in the light of subsequent assistance from Julian Ellis and Peter van Dijk of WRc. Considerable assistance was given by the Institute of Freshwater Ecology (IFE), in particular from Rick Gunn on the procedures for auditing. Bob Dines has steered and assisted with all aspects of the work from its inception. The work has also benefited from the experience of biologists working in and for the Agency.

## 1.2 Terms used in this document

Four degrees of prescription are recognised in this document:

suggestions, indicated by the phrase *may be* or *can be*;
recommendations, indicated by the phrase *is recommended*;
mandatory when possible, indicated by the phrase *should be*;
mandatory under all circumstances, indicated by the phrase *must be*.

All technical terms are defined in the glossary at the end of this document. Some of the more widely used terms are described below.

Samples collected from the environment to provide information about it are called *primary samples* (cf. *AQC sample* and *audit sample*). The person analysing the primary sample is called the *primary analyst*, their analysis is called the *primary analysis*, and the data which they obtain from it is the *primary data*. Samples selected from the primary samples to provide information about the quality of the primary analysis for AQC are called *AQC samples*. The person analysing the AQC samples is called the *AQC inspector* and their analysis is called the *AQC inspection*. Samples selected from the AQC samples (or if necessary, from primary samples) to obtain information about the AQC inspection or the primary analysis for the audit are called *audit samples*. The person analysing the audit samples is called the *auditor*, and their analysis is called the *audit*. The audit of the primary analysis is called the *primary audit*, and the audit of the AQC inspection is called the *AQC-audit*.

Throughout this document, others in the same series have been referred to by their series number: BT001 = Environment Agency 1999 and BT002 = Environment Agency 1996c. References to Sections in BT001 relate to Version 2 of that document.

## 1.3 Using this document

Considerable progress has been made in the development and refinement of biological monitoring techniques over the last five years. The adoption of these standard methods is not intended to stifle this development; it should facilitate it. This document will inevitably need to be revised from time to time, and has been designed with this in mind. Please let the author know if you have any ideas for improving either the procedures or this document.

# 2 LITERATURE

When using these procedures, you will also need to consult the following documents.

## 2.1 Internal Environment Agency sources

**Quality management systems for environmental monitoring: biological techniques, BT001**. Procedure for collecting and analysing macro-invertebrate samples. (Note that references in the text relate to Version 2 of BT001)

**Quality management systems for environmental monitoring: biological techniques, BT002**. Procedure for collecting and analysing river macro-invertebrate samples for GQA surveys.

## 2.2 External sources

None.

# 3 PROCEDURE

## 3.1 Staff

Staff analysing samples of the type encompassed by this document must be trained in the procedures for analysing samples described in BT001 and the additional procedures required for the AQC and audit (Section 3.2.1). Staff responsible for implementing the AQC and audit (AQC inspectors and quality controllers), staff managing biological laboratories, and those responsible for implementing corrective action must have read and understood all the instructions in this document.

Whereas the AQC scheme is the responsibility of individual laboratories, the overall responsibility for the audit is with Regional staff (the Regional Biologist).

Each laboratory will have to appoint its own quality controller, to be responsible for the administration of the AQC and audit, as well as one or more of their biologists to act as AQC inspectors, to be responsible for reanalysing samples to determine the errors in the primary analysis. Regions may appoint a Regional quality controller to be responsible for the audit.

The quality controller does not need to be a member of the laboratory staff, nor do they need to be a biologist. For the AQC scheme they will be responsible for selecting the AQC samples; providing the AQC inspectors with the AQC samples, including the vial of identified specimens, and the original list of taxa in a way that maintains the anonymity of the primary analyst; calculating and, if necessary, re-setting the AQC parameters; updating the cusum record; ensuring that the laboratory manager is informed as soon as the control state changes (Section 3.3.7); ensuring that the laboratory manager and AQC inspectors are informed when an alarm is signalled; and controlling the relevant paperwork. They will also be responsible for maintaining the laboratory's record of net gains (Section 3.3.11). For the audit, they will be responsible for selecting the audit samples, preparing the audit data sheets (Section 3.4.2), and arranging for the audit samples to be sent to the auditors. See summary in Appendix B.

The AQC inspectors must be experienced in detecting and identifying all the taxa likely to be observed in the samples analysed in the laboratory. They must be capable of analysing samples to a consistently high quality, and should be selected on the basis of having good AQC and audit results.

The Regional Biologists of each Region comprise the Project Executive for the audit, for which they have overall responsibility. The National Audit Manager is responsible for managing the audit contract and coordinating the audit.

## 3.2 Common procedures for AQC and audit of sample analysis

### 3.2.1 Modifications to primary sorting for AQC and auditing

During primary analysis, examples of every taxon found in the sample should be placed in a vial. This makes it easier for the AQC inspector and auditor to check the sample, and helps to identify the cause of errors.

Primary analysts must place up to three specimens (if available) of every invertebrate family in a small vial containing alcohol preservative. This includes representatives of families that are not included in the BMWP-score system, to help determine the causes of errors, although they do not contribute to the error measurements. These specimens should be good quality examples and not simply the first specimens encountered during sorting. It is recommended that at least three specimens of each family of flatworms are included in the vial whenever possible, because they are prone to disintegration. It is also recommended that representatives of each of the aquatic life stages of each taxon present in the sample are put in the vial. Pupae must be put in the vial if larvae of the same taxon are not present in the sample. Only large pearl mussels (*Margaritifera margaritifera)*, medicinal leeches *(Hirudo medicinalis)* and crayfish (Astacidae) do not have to be supported by voucher specimens. Use more than one vial if necessary, but label them as being vial number 'x' of 'n' vials. If a specimen is too large for a vial (a large mussel, for example) place a note to this effect, in pencil on waterproof paper, in the vial so that the auditors do not record an omission (defined below).

When sorting has been completed, put the vial into a standard sample container with the rest of the sample. Any labelling on its outside must be alcohol proof, because it will be immersed in preservative.

### 3.2.2 Storing samples

It is unlikely that samples will be re-analysed for AQC and virtually impossible for them to be audited within 48 hours of collection, so they will have to be fixed and preserved. Fixative or preservative should be added to the sample in a fume cupboard, following the procedures described in Section 3.9.3 and Section 3.9.4 of BT001. Note that alcohol is an inadequate fixative and samples which have not been fixed previously with formalin should be re-analysed as soon as practicable.

Samples in formalin are harmful, and samples in industrial methylated spirit (IMS) are flammable and harmful. Every container must be labelled with the appropriate warning labels (see BT001, Section 4.2.10).

### 3.2.3 Measures of quality

Taxa found in the sample or vial by the AQC inspectors or auditors, that were not recorded by the analyst whose data is being checked, are termed **gains**.

Taxa recorded as present by the analyst whose data is being checked, that are not found in the sample or vial by the AQC inspectors or auditors, are termed **losses**. The only exception to this are records of the few taxa that do not have to be supported by voucher specimens, listed in Section 3.2.1.

Taxa recorded on the sample data sheet and found in the sample by the auditors, but which were absent in the vial are termed **omissions**. These are recorded in the audit only, to help trace the source of errors.

**Net gains** (gains minus losses) are used by RIVPACS to calculate confidence limits for EQIs and significant differences between them.

Performance is measured in terms of arithmetic means of these parameters. Confidence limits can be placed around the averages derived from the AQC or audit to take account of sampling error. This is explained in Section 3.2.5, and assumes that the measurements follow a Poisson distribution. It is strongly recommended that you always determine the confidence limits as well as the mean, as this recognises more of the information inherent in the samples.

Only BMWP-scoring taxa contribute to these measures of quality. For the Environment Agency, this excludes Chrysomelidae, Clambidae and Curculionidae. Also excluded are larval and pupal exuviae, empty caddis cases, empty mollusc shells, and specimens present only as posterior ends. For insects, a thorax plus abdomen is acceptable, but a head or abdomen alone is not. Pupae of Diptera and Trichoptera are included. See BT001 Section 3.10.4.

The performance of a laboratory, or the quality of its data, should be based on the results of the primary audit. There is no advantage in using the results of AQC inspections, because errors in AQC inspection would have to be taken into account. This information is available only from the AQC audit, the number of which will further reduce the precision of estimates based on AQC inspections. This is explained more fully in Section 3.2.5. It is envisaged that, once the quality of AQC inspection reaches a similar quality to our current auditors and is stable throughout the Agency, data derived from the AQC inspections will be used to measure laboratory performance and the data quality.

### 3.2.4 The target quality

Throughout the Environment Agency, the target quality for samples used to assess the biological quality of rivers using the BMWP-score system is an average of no more than two gains (actual not net gains), measured over the year. This target was established in 1992, following an analysis of the quality of the 1990 National River Quality Survey by Kinley and Ellis (1991), and it was reviewed in 1994 (van Dijk 1994a). Although the quality of individual samples will vary naturally around this mean target, extreme variations in quality also need to be controlled, so individual samples with five or more gains, two or more losses, or six or more errors in total must always be investigated.

You should always determine confidence limits around estimates of mean quality. Remember though that the target is the mean (which is the best estimate of true quality) and not the lower 95% confidence limit.

### 3.2.5 Determining analytical quality

The analytical quality of a laboratory or Region (by whatever measure, see Section 3.2.3) can be determined easily from the results of the primary audit. There is no advantage in basing it on AQC inspections because it is computationally more difficult and the precision is unlikely to be better even if there are substantially more AQC inspections than primary audit results.

You should base estimates on a minimum of 20 samples, and preferably more. A large number is needed because the of the highly skewed (Poisson) distribution of the data. Remember that the annual audit is based on at least 60 samples per Region to obtain a reasonably accurate and precise estimate of Regional quality. The precision of the estimates depend on the number of samples on which they are based (audit samples or AQC inspections), not the proportion of primary samples that are audited or inspected. If you have insufficient samples for the period that you are interested in, you should estimate the analytical quality over a longer period.

It is more difficult to base estimates of quality on the results of the AQC inspections, particularly if you want confidence limits around them, because the quality of the AQC inspections will have to be taken into account. Information on the quality of AQC inspection can only be obtained from the AQC audit. It is unlikely that there will be an improvement in precision when there is more AQC inspection data than audit data for the period of interest. The amount of AQC audit data will compromise any potential increase in precision, as will the fact that the results will inherit the statistical errors of two estimates (average quality of primary analysis based on AQC inspection and average quality of AQC

inspection based on AQC audit) rather than just one. These errors are additive. You should base *both* estimates on a minimum of 20 samples (i.e. 20 AQC inspections and 20 AQC audit results), and preferably more. Both sets of data should relate to the same period of time.

It may be useful to estimate analytical quality from AQC inspections for special surveys when the primary audit is inappropriate, for example if samples are analysed beyond the level required for the BMWP-score system, but you wish to use the error module in RIVPACS to determine the significance of changes in BMWP indices derived from them. The AQC inspection must be to family level, and undertaken to the same standard as AQC inspections for standard samples analysed according to BT001. If this is done, the quality of AQC inspection can be assumed to be the same as the quality of AQC inspection of standard samples, and no additional AQC audit will be necessary. This assumption cannot be made if the quality of AQC inspection is likely to be different, for example if its quality varies or a different AQC inspector is used. In that case, you will need a minimum of 20 AQC audit samples relating to the survey. More intensive AQC inspection (and AQC auditing) may be necessary than usual to obtain sufficient AQC inspection data: if 10% of primary samples are selected for AQC inspection, you will need 200 primary samples, which is more than are analysed in most special surveys. Wherever possible though, base analytical quality on the primary audit.

The average analytical quality is simply the total number of gains divided by the number of audit samples. The period to which this relates is the period from which the audit samples were selected (i.e. the earliest to last date of analysis of primary samples, which is not necessarily the same as the earliest to latest date of analysis of the audit samples). The quality controller should have information about the dates of primary analyses to which each audit sample (and AQC sample) represents, see Section 3.2.6.

The lower and upper confidence limits are calculated separately, because confidence limits for Poisson distributions are not symmetrical. Upper and lower confidence limits for the total number of gains observed in the primary audit are determined from the table in Appendix A. These confidence limits are divided by the number of samples on which they are based (primary-audit samples) to obtain the confidence limits for the mean number of gains.

Worked example 1

In the period in which we are interested (say a year), 20 primary audit samples were analysed in the laboratory. In these, a total of 30 gains were recorded. The average analytical quality during this period was therefore:

Average analytical quality = total number of gains ÷ number of (audit) samples

= 30 ÷ 20

= **1.50**

The upper and lower confidence limits are determined using the table in Appendix A. In the first column, total number of gains are listed, and in the second and third, the lower and the upper 95% confidence limits for $\lambda$. In this example, a total of 30 gains were recorded, so reading off the table, the confidence limits for $\lambda$ are:

| Total No. of gains | Observed 95% confidence limits for $\lambda$ | |
|---|---|---|
| | lower | upper |
| 30 | 20.24 | 42.84 |

The upper and lower 95% confidence limits are obtained by dividing the values for $\lambda$ by the number of samples (in this example, 20).

Lower 95% confidence limit = lower 95% confidence limit for $\lambda \div$ number of samples

$= 20.24 \div 20$
$= 1.01$

Upper 95% confidence limit. = upper 95% confidence limit for $\lambda \div$ number of samples

$= 42.84 \div 20$
$= 2.14$

Confidence limits for losses, omissions and net gains can be estimated in a similar way, by substituting analogous statistics for the parameter of interest into the equations above.

If you want to determine the overall quality of a laboratory or Region's data, some of which has been provided by another laboratory (whether another Agency laboratory or contractors), you will have to take their analytical quality into account. How to do this is explained in Section 3.4.7 (see also Section 3.4.8). Each laboratory's analytical quality should be based on at least 20 audit samples: if you have less than this for the other laboratory, your estimate of overall quality will be less precise, particularly if the other laboratory undertook a large proportion of the primary analyses.

It is strongly recommended that you always base estimates of the quality of primary analyses on the results of the primary audit. The audit only covers samples analysed to BMWP-level, so if you want to know the quality of primary analysis for special surveys (for instance analysed to species) you will have to use internal AQC inspection data. You will also have to use AQC inspection data if you want to determine the quality an individual season or analyst over a couple of years.

Whenever you estimate the quality of primary data from AQC inspections, you will have to take account of the quality of the AQC inspection. Information on

the quality of AQC inspection is provided by the AQC-audit. *If* the AQC inspection of the samples is undertaken in precisely the same way as for other samples analysed to BMWP-level, and *if* the quality of the AQC inspections consistent, you can use data from the AQC-audit even if the AQC-audit covers a different set of samples.

The average analytical quality of the primary data is simply the sum of the average number of gains observed in the AQC inspection, and the average number of gains observed in the AQC audit.

Confidence limits for the average number of gains observed in the AQC inspection and for the average number of gains observed in the AQC audit can be calculated in the same way as for the average number of gains observed in the primary audit, using the table in Appendix A (see Worked Example 1). These two sets of confidence limits have to be combined to obtain the confidence limits for the average number of gains the primary data. Unfortunately, combining confidence limits for two means based on Poisson distributions is not straightforward. There is no statistical literature covering the combination of confidence intervals when adding estimates of population means, so an 'informal' method has been proposed by Julian Ellis and Peter van Dijk of WRc, which involves summing the confidence interval half-widths in quadrule, as explained below.

The confidence interval half widths are determined by subtracting both the upper and lower confidence limits from the means. The upper confidence interval half widths of the gains observed by AQC inspectors and the gains observed in the AQC-audit are then summed in quadrule, as are the lower confidence limits. Summing in quadrule means squaring the values, adding the squares, and then taking the square-root of the sum. For example adding 0.3 and 0.7 in quadrule is $\sqrt{(0.3^2 + 0.7^2)} = 0.76$.

**Worked example 2**

The AQC inspectors found a total of 15 gains in 20 AQC samples, giving an average of 0.75 gains with a lower 95% confidence limit of 0.42 and upper 95% confidence limit of 1.24.

The AQC inspection was undertaken to BMWP family level, and to the same standard as other samples analysed to this level, and by the same inspectors. The quality of AQC inspection in the laboratory remained relatively constant. It was therefore considered acceptable to use AQC audit data obtained from the audit of standard samples analysed for BMWP indices and GQA during that year.

The auditors found a total of 5 gains in the 20 AQC-audit samples, giving an average of 0.25 gains, a lower 95% c.l. of 0.08 and an upper 95% c.l. of 0.58 gains.

Average gains in primary data = average gains observed in AQC inspections + average gains observed in AQC audit

$= 0.75 + 0.25$
$= \mathbf{1.00}$

Determining the confidence limits around this is more difficult.

| | Gains observed by AQC inspector | Gains observed in AQC-audit |
|---|---|---|
| average | **0.75** | **0.25** |
| lower confidence limit | **0.42** | **0.08** |
| upper confidence limit | **1.24** | **0.58** |

The upper and lower half widths are calculated as the mean − confidence limit

| | | |
|---|---|---|
| average − lower confidence limit | 0.33 | 0.17 |
| average − upper confidence limit | -0.49 | -0.33 |

The two upper and the lower values are each summed in quadrule

| | | |
|---|---|---|
| lower half width | $= \sqrt{(0.33^2 + 0.17^2)}$ | $= 0.371$ |
| upper half width | $= \sqrt{(-0.49^2 + -0.33^2)}$ | $= 0.590$ |

Then:

lower confidence limit of process average = process average − lower half width

$= 1.00 - 0.371$
$= \mathbf{0.63}$

and

upper confidence limit of process average = process average + upper half width

$= 1.00 + 0.590$
$= \mathbf{1.59}$

### 3.2.6 Selecting samples for AQC or audit

Samples selected for AQC inspection are termed AQC samples (control samples in van Dijk 1994b). Samples selected for audit are known as audit samples. All samples collected in accordance with RIVPACS sampling methods and analysed to the level required for the BMWP-score system must have a chance of being selected for inspection as an AQC sample. The samples from which these are selected are called primary samples. The AQC samples and audit samples are chosen by random selection.

Ten percent of all primary samples will be selected as AQC samples.

Every primary sample must have an equal chance of being selected for the AQC. They must be selected at even intervals and sequentially from the primary samples as they are analysed, so that changes in quality throughout the year are taken into account. This includes samples that have been re-analysed because the initial analysis was considered to have been of unacceptable quality in a previous

AQC inspection. AQC samples should be chosen by randomly selecting one sample from every batch of ten primary samples.

The procedure for selecting AQC samples must not only be random, it must be seen to be random. The second point is particularly important because it provides an assurance that the selection is truly representative. The selection should therefore be done in front of witnesses. Rejecting an outcome once it has been observed is not permitted.

Batch membership and the order of samples within batches should be determined by the date and time at which the primary sorting was completed. Nine white and one coloured ball which indicates the sample to be checked (or similar objects indistinguishable by touch) are placed in a bag. Each consecutive ball removed represents a consecutive sample in the batch. The balls are removed from the bag, *and not replaced* until the red ball is selected and thus the sample to be checked has been determined.

Alternatively, the balls can be marked with numbers from one to ten to represent consecutive samples in the batch: the first ball removed represents the sample to be checked. The quality controller can determine the sample to be checked without physically placing them in batches if they know the date (and time) that each sample was sorted, for instance by referring to the sample recording log (see BT001 Section 4.1.1).

The quality controller must keep a record of the earliest and latest date of analysis for the batch of primary samples represented by each AQC sample, from which they will determine the dates of primary analyses represented by each audit sample. These dates must be recorded on the net gains record sheet (see Section 3.3.11 and Figure 4.3) for use with the RIVPACS error modules.

If samples are subject to a repeat primary analysis following an alarm, data from the original primary analysis must be removed from all data archives, but notes pertaining to the state or nature of the sample and sampling conditions should be retained. Remember also to retain any records of crayfish or other rare taxa not retained in the sample (see Section 3.2.1). It is recommended that the original sampling notes are consulted to ensure that nothing is omitted. Remember also to replace the original date of analysis and the sorters' initials by those of the re-analysis.

When samples are reanalysed or their data scrapped following an alarm, all the remaining samples analysed during the terminating sequence must be re-allocated to batches, and new AQC samples selected from them. AQC samples representing samples that are retained must be inspected before those representing newly analysed samples, to maintain the date order of the cusum record and of

the audit samples, see Figure 3.1. These AQC samples are necessary to assure the quality of the samples that are retained and to ensure that the samples are properly represented by the audit, so that the measures of quality truly reflect the data that is used and archived. If an AQC sample is retained and, by chance, it is selected again as an AQC sample, there is no need to re-inspect it. The results of its AQC inspection can be entered onto the cusum record from its original AQC sample record sheet.

Thirty samples per laboratory from Regions with two laboratories, or twenty samples per laboratory from Regions with three or four laboratories will be subject to the independent audit. This provides a minimum of 60 AQC samples per Region for the primary audit, and a minimum of 20 AQC samples per laboratory for the AQC-audit.

Audit samples must be selected from the AQC samples, unless insufficient AQC samples are inspected during the year because insufficient primary samples are analysed, in which case the numbers should be made-up by primary samples not subject to AQC inspection, but evenly distributed throughout the year. Every AQC sample must have a chance of being selected for audit. However, the audit samples must also be a true representation of the primary data that is used or archived. This is why it is important that when any data is scrapped, the AQC samples must be re-allocated to the samples that are retained (see paragraph above), and the audit samples re-selected. Samples that have been re-analysed re-enter the selection procedure for AQC inspection, and therefore have a chance of being audited after their re-analysis.

Audit samples should be distributed evenly amongst that year's primary samples so that changes in quality throughout the year can be observed, and they must be chosen randomly. It is recommended that the audit samples are selected in the same way as the AQC samples, to ensure that they are the best practical representation of the primary data. Because a fixed number have to be selected for audit, laboratories will have to estimate the total number of samples that they intend to analyse in the year in order to determine the appropriate batch size. It is likely that the batch size will have to be altered periodically to ensure that all AQC samples have a chance of selection. It is recommended that this is done at least quarterly. Batch membership should be based on the consecutive date and time that the definitive primary sorting was completed.

Batches of AQC samples from which audit samples are selected must be stored until the all the AQC samples in the batch have been inspected and the audit sample sent to the auditors. This is in case the audit sample has to be reselected, following the reselection of AQC samples after an alarm, as in the example shown in Figure 3.1.

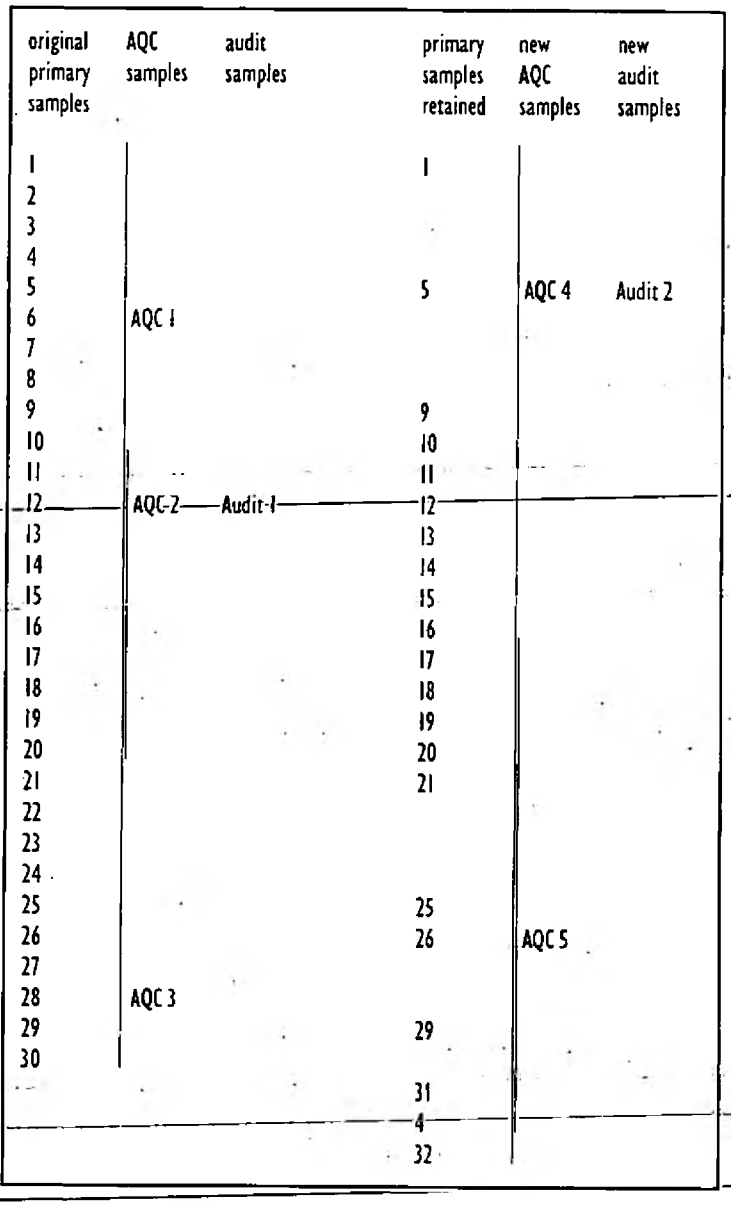



**Figure 3.1 Example of re-allocated AQC batches after scrapping and re-working primary data following an alarm.** This figure is to help explain why AQC and audit samples have to be re-selected and how to do this. Single and double vertical lines represent batches of 10 samples represented by each AQC sample. Data from samples 2, 3, 6, 7, 8, 22, 23, 24, 27, 28 and 30 were scrapped, and sample 4 was re-worked. The remaining samples were put into new batches from which new AQC samples were selected, including the batch represented by AQC2, even though all samples in this batch were retained. Samples 1, 5, and 9 to 16 were now represented by AQC4, and 17 to 21, 25, 26, 29, 31, and re-analysed sample 4, by AQC5. Sample 4 was re-analysed after sample 31. Its date of re-analysis becomes its new date of primary analysis, which is why it is included in the batch represented by AQC 5 and not AQC4. It could have been re-analysed at any time. Although sample 12 was retained, it could no longer be an audit sample, because it was not re-selected as an AQC sample. The audit sample had to be re-selected from the AQC samples by the same procedure as the original selection.

Analysts must not know which of their samples are to be re-analysed for AQC or audit. There must be no further analysis of the sample once it has been selected for AQC inspection or auditing.

### 3.2.7 Likely causes of errors

The most common causes of errors are described here to help you to decide the most appropriate action.

Gains usually occur because an analyst fails to notice a taxon in a sample. They are also caused by errors in recording. This will be the case when an analyst recognises the presence of a taxon and places an example in the vial, but fails to record its presence on the sample data sheet. Gains can also occur as a result of misidentifications.

Gains often involve taxa which are represented by only a single individual in a sample (singletons). Although missing one singleton in a sample does not warrant concern, missing several singletons in a sample may indicate a problem.

Losses usually occur because of misidentifications, which usually (but not always) cause a corresponding gain involving a similar taxon. Losses also occur when an analyst finds a taxon in the sample, but fails to return it to the vial or sample. A common cause is that the analyst thinks that all specimens have been put in the vial, but a small individual is left in the Petri dish and gets washed out. A loss will also occur when a taxon is noticed at the site, and the observation is entered into the results instead of the sample notes: such observations are not part of the sample. All results must be supported by voucher specimens except for the few rare taxa mentioned in Section 3.2.1.

Omissions are recorded only in the audit. They occur when the auditor finds a taxon in the sample which the primary analyst or AQC inspector correctly recorded as being present, but omitted to place a specimen in the vial, or put an empty shell in the vial instead of a complete specimen. An omission could also indicate the combination of a misidentification and a gain by the primary analyst or AQC inspector, when the auditor finds an example of the named taxon in the sample.

## 3.3 The AQC for sample analysis

The chief objective of the AQC is to enable the laboratory to ensure that the average number of gains remains at an acceptable level, and to reduce the number of samples with

particularly poor quality. Ultimately, the aim of the AQC is to provide a mechanism for ensuring that appropriate effort is devoted to the analysis of samples.

Unacceptable quality must be detected promptly so that corrective action can be taken as soon as possible. Experience has shown that the AQC scheme works best when an interval of no more than two weeks is allowed between the primary analysis and the AQC inspection of a sample.

### 3.3.1 Staff covered by the AQC scheme

In a biology laboratory, it is impractical to apply AQC independently to each analyst, because the low throughput would make the response times inordinately long. Therefore, AQC is applied to the laboratory as a whole.

Inexperienced analysts should not be included in the main scheme until they have reached the desired level of competence. The procedure for new or inexperienced analysts is explained in Section 3.3.9.

### 3.3.2 Anonymity

The anonymity of the primary analyst must be maintained until the inspection is complete. However, when the causes of quality failure are being investigated, it must be possible to discover the identity of the primary analyst. The quality controller will maintain the anonymity, either by removing the original labels from the sample container, or by using fresh containers. Labels inside containers and on the vial should also be changed. Labels on the AQC samples should give only a code identifying the AQC sample.

### 3.3.3 Quality of AQC inspection

It is most important that the inspection of AQC samples is performed with sufficient care to ensure that, as far as is practicable, all taxa in the samples are detected and identified correctly. AQC inspectors must be capable of consistent, as well as good, analytical quality. More time may be needed for the AQC inspection than for the primary analysis, although this will be partly offset by the provision of the primary data and the vial of identified specimens.

The AQC-audit provides information about the quality of AQC inspection. The precision of this information will be limited, because of the small number of samples audited for each laboratory: a minimum of 20. It is therefore important

that all the audit samples are selected from the AQC samples, so that the number of samples on which the AQC-audit is based is as large as possible.

If the audit indicates that the AQC inspectors are not performing satisfactorily, corrective action must be taken, for instance, retraining, taking more time for a more thorough AQC inspection, or selecting a different AQC inspector. As a guide, it was originally anticipated that the quality of AQC inspection should be no worse than an average of 0.5 gains.

The quality of AQC inspection affects the working acceptable quality level ($AQL_A$) of the laboratory's AQC scheme, see Section 3.3.5.

### 3.3.4 AQC inspection

AQC samples must be inspected in order of the date of the completion of their primary analysis and after all the samples in the batch from which they were selected have been analysed. This is to ensure that the primary analysts are never aware whether the sample that they are analysing will be inspected for AQC (and audit) or not.

The quality controller will complete a separate AQC sample record sheet (Figure 4.1) for each AQC sample, and either replace the labels or re-pot the sample. Before inspection, the quality controller should record the AQC sample identification code on the control sample record. They must keep a record of the samples to which the AQC sample codes are associated. This record must not be available to the AQC inspectors, so that they cannot determine the identity of the samples or primary analysts.

The AQC inspectors should re-analyse the samples with at least the same care in washing and sorting as the primary analysts (see BT001). The inspector's task will be helped by the vial of specimens, and the primary analyst's data. Having checked the identity of the taxa in the vial, they should search the rest of the sample carefully for additional taxa. AQC inspectors must not alter the contents of the primary analyst's vial(s), but must use a separate labelled vial for any additional taxa that they find. Specimens in a primary analyst's vial that the AQC inspector believes to have been misidentified must be left in the primary analyst's vial. Misidentified taxa must be recorded as such on the AQC sample record sheet. When the inspection has been completed, the vial must be returned to the standard sample container with the rest of the sample, so that it can be audited.

During inspection, the AQC inspector will enter the names of the taxa involved in gains and losses on the AQC sample record sheet. The inspector will also record comments on the state of the sample, such as the amount of detritus, that

could affect the difficulty of the primary analysis and AQC inspection, together with information about the taxa involved in errors (whether they are juveniles, damaged specimens, singletons, etc.). The AQC inspector must also record their name and the date on which they completed the AQC inspection. This information will be used for analysing the results of the AQC-audit if the sample is selected for audit. It is mandatory on the audit data sheets (Section 3.4.2).

After inspection, the quality controller will complete Part C of the AQC sample record sheet, and transfer the number of gains to the cusum record (Section 3.3.7). They must also restore the original labelling to the AQC samples which are chosen to be audit samples. If the sample is selected for audit, they will also have to complete an audit data sheet, as explained in Section 3.4.2.

### 3.3.5 Running the AQC procedure: setting the AQC parameters

The target quality for primary analyses is an average of no more than 2 gains (acceptable quality level, AQL = 2.0). An AQC scheme has been chosen that causes a false alarm approximately once in every 100 AQC samples (i.e. the target average run length, ARL ≈ 100) when the average analytical quality is 2 gains (i.e. it is equal to the AQL). This should give a good balance between providing early warnings of poor quality and low frequency of false alarms.

The desired combination of AQL and ARL is achieved by adjusting two parameters: the reference value (R) and the decision interval (D). It is not necessary to understand R and D to run the AQC procedure; however, an explanation is given in Section 3.3.6.

The quality of the AQC inspection also affects the AQC procedure. It is taken into account by adjusting the AQL. The adjusted AQL ($AQL_A$) is the AQL less the average number of gains made by the AQC inspectors.

$$AQL_A = AQL - y$$

where: $AQL_A$ = adjusted AQL for a particular laboratory AQC scheme
AQL = 2
y = average gains according to the laboratory's AQC-audit

The value of y should be based on the previous 20 AQC-audit results for the laboratory (i.e. the external audit of samples subject to internal AQC, which measures the quality of the AQC-inspection), re-calculated on a rolling basis after every batch of AQC-audit sample results. The value of y should be based on the gains recorded in the laboratory's AQC-audit only. It should not be altered to take account of the quality of primary analyses undertaken by the AQC inspectors, or changes in the personnel employed as AQC inspectors.

Re-calculate y and determine $AQL_A$ as soon as you get the audit results.

Values of $AQL_A$ to the nearest 0.25 taxa (known as the working AQL, $AQL_w$) are used to determine the appropriate values of R and D (see Table 3.1). This is so that they do not have to be re-adjusted every time that y is re-calculated. If it is necessary to adjust the values of R and D, do so immediately. If the laboratory's analysis is in the defer state (see Section 3.3.7), re-calculate the scores and the cusum scores from the first sample when the defer state was entered (as in the example in Figure 3.2).

The quality controller is responsible for determining $AQL_A$, R and D.

When the quality of the laboratory's primary analysis is continually poorer than the AQL, alarms will (and should) be triggered frequently until the analytical quality improves.

**Table 3.1** **Values of R and D to be used for different values of $AQL_A$**, derived from a table prepared by P. van Dijk and J. Ellis of WRc.

| Adjusted acceptable quality level ($AQL_A$) | 0.125-0.374 | 0.375-0.624 | 0.625-0.874 | 0.875-1.124 | 1.125-1.374 | 1.375-1.624 | 1.625-1.874 | 1.875-2.000 |
|---|---|---|---|---|---|---|---|---|
| Reference value (R) | 0.4 | 0.8 | 1.1 | 1.4 | 1.7 | 2.0 | 2.3 | 2.5 |
| Decision interval (D) | 2.6 | 3.0 | 3.6 | 4.0 | 4.4 | 5.0 | 5.0 | 6.0 |

### 3.3.6 Further information about the AQC procedure

It is not necessary to read or understand this section in order to administer the AQC procedure. This section includes more information about the AQC parameters R and D, and the ARL.

The reference value (R) triggers the defer state, see Section 3.3.7, and is slightly greater than the AQL. The decision interval (D) triggers an alarm, i.e. it determines the size of the defer state. The value of D affects the ARL. When D is smaller, ARL is smaller, but there is a greater chance of false alarms.

The impact of errors in AQC inspection on $AQL_A$ (and therefore on R and D, see Table 3.1) demonstrates the benefits for laboratories if they employ experienced biologists as AQC inspectors so that their AQC inspection is of a high quality.

**Table 3.2** **Average run lengths for given values of $AQL_A$**, derived from a table prepared by P. van Dijk and J. Ellis of WRc, ARLs based on computer simulations yielding 2000 alarms. $AQL_w$ is the $AQL_A$ to the nearest 0.25 taxa, and is the actual value on which ARL (and values of R and D in Table 3.1) were based.

| Adjusted acceptable quality level ($AQL_A$) | 0.125-0.374 | 0.375-0.624 | 0.625-0.874 | 0.875-1.124 | 1.125-1.374 | 1.375-1.624 | 1.625-1.874 | 1.875-2.000 |
|---|---|---|---|---|---|---|---|---|
| Working acceptable quality level ($AQL_w$) | 0.25 | 0.50 | 0.75 | 1.00 | 1.25 | 1.50 | 1.75 | 2.00 |
| Deterioration from $AQL_w$ | Average run length (ARL) | | | | | | | |
| 0.00 | 98.8 | 104.2 | 101.1 | 97.5 | 104.3 | 96.1 | 99.7 | 99.7 |
| 0.25 | 15.7 | 24.5 | 28.8 | 31.4 | 36.0 | 39.3 | 43.2 | 42.7 |
| 0.50 | 7.8 | 11.0 | 13.7 | 15.6 | 17.8 | 19.6 | 22.0 | 23.7 |
| 0.75 | 5.0 | 7.0 | 8.5 | 9.7 | 11.0 | 12.1 | 13.6 | 14.4 |
| 1.00 | 3.9 | 5.1 | 6.1 | 7.0 | 8.0 | 8.5 | 9.7 | 10.5 |
| 1.25 | 3.1 | 4.1 | 4.7 | 5.5 | 6.2 | 6.6 | 7.4 | 7.9 |
| 1.50 | 2.6 | 3.3 | 3.8 | 4.4 | 5.0 | 5.4 | 6.0 | 6.5 |
| 1.75 | 2.3 | 2.9 | 3.3 | 3.8 | 4.2 | 4.5 | 5.0 | 5.4 |
| 2.00 | 2.0 | 2.5 | 2.9 | 3.3 | 3.7 | 3.9 | 4.2 | 4.6 |
| 2.25 | 1.8 | 2.2 | 2.6 | 2.9 | 3.2 | 3.5 | 3.8 | 4.1 |
| 2.50 | 1.7 | 2.1 | 2.3 | 2.7 | 2.9 | 3.1 | 3.4 | 3.7 |
| 2.75 | 1.6 | 1.9 | 2.1 | 2.5 | 2.7 | 2.8 | 3.1 | 3.4 |
| 3.00 | 1.5 | 1.8 | 2.3 | 2.3 | 2.5 | 2.6 | 2.8 | 3.1 |

For the combination of $AQL_A = 2$ and target ARL = 100, the reference value, R, is 2.5, and the decision interval, D, is 6 (Table 3.1). If the laboratory is really performing at this level, the results of the AQC will lead to a decision to take corrective action on average once in every 99.7 AQC samples (these will be false alarms), see Table 3.2. When the quality indicated by the AQC is worse than $AQL_A$, the frequency of alarms increases (the ARL becomes smaller).

The inherent variability used to calculate the average run length was assumed to follow a Poisson distribution (based on Kinley & Ellis, 1991). If the true

variability differs from this assumption, the average run length may be incorrect. In particular, false alarms may be more frequent. Experience will show whether an AQC scheme with a higher ARL needs to be chosen to obtain satisfactory run lengths in practice. This aspect of the scheme will be reviewed after its first full year of operation. If frequent alarms are caused by the primary analysis being continually poorer than the AQL, the ARL should not be adjusted.

### 3.3.7 Running the AQC procedure: determining the state of the analysis

The cusum record is an ongoing record and is displayed on a special form (see Figure 4.2). After each AQC sample has been inspected, the quality controller must transfer the number of gains from the control sample record to the cusum record, and, if necessary, update the cusum score. The entries in the record must follow the order of completion dates of the AQC inspections, which must themselves follow the order of completion of primary analyses.

The scheme must be followed rigorously, and no other interpretation of the results is permitted. Alarms must always be acted on immediately and no formal corrective action should be taken unless an alarm is triggered. However, individual AQC results may be investigated outside the scheme if it is felt that this will be helpful, and the causes of individual AQC samples with many errors must be investigated.

When applying the AQC scheme, start at Stage 1, the accept state.

*Stage 1 (accept state)*

Enter the number of gains observed on the cusum record form (together with the AQC sample codes and inspection dates). If a result is less than or equal to the reference value (R), no further action is required until the next AQC sample has been inspected.

If the next result is also equal to or less than R, the scheme remains in the accept state. This continues until a result is obtained that is greater than the reference value (as at Samples 5, 7, 18 and 22 in the example in Figure 3.2). This triggers the start of Stage 2, the defer state.

*Stage 2 (defer state)*

Having observed a result greater than the reference value, subtract the reference value from the result to give the score. Enter the score into the appropriate column and into the next column to start the record of its cumulative sum (cusum). Continue in the defer state, entering results, subtracting R from the result to obtain the score and maintain its algebraic sign (+ or -). The cusum of the score is updated after each AQC sample, by adding the latest score to the

**AQC Cusum Record Form**

**Laboratory:** EXAMPLE

**Reference Value, R =** ~~1.7~~ 2.0 **Decision Interval, D =** ~~4.4~~ 5.0

| AQC sample code | Inspection date | Gains T | Score T - R | Cusum of Score | State | Action |
|---|---|---|---|---|---|---|
| 1 | 6.1.97 | 0 | | | ACCEPT | |
| 2 | 5.2.97 | 1 | | | ACCEPT | |
| 3 | 6.2.97 | 1 | | | ACCEPT | |
| 4 | 10.3.97 (am) | 0 | | | ACCEPT | |
| 5 | 10.3.97 (pm) | 2 | 0.3 | 0.3 | DEFER | |
| 6 | 11.3.97 | 0 | -1.7 | 0 | ACCEPT | |
| ~~7~~ | ~~18.3.97~~ | ~~3~~ | ~~1.3~~ | ~~1.3~~ | ~~DEFER~~ | VALUE OF R RE-SET TO 2.0 VALUE OF D RE-SET TO 5.0 |
| ~~8~~ | ~~5.4.97~~ | ~~2~~ | ~~0.3~~ | ~~1.6~~ | ~~DEFER~~ | |
| 7 | 18.3.97 | 3 | 1.0 | 1.0 | DEFER | RE-CALCULATE CUSUM FROM FIRST SAMPLE IN THE CURRENT DEFER SEQUENCE. |
| 8 | 5.4.97 | 2 | 0 | 1.0 | DEFER | |
| 9 | 6.4.97 | 4 | 2.0 | 3.0 | DEFER | |
| 10 | 25.4.97 | 1 | -1.0 | 2.0 | DEFER | |
| 11 | 1.5.97 | 3 | 1.0 | 3.0 | DEFER | |
| 12 | 2.5.97 | 3 | 1.0 | 4.0 | DEFER | |
| 13 | 6.5.97 | 2 | 0 | 4.0 | DEFER | |
| 14 | 9.5.97 | 4 | 2 | 6.0 | ALARM | REVIEW PROCEDURES *ALLOW MORE TIME FOR SORTING |
| 15 | 19.5.97 | 1 | | | ACCEPT | |
| 16 | 26.5.97 | 1 | | | ACCEPT | |
| 17 | 30.5.97 | 2 | | | ACCEPT | |
| 18 | 2.6.97 | 4 | 2.0 | 2.0 | DEFER | |
| 19 | 6.6.97 | 1 | -1.0 | 1.0 | DEFER | |
| 20 | 9.6.97 (am) | 2 | 0 | 1.0 | DEFER | |
| 21 | 9.6.97 (pm 1) | 1 | -1.0 | 0 | ACCEPT | |
| 22 | 9.6.97 (pm 2) | 4 | 2.0 | 2.0 | DEFER | |
| 23 | 17.6.97 | 4 | 2.0 | 4.0 | DEFER | |
| 24 | 18.6.97 | 3 | 1.0 | 5.0 | ALARM | REVIEW PROCEDURES TRAINING SESSION ON DIPTERA PUPAE AND SMALL STONEFLY LARVAE RECOGNITION |

Version 1.2 23 April 1997 JM-B

**Figure 3.2** **Example of a completed cusum record form**, to show how the AQC scheme operates. The AQC parameters were re-set part way through this record because of a change in $AQL_1$ from 1.20 to 1.45, causing $AQL_2$ to change from 1.25 to 1.50. Note that the AQC samples are listed in consecutive inspection date (and time) order.

previous cusum. Continue in the defer state, taking samples and accumulating the cusum score, until one of two points is reached.

1. The cusum returns to zero or becomes negative. If so, the defer state is terminated and the process average is considered to be satisfactory. Reset the cusum score to zero and return to Stage 1, the accept state.

2. The cusum reaches or exceeds the decision interval (D), as at AQC samples 14 and 24 in the example shown in Figure 3.2. This triggers the alarm (Stage 3).

*Stage 3 (alarm state)*

When an alarm is signalled, the average quality is considered to be unsatisfactory. The deterioration in the process average will be deemed to have existed since the beginning of the final defer state, and the current process average will be the average over this period.

AQC inspection must cease as soon as an alarm is signalled, and must not recommence until the corrective action has been decided. There is no point in continuing to inspect outstanding AQC samples because, if some of the primary data during the last defer period is scrapped or re-worked, the AQC samples representing the primary samples that are retained will have to be re-selected and inspected beforehand.

After corrective action has been taken following an alarm, re-set the cusum to zero and return to Stage 1.

The quality controller must inform the laboratory manager as soon as the control state changes, because they will have to arrange for all the primary samples to be stored whilst the analysis is a defer state, to facilitate corrective action (see Section 3.3.8).

At any time, the scheme is in one of the three states: accept, defer or alarm. Whilst in the accept state, analytical quality is considered to be satisfactory. When in the alarm state, quality is judged to be unacceptable and some corrective action must be initiated. During the defer state, no decision can be made as to whether the quality is acceptable or not; the quality of the process is under probation.

Note that the scheme may go into the defer state even if the quality is better than the AQL. The fact that the scheme is in the defer state at any time should not, therefore, be taken to indicate that the process needs tightening up, although management has the freedom to investigate further any AQC sample results.

If the values of D and R are altered as a result of a change in $AQL_A$, and the scheme is in a defer state, re-calculate the cusum from the first sample in the current sequence (see example in Figure 3.2), and re-determine the state of analysis.

### 3.3.8 Corrective action

The corrective action that is appropriate will depend on the cause of the quality failure and the particular circumstances that led to it. This section is, therefore, advisory rather than prescriptive. However, it is mandatory for appropriate action to be taken after an alarm.

There is no such thing as a freak result. If the AQC samples have been chosen correctly, and the primary analysts do not know which samples will become AQC samples, the AQC samples must be considered to be truly representative of the primary samples. If an AQC sample has particularly poor quality, you must assume that a proportion of the primary results suffer a similar problem. Major and obvious errors are usually caused during data handling, and when this is found corrective action must be taken to eliminate or minimise the chances of its reoccurrence.

All instances where the AQC indicates 5 or more gains, 2 or more losses, or 6 or more errors in total (excluding AQC inspectors' errors) must be investigated fully, regardless of the action state of the AQC. Such instances should be treated seriously.

When an alarm is signalled, and not before, corrective action should be taken to ensure that the quality becomes acceptable again. It is a management responsibility to decide what action to take. Clues about what has caused the alarm may be obtained by referring to the list of taxa that caused the errors, by considering the state of the sample, and who the primary analysts were, particularly those whose AQC results caused the cusum to rise. At the taxonomic level required for the BMWP-score system, most errors are a result of shortcomings in sorting rather than identification.

If the alarm appears to have been caused by a single analyst, as indicated by their having several samples with high cusum scores during the defer state, then the appropriate action ranges from a quiet word of encouragement, through guidance on the identification of taxa which they find difficult, to withdrawal of that analyst from the scheme for re-training. The decision about which is appropriate depends on the experience or previous training of the analyst, the difficulty of analysing the samples, and the particular taxa causing the errors.

Pay particular attention to the taxa that cause most errors, including those reported in the annual audit reports. It may also be helpful to consider the provenance of samples causing most errors. If the alarm was because of a series of particularly unusual or difficult samples, some specialised retraining or a re-appraisal of the methods used for washing and sorting (see BT001) is likely to be necessary.

If the cause was a general decline in average quality by all analysts, perhaps because of an increased workload or excessive numbers of interruptions, action must be taken to relieve the situation. This may include allowing more time per sample with a consequent reduction in the number of samples which can be analysed.

As well as taking decisions to improve the analysis of subsequent samples, managers also need to decide what to do about the results that have been declared to be below the acceptable quality level. The affected results include all the primary samples analysed during the period when the scheme was in the final defer state, and not just those which were checked. There are three possibilities:

1. rework some or all of the primary samples;

2. scrap some or all of the results;

3. accept the results with the proviso that their quality is worse than is normally acceptable.

Only the first two options will ensure that the acceptable quality standard is met for the year. When meeting the quality standard is a priority, these will be the only practicable options, unless the failure represents a few samples only, or the overall process average is much better than the acceptable quality limit. These two options provide the mechanism by which the AQC *controls* quality.

If only one or two analysts are implicated in the decline in quality, only their work may need to be reworked or scrapped.

If it is decided that data will be re-worked or scrapped, only samples analysed whilst the scheme was in the final defer state should be affected. Samples from previous batches should not be altered, because this would make our measurement of analytical quality erroneous.

The best option may be to re-analyse the relevant primary samples, taking more care or using more experienced primary analysts. This option is available because the process of biological enumeration is repeatable. However, it is time-

consuming and therefore costly, so there must be good justification for taking this course.

To keep this option open, (and the option of scrapping only some of the data) the primary samples will have to be stored whenever a defer state is entered, and kept until the analysis re-enters the accept state, or until corrective action has been taken following an alarm. Usually, no more than about 30 samples will have to be stored, based on the average terminating sequence lengths given in van Dijk (1994b) for an AQC scheme with AQL = 2 and ARL = 100, and one in ten primary samples being an AQC sample.

Selecting the second option implies loosing the information gathered in the samples that are scrapped. Management must accept having no information, or collect new samples. This is a costly option that will rarely be feasible except when there are legal requirements to achieve pre-set targets for quality, where the data is thought likely to be misleading and it is not possible to re-analyse the samples because they have been discarded (all primary samples should be retained during the defer state) or damaged, or there are concerns that some taxa may have been lost because of poor sieving technique, thus precluding their re-analysis.

When particular analysts' samples are scrapped, or some or all of the samples are re-analysed, all the primary samples that are retained or re-analysed must be re-submitted to the AQC scheme, to assure their quality. The audit samples must be re-selected to ensure that the remaining samples are represented equally by the audit.

The best solution may be to go for the third option and simply accept that the estimate of quality is worse than the target, although this is not strictly corrective action. Whether or not this is reasonable depends on what is subsequently done with the results. Given the inherent variability of the sampling process (see Furse *et al.*, 1995), it may be acceptable occasionally to have greater analytical error than is targeted. It can be argued that it would be wrong simply to discard the results when some useful information can still be obtained, albeit at a poor quality, so long as they are not likely to be misleading. The confidence limits around the process average (see Section 3.2.5) should be calculated to see whether the analytical quality was so poor that it is not possible to detect significant differences in biological quality when comparing samples.

### 3.3.9 New or inexperienced analysts

Inexperienced biologists must not simply be included in the formal AQC scheme, because it was designed to apply to groups of analysts whose performance is of

a similar standard and is generally satisfactory. If inexperienced staff are included, the statistical distribution of the results on which the scheme is based will change. In any case, such staff need much closer supervision and training. Analysts whose analytical quality is considered to be inadequate on the basis of the AQC results (see Section 3.3.8) should be treated in the same way, as should experienced biologists either new to an Area, or analysing samples from a part of the country with which they are unfamiliar, because of the differences in the taxa that are likely to be found.

Inexperienced biologists will go through a three-phase process: whilst in the first two they will be considered to be in training.

*Phase 1*

The first five samples sorted by an inexperienced biologist will be checked in full by an experienced analyst from the laboratory. Sorting will be checked tray by tray, as will the identifications and other aspects of the analysis such as washing and sieving. In this phase, the inexperienced biologists should sort samples with a wide range of characteristics and different taxa.

Similar tuition will be needed for sampling, for which inexperienced biologists should be accompanied to a wide range of site types, and other aspects of the work. Inexperienced biologists should also read the relevant procedural manuals.

*Phase 2*

After the first five samples, the amount of checking can be reduced progressively. During this stage, an experienced biologist must screen the samples for errors. They must confirm the identity of the specimens in the vial, re-sort part of the remaining material to check that all the taxa have been recognised, and check all other aspects of the sample analysis, especially washing and sieving. The trainee must analyse a variety of sample types, and supervision should be more rigorous when the trainee encounters a type of sample which they have not analysed before.

Supervision whilst sampling can also be reduced, although biologists in training should not sample sites on their own that are very different in character from those which they visited in Phase 1.

As a guide, this phase is likely to involve the next 1-2 months of sorting, or longer if sorting is undertaken intermittently.

*Phase 3*

The trainee will enter the AQC scheme fully when they are considered to be capable of achieving the current target performance (an average of no more than two gains).

Whilst inexperienced biologists are in training, their data sheets should be amended by their trainer if the data is to be used operationally.

Particular attention should be given to the taxa which cause errors most frequently in the laboratory, as identified by the AQC and the audit, and nationally as indicated by the audit. Biologists in training must also be made aware of the taxa which cause frequent errors nationally but not regionally. These taxa may simply be less common in the Region or Area, but are even more likely to cause errors when they are present.

Any data from biologists in training that are used operationally must be included in the formal AQC and audit schemes, and should be identified as being from training samples. The trainer will be responsible for the quality of the analysis, and they will be the 'main analyst' for any samples analysed by the trainee. The errors will not contribute to the trainer or trainee's personal AQC or audit results, although both of them must be identifiable. These samples must be labelled with the trainers identity code, followed by the word "training", followed by the trainee's identity code.

### 3.3.10 Analysing samples from a different Area or Region

Samples from different Areas or Regions are likely to contain taxa with which the analysts are unfamiliar. The differences will be greatest when there are differences in geology and topography. The nature of the samples may also differ. Analysts used to sorting samples from mountain streams may have difficulty when they start to sort samples from lowland rivers, particularly if they contain a large amount of silt, algae or weed. It may take a while for them to adjust their visual perception to recognise all the taxa present. They are also likely to have to alter the way that they wash samples. Similar problems will be encountered when analysts used to sorting samples live begin to sort preserved samples, and vice versa. These problems are the same as those that occur when an experienced biologist joins a laboratory from another Area.

Analysts must contact biologists in the laboratory from which the samples originated, to find-out which washing and sorting procedures they consider to be most effective. A decision will have to be made whether the analyst uses a procedure with which they are familiar, or the method used by the other laboratory.

When analysing these samples, the analyst should undergo the same AQC procedures as inexperienced analysts (see Section 3.3.9). Although the biologist who checks their work may themselves be unfamiliar with some of the taxa, or the type of sample, a second opinion from an experienced biologist will speed the learning process. Unlike samples analysed by inexperienced biologists in training, the analyst, not the checker, is the main analyst for the sample, and their code rather than the checker's code should be associated with the sample for the purposes of the AQC and audit.

It is recommended that samples analysed from a different Area are not included in the AQC scheme for samples from the laboratory's own Area, but are subject to a separate AQC scheme, particularly when there are large differences between the samples from the two Areas. This is so that they do not upset the laboratory's AQC scheme, which will be optimised to controlling the errors in its own samples. Corrective action, if necessary, is likely to be different.

If possible, the AQC should be undertaken by the AQC inspector of the laboratory from which the samples originated, because they will be familiar with the samples and the taxa in them, and they will know their own error rate in inspecting such samples; therefore they will be able to apply a realistic $AQL_A$. The analyst's own AQC inspector is likely to be equally unfamiliar with these samples. If the analyst's own AQC inspector has to do the AQC inspection, as is most likely, it is recommended that their own laboratory's existing $AQL_A$ is used. The $AQL_A$ applied to these samples should be modified if AQC-audit results are obtained for these samples.

Analysing samples from another Area provides an opportunity for improving a laboratory's skills. It is useful for biologists to see taxa that do not, or very rarely, occur in samples from their own Area.

If a biologist from another Area is to collect samples, they should be accompanied by a biologist from that Area to at least five sites, covering the range of characteristics likely to be encountered, as would new or inexperienced biologists.

### 3.3.11 Maintaining the record of net gains

The quality controller should maintain a record of the net gains (gains minus losses, known as bias in RIVPACS error modules) observed in the AQC inspections, so that this error term can be used in RIVPACS to determine confidence limits for EQIs and the probability of changes in GQA biological class. This should be done using the form shown in Figure 4.3.

For most purposes, including GQA reporting, the estimate of average net gains reported in the annual primary audit report will be sufficient. However, for operational investigations, there may be advantages in using estimates of net gains based on AQC inspections (as recorded on the net gains record sheet) over a shorter period more closely relating to the period of analyses for the survey in question, or towards the end of the year if the laboratory's analytical quality has changed. The advantages of basing net gains on the more numerous AQC samples over a given period will have to be balanced against the smaller number of AQC audit samples.

Net gains observed in AQC inspections will have to be corrected ot take account of errors in AQC inspection that are detected in the AQC audit. On the Net Gains Record Sheet (Figure 4.3), values in the column "net gains in AQC inspection observed by AQC-audit" is the net gains observed in the AQC audit and the same value should be entered against each AQC sample in the batch of AQC samples from which the audit sample was chosen. Net gains in primary data is the sum of net gains observed by AQC inspection and net gains in AQC inspection observed by AQC-audit.

Confidence limits of average net gains can be estimated in precisely the same way as for gains, which is explained in Section 3.2.5.

The precision of these estimates will be important when using the error module of RIVPACS. The more precise your estimates of analytical performance are (i.e. the tighter the confidence limits around a given average), the smaller will be the differences in EQIs that will be statistically significant.

## 3.4 Audit of sample analysis

One of the aims of the audit is to measure the quality of the data used operationally and held on the data archive. The audit samples must be representative of this data. For this reason, AQC samples representing data that have been discarded because of unacceptable quality must not become audit samples, and audit samples must be re-selected (as must AQC samples) if samples are re-analysed or data is discarded (see Section 3.2.6).

The audit is analysed and reported annually. The audit is based on samples that have undergone AQC analysis before the end of February. Any shortfall in the number of samples (see Section 3.2.6) must be made-up from samples subject to primary analysis only. This allows the audit reports to be produced in May.

### 3.4.1 Analyst's identity

Every primary analyst and AQC inspector must be identified by a unique four digit numerical identification code. Biologists will retain the same identifying codes when they move to another laboratory. This will enable the relative difficulty of analysing samples from different parts of Britain to be assessed, and may give an objective insight into the merits of different laboratory practices. The codes will overcome the problem of duplicate initials within the same Region or laboratory, and changes in initials, for instance because of changed martial status. They will also improve anonymity on annual audit reports.

All codes must be confirmed with the national audit manager, in particular new codes for new members of staff, to ensure that they are all unique. The national audit manager will maintain a record of the codes and who they relate to.

If more than one person sorts a sample, all sorters' codes must be stated on the audit data sheet (Section 3.4.2). The biologist who did most of the analysis should be considered to be the main sorter, and take responsibility for the quality of the analysis of the sample: the results of the audit will contribute to their performance only. On data sheets, the main sorter's code should be given first, followed by the other sorters' codes in parentheses. The same protocol should be followed with AQC inspectors (see Figure 3.4).

Samples analysed by a biologist in training and which are used operationally should be identified by the trainer's code, followed by the word "training", followed by the trainee's code. The results of the audit will not contribute to either the trainer's or trainee's performance, see Section 3.3.9 and Figure 3.4.

### 3.4.2 The audit data sheet

The quality controller must complete a standard audit data sheet (Figure 4.4) for every audit sample. This must include the dates on which the primary analysis and AQC inspection were completed so that changes in analytical quality can be graphed against time. The date of sample collection may be unrelated to the date of analysis. Both the primary analyst's and the AQC inspector's identity code(s) and laboratory must be included on the form.

Mark the boxes in the 'primary' column with what the primary analyst recorded as being in the sample, and the boxes in the 'AQC' column with what the AQC inspector thought it contained.

Where the AQC inspector considers that the primary analyst has misidentified a taxon, an asterisk should be placed against the boxes relating to the correct and

the incorrect taxon (or taxa) in the 'AQC' column. Only the box(es) in the 'AQC' column representing what the AQC inspector believes to be the correct identity should be filled-in: the other, or others, in that column should be left blank. Taxa representing other misidentifications should be marked "2*", "3*", etc. in a similar way. See the example in Figure 3.3.

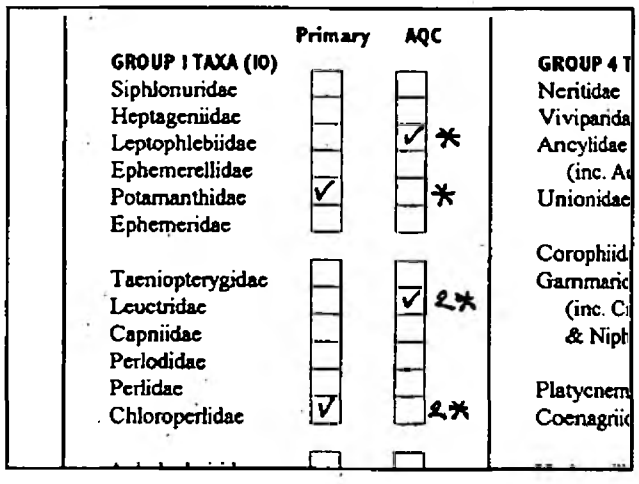
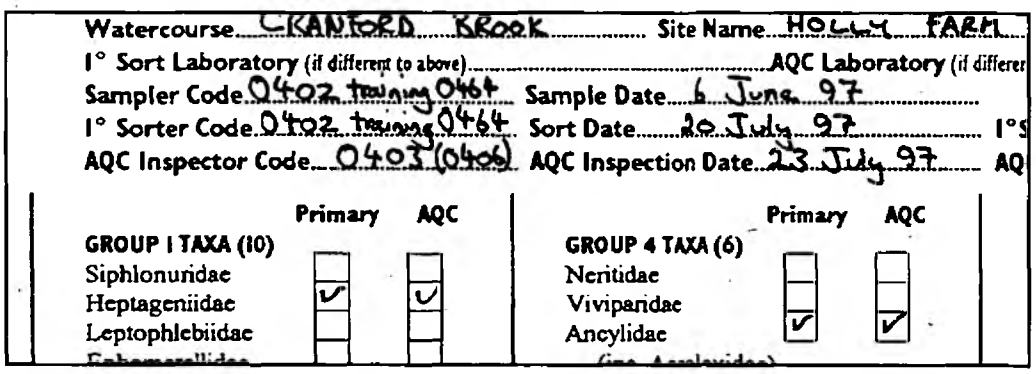



**Figure 3.3 Recording misidentifications on audit data sheets.** In this extract from an audit data sheet, the AQC inspector suspected two misidentifications: in the first they considered that the primary analyst misidentified Leptophlebiidae as Potamanthidae (signified by "*" opposite the 'AQC' column), and in the second Leuctridae as Chloroperlidae (signified by "2*").

Under Area/Laboratory, enter the name of the Area from which the sample was collected, if that Area has one biological laboratory of its own. If the Area is served by a laboratory which serves more than one Area, enter the name of the laboratory. If the Area has more than one laboratory you should enter both the name of the Area and the laboratory. To save time, these parts of the audit data sheet can be filled-in before it is duplicated. If the sample is analysed or inspected by a different laboratory, this should be stated in the appropriate space. This information will ensure that the auditors collate the results correctly.

Audit samples analysed by a biologist in training should be identified by the trainer's code under "1° Sorter Code", followed by "training", followed by the trainee's code. Secondary analysts' codes should be recorded in parentheses, see Figure 3.4.

A copy of each audit data sheet should be despatched to the auditors with the corresponding audit samples. A copy must be retained by the quality controller.



Watercourse CRANFORD KBrook Site Name HOLLY FARM

1° Sort Laboratory (if different to above) AQC Laboratory (if differer

Sampler Code 0402 training 0464 Sample Date 6 June 97

1° Sorter Code 0402 training 0464 Sort Date 20 July 97 1°S

AQC Inspector Code 0403 (0406) AQC Inspection Date 23 July 97 AQ

| GROUP 1 TAXA (10) | Primary | AQC |
|---|---|---|
| Siphlonuridae | | |
| Heptageniidae | ✓ | ✓ |
| Leptophlebiidae | | |

| GROUP 4 TAXA (6) | Primary | AQC |
|---|---|---|
| Neritidae | | |
| Viviparidae | | |
| Ancylidae | ✓ | ✓ |

**Figure 3.4 Recording sorters' codes on audit data sheets.** Note that a second AQC inspector, 0406, undertook some of the AQC inspection, but that 0403 was the main AQC inspector, and that the primary analysis was done by 0464 whilst being trained by 0402.

### 3.4.3 Despatching samples to the auditors

When the analytical process is in the accept state, audit samples should be sent to the auditors in batches of five consecutively analysed samples, within two weeks of the date on which the last sample was analysed for AQC. Do not allow more than five audit samples to accumulate before sending them to the auditor.

When the analysis is in the defer state, audit samples representing primary samples whilst the analysis was in the final defer state should be stored in case an alarm is signalled and corrective action results in samples having to undergo AQC again, and the audit samples having to be reselected (see Section 3.2.6).

Send these audit samples to the auditors when the AQC scheme re-enters the accept state.

Consignments of samples must be labelled in accordance with the Chemicals (Hazard Information and Packaging) Regulations 1993, and be accompanied by an appropriate Transport Emergency (TREM) card (see Figure 3.5). Samples in formalin are harmful, and samples in IMS are flammable and harmful. Every container must be labelled with the appropriate warning labels.

The procedures for storing and transporting samples in Section 3.9 of BT001 must be followed. This gives instructions on labelling and packaging as well as fixing and preserving samples.

Address audit samples, audit data sheets, and enquiries about the audit to:

## TREM CARD

ENVIRONMENT AGENCY
TRANSPORT EMERGENCY CARD (Road)

| | |
|---|---|
| CARGO | FORMALDEHYDE SOLUTION 4% (IN PLASTIC CONTAINERS)<br><br>Colourless solution, odour of formaldehyde. |
| NATURE OF HAZARD | Harmful if ingested in quantity or if exposure is prolonged.<br>Irritating to skin and eyes. |
| PROTECTIVE DEVICES | Goggles or face shield.<br>Rubber or plastic gloves. |
| EMERGENCY ACTION | Move people away from vapours as soon as possible. |
| SPILLAGE | Dilute with plenty of water and run to waste. |
| FIRST AID | Eyes: irrigate thoroughly with water for at least 10 minutes.<br>Lungs: remove from exposure, rest and keep warm.<br>Skin: wash off thoroughly with water. Remove contaminated clothing.<br>Mouth: wash out mouth thoroughly with water and give plenty of water to drink.<br><br>In severe cases<br>OBTAIN MEDICAL ATTENTION. |

PLEASE CONTACT _____
THROUGH THE REGIONAL COMMUNICATIONS CENTRE
TELEPHONE NUMBER _____ FOR FURTHER
ASSISTANCE IN DEALING WITH AN EMERGENCY.

APPLIES ONLY DURING ROAD TRANSPORT

**Figure 3.5** **Transport Emergency Card (TREM Card) for samples in fixative carried by road**; based on a card used in Southern Region to accompany samples to the auditors.; the relevant communications centre telephone number must be entered by the laboratory.

Rick Gunn
Institute of Freshwater Ecology
River Laboratory
East Stoke
Wareham
Dorset BH20 6BB

Send them to Rick Gunn in person, not simply to IFE.

### 3.4.4 The analysis undertaken by the auditors

The auditors will sort the sample without reference to the sample data sheet or vials, and will list all the BMWP-scoring taxa that are present. They will then identify and list the BMWP-scoring taxa present in the primary analyst's and the AQC inspector's vials. They will compare their lists with the taxa recorded as being present by the primary analyst and by the AQC inspector on the audit data sheet. They will also compare the primary analyst's and AQC inspector's results with the contents of the vials. The auditors will record the losses, gains and omissions in the primary analyst's and the AQC inspector's results. They will also record the status of any gains in the sample, such as their stage of development (larva, pupa, adult) and whether they are present as singletons. The auditors will identify the gains further, where possible to species, to help identify the species which cause repeated problems.

When they have audited the sample, the auditors will pool the contents of the primary analyst's and AQC inspector's vials, to which they will add any taxa not found by either the primary analyst or the AQC inspector. The auditors will keep the vials and their contents for future reference, but will not necessarily retain the rest of the sample.

### 3.4.5 Audit results

The results of the audit of individual samples will be sent to the Regional Biologists by the auditors within six weeks of the auditors receiving the samples. Copies will also be sent to the manager of the national audit (John Murray-Bligh), to be used for managing the audit: this copy will be archived. Regional Biologists should send copies of these to the Area from which the sample originated (and the laboratory that analysed the sample, if different). Regions and Areas must file these carefully, because they will not be copied in reports from the auditors.

The results of the primary audit and the AQC-audit of each sample will be reported on separate results sheets. These will record whether any BMWP taxa in the vial(s) have been omitted from the primary analyst's or AQC inspectors results or misidentified; whether any BMWP taxa have been missed in sorting; the taxa involved and the further identity, if possible to species, of any gains, and the probable reasons for errors involving BMWP taxa.

At the end of the year, the auditors will produce two national reports: one for the primary audit and one for the AQC-audit. These will contain summaries of the results and statistics relating to individual analysts, laboratories, Regions, and the Environment Agency as a whole. These statistics will include the mean number and standard error of gains, losses and omissions, the effect of errors on BMWP-score and N-taxa, the numbers and percentage of samples with more than 2 gains, shortfall in BMWP-score of more than 13 and shortfall in N-taxa of more than 2. They will also list the frequency with which each BMWP-taxon and individual species were missed by each laboratory and the Environment Agency as a whole.

The auditors will send a single master copy of each report to the national audit manager, who will check them for obvious errors and obtain an Agency National Library and Information Index Code (IC code) for each of them. The national audit manager will distribute copies to the National Biology Technical Group and to Area Biology Laboratories. Copies will also be sent to Regional libraries.

Confidence limits can be applied to the results of the audit, in the same way as from the results of AQC inspections, using the table in Appendix A (see Section 3.2.5). This is more straightforward for the audit, because only one distribution is involved, and it provides more precise results.

### 3.4.6 Corrective action

Corrective action is not an integral part of the audit in the same way that it is in the AQC procedure. However, it is prudent to take some action to improve the quality in the future.

All instances where the audit indicates 5 or more gains, 2 or more losses, or 6 or more errors in total must be investigated fully, and should be treated seriously.

If your Region or laboratory fails to meet the quality specification of an average of no more than two gains, corrective action must be taken if it has not been done already. The laboratory AQC scheme, which should have prevented such a failure, will have to be reviewed. If few or no alarms have been triggered, you will probably need to adjust the AQC parameters. In other cases, you should review the effectiveness of the corrective action that followed AQC alarms. To

ensure that you meet the quality specification, it is necessary to *aim* for somewhat better quality than the standard, so you may need to reconsider the quality that is regarded as acceptable in the laboratory. More time will have to be allocated to the analysis of samples, particularly whilst quality is being improved. It may be helpful to reconsider the procedures used to wash and sort samples: those recommended in BT001 have been found to be the most effective, and most efficient from experience in a number of laboratories, even though they may seem complicated. It is strongly recommended that you visit a laboratory which is responsible for similar types of rivers and meets the quality specification to help you to find the best course of action.

It is important that all biologists are aware of the taxa which cause errors most frequently, both nationally and locally. Their notice should be drawn to the taxa identified in the auditors reports. These taxa should receive particular attention in training sessions. At the taxonomic level required for the BMWP-score system, most errors are a result of shortcomings in sorting rather than identification.

Primary data must not be altered in the light of the audit results. The audit samples represent a very small fraction of the total number of samples, and correction would render the results of the audit erroneous.

It is recommended that the results of the audit are archived on computer databases in such a way that both corrected and uncorrected data can be retrieved. Whilst it is inappropriate for corrected data to be used for most purposes, for instance water quality analysis, there are instances when corrected data is useful, for instance, when mapping taxonomic distributions.

### 3.4.7 Auditing samples analysed by another Environment Agency laboratory

The audit of these samples should be managed and funded by the laboratory for whom the samples were analysed, as they will be the main beneficiaries.

Samples analysed by another Environment Agency laboratory should be audited in the same way as other primary samples, (with both a primary and an AQC-audit) and by the same auditor. Samples analysed for you by each different laboratory must be audited separately. They cannot be included in the audit of samples which your own laboratory analyses, because you will need a minimum of 20 samples for estimating your own primary sorters' and AQC inspectors' performance (the latter to calculate $AQL_A$ for the AQC scheme). A minimum of 3-5 samples should be audited, but no more than 20 in total. The precision of audit results is determined by the number of samples audited. It is particularly

important that precision is as high as possible, because errors are likely to be more frequent.

This audit will provide you with information on the quality of the other laboratories' analysis of your samples.

If data from samples analysed by another laboratory is to be archived or used with data from your own analyses, you must include these results when determining the overall quality of your *data*. If a similar proportion of samples have been audited, simply pool the audit results and take an overall average: calculate the confidence limits in the usual way, see Section 3.2.5. If, as is more likely, a different proportion of samples have been audited, determine the overall quality using the general formula below. Data analysed by consultants can also be included in this formula. The formula can also be used to estimate the upper and the lower confidence limits (by substituting either of these parameters for $t_i$), but only if the quality of each laboratory's data is based on the same number of audit samples. It is recommended that you base the quality of data from each laboratory on as many audit samples as possible for this, but remember that if it is based on substantially less than 20 audit samples from each laboratory the results are likely to be unreliable.

$$\bar{T} = \frac{(\frac{t_1}{n_1} \times N_1) + (\frac{t_2}{n_2} \times N_2) + \ldots (\frac{t_i}{n_i} \times N_i)}{N_1 + N_2 + \ldots N_i}$$

where $\bar{T}$ = overall average number of gains (or losses) $t_i$ = number of gains in samples audited from laboratory i
$n_i$ = number of samples audited from laboratory i $N_i$ = number of samples analysed by laboratory i
1, 2, ... i = each laboratory which analysed the samples, including your own

If the AQC inspection was done by the laboratory which analysed the samples, as is likely, they should be given the results of the AQC-audit of the samples which they analysed as soon as possible. This will enable them to identify problems in their AQC inspection, and to apply a more realistic $AQL_A$. The audit of samples analysed by different Environment Agency laboratories should be arranged under the IFE Technical Services Agreement.

### 3.4.8 Auditing samples analysed by contractors

Samples analysed by contractors must be audited in the same way as other primary samples, although only a primary audit will be appropriate. Although samples may be audited by Environment Agency laboratories to determine the

quality of the contractors analyses, audits to determine the quality of the data should be undertaken by the same auditor that audits the Environment Agency's own analyses, so that the results are consistent and compatible. The audit should be managed and funded by whoever in the Environment Agency commissioned the work, although the management of it may be delegated to the laboratory in the Area from which the samples originated. Samples analysed by consultants must be audited separately from the samples analysed by Environment Agency laboratories for the same reasons that samples analysed for you by other Agency laboratories should be audited separately, see Section 3.4.7.

It is important that contractors never know beforehand which samples are to be audited. If they do there will be a strong temptation for them to check the samples before they are audited, and to alter either the results or the samples. If they do this, the results of the audit will not be representative of all the samples which they have analysed. To avoid these problems, the Agency manager for the contract should choose which samples are to be audited. The best option is to ask the contractor either to deliver all the samples to their nearest Agency laboratory and for that laboratory to send the chosen samples to the auditor, or to ask them to send all the samples to you or to the auditor. A less satisfactory option is ask the contractor to retain all samples. After you have received the results sheets for a sample which you have chosen to be audited, ask them to send it to the auditor. This option may be easier to organise, but although they will not be able to alter their results, they could still re-sort the sample and remove any additional taxa which they find before sending it to the auditor.

It is strongly recommended that some (if not all) samples analysed by outside contractors are audited before being sent to them for analysis. It will not be possible to check whether the contractor looses specimens (for instance by careless washing or sieving) if they are audited after they have been analysed by the contractor. Some of these audited samples should be re-audited after the contractor has analysed them, if they are to be retained for further use, to check that the auditors have replaced the whole sample intact.

The guidelines for determining the number of samples analysed for you by another Environment Agency laboratory that should be audited should also be followed for samples analysed for you by contractors, see Section 3.4.7.

When determining the quality of the Area's *data*, some of which has now been analysed by the contractor, the number of samples analysed by the contractor that are audited, and the proportion of samples that the contractor analyses in relation to samples analysed by other laboratories, must be taken into account, see formula in Section 3.4.7.

## 3.5 Quality assurance for sample analysis to species

Methods for assuring the quality of analyses beyond family have not been adopted yet.

Standard methods for AQC have not been developed.

The auditors at IFE have undertaken a number of audits of analyses at species level for the Agency and others. These are undertaken in a similar manner to audits of analyses to BMWP-scoring taxa. Primary analysts place examples of every taxon in vials, but it is particularly important that enough material is provided to the auditors to confirm the identifications. Currently, they cover the species included in the current RIVPACS list (see Appendix C of BT001) with the exception of Sphaeriidae, Chironomidae and Oligochaeta. Gains and losses are reported, but are not measured against a target. There is insufficient information to determine an acceptable target. In some cases, the auditors take identification further than the RIVPACS list to provide additional information to the analyst. It is hoped that a standard procedure will be finalised in the next year or so.

## 3.6 Quality assurance for sample collection

Quality control and auditing for sample collection (including the collection of environmental data for RIVPACS), equivalent to that for sample analysis, would involve the collection and analysis of replicate samples by the auditor or AQC inspector. This is costly and impractical. The errors associated with sample collection and the collection of environmental data for RIVPACS have been estimated and are reported in Furse *et al.*, 1995. These errors have been incorporated in the error module in RIVPACS (versions III+ and higher).

The procedures manual BT001 in this series, the training video (National Rivers Authority, 1990) and training workshops on sample collection comprise the quality assurance of sample collection. If the sampling procedures described in BT001 are followed precisely, errors from variations in sample collection and examination will be minimised and should be equivalent to those reported in Furse *et al.*, 1995.

Documentation is an important component of quality assurance. It is important that the precise location of the sampling area can be identified within the survey area. Different staff must be able to find the survey area without error or uncertainty, to ensure that comparisons of samples reflect environmental changes rather than differences between sites. This is why the site manual described in Section 4.1.4 of BT001, or documentation equivalent to it, is so necessary.

## 3.7 Quality assurance for sample traceability

Clear sampling schedules must be maintained, as described in Section 4.1.1 of BT001. Documentation indicating what stage of analysis a sample has reached is an important component of quality assurance, so that samples which are damaged, destroyed or subject to other accidents, can be identified and traced. The instructions in BT001 must be followed.

## 3.8 Quality assurance for data archiving and analysis

All data should be entered onto the laboratory's standard data recording sheets in the first instance, if not directly onto computer. The data recording sheets are described in BT001. Data sheets should be filed properly as soon as they are completed.

All data entered onto Regional databases manually must be checked for transcription errors against the original data records. This is most easily done by two people; one reading out the original data records, the other checking against a listing from the database. A record of this checking must be kept (see Section 3.7). These checks must be made whenever data is entered or amended on local databases or the national biological database held at Thames Region. Regions or Areas are responsible for this checking. Regions are responsible for the accuracy of their entries on the National Database. Changes made to entries on the local database for samples which are also held on the national database *must* be reported to those maintaining the national database.

## 3.9 Quality assurance of samples collected or analysed by other Environment Agency laboratories

Treat biologists from other Areas who are to collect samples in your Area as you would an experienced biologist new to your laboratory. Accompany them to the first five sites that they sample, in accordance with Section 3.3.9. This is particularly important if watercourses in their Area are very different owing to geology or topography.

The laboratory analysing the samples is responsible for their AQC. Although not always feasible, it would help if your AQC inspector undertook the AQC inspection of these samples. This is so that a realistic AQLw could be used, and because the AQC inspector in the laboratory undertaking the analysis is likely to be unfamiliar with samples from your Area. See Section 3.3.10 for guidance.

Samples analysed in other laboratories should be audited separately, see Section 3.4.7. Although information is available about the quality of their work from the audit, remember that analysts in other laboratories may not be familiar with the taxa or type of samples from your Area.

## 3.10 Quality assurance of samples collected or analysed by contractors

This is not easy. Most Regions have had work of poor quality from contractors, so always be cautious and wary.

Guidance on quality assurance for projects, including those undertaken by contractors, is provided in Section 5.2 of Procedures Manual Volume 14 (Environment Agency, 1996a) and summary guide (Environment Agency, 1996b), which should be consulted for general advice.

All laboratory analyses undertaken by contractors must be audited, and its cost allowed for. Although analysis of samples to the degree required for the BMWP-score system is not particularly taxing, bear in mind the time it took your laboratory to become proficient, as well as the time it takes your laboratory to analyse samples to an adequate standard. For analyses to the level required for the BMWP-score system, contractors should be required to meet the same quality as the Environment Agency's laboratories. They will usually have to charge more to guarantee to meet a particular quality, even if it is a quality which they normally achieve. Be especially cautious of particularly low prices: however experience has shown that cost is not always related to quality. Allow sufficient budget for these samples to be audited by our auditors. These samples should be audited by a different auditor only in exceptional circumstances. The auditor must be competent, and should comply with the same specifications as required by our main auditors. Auditing should be the subject of a separate contract from the Environment Agency (as described in Section 3.4.8) even though it will involve more work, because there may be commercial conflicts of interest if the auditor is sub-contracted to the contractor, particularly if the contractor's work is poor.

It is strongly recommended that some (if not all) samples analysed by outside contractors are audited before being sent to them for analysis (see Section 3.4.8).

If consultants are to collect samples and are unfamiliar with the methods, they must be given detailed instructions. It is more effective to arrange for an Environment Agency biologist to accompany them before (or even when) they collect the first samples than to rely on documentation alone. They should be shown the video on collecting pond-net samples for RIVPACS (National Rivers Authority, 1990), but must be told about the errors in it (such as squeezing a large sample into a small pot using a poly bag). They must have read the relevant procedures manuals BT001, BT002 (if relevant), and this manual, if they are to undertake the procedures covered by them.

Ensure that prospective analysts are capable of undertaking the work. It is useful to ask for the *curriculum vitae* of those who will be undertaking the analysis, including sub-contractors, before the contract is awarded, and to demand that only analysts that you approve undertake the work if there is a change after the contract has been awarded. You should ask about other work that the contractor has done, or is doing, for the

Environment Agency or its predecessors, including the titles of any reports and the name of the Environment Agency project managers. You are advised to contact the project managers to verify the quality of the contractor's work, and to read any quality review panel reports of the reports and other outputs produced by the contractor.

With large or important contracts, it may be best to issue a small initial contract to test the contractor's competence, and to award the main contract only if the initial work is of acceptable quality.

Do not sign-off any work with which you are unsatisfied without attempting to get it resolved. This may conflict with pressures to achieve your financial targets. Simply accepting poor quality work, without question, does not help the Environment Agency, nor is it fair to those contractors that do produce work of good quality. Remember that statistical measures of quality will not be precise unless a large number of samples are audited, which is unlikely to be practical given the relatively small number of samples covered by most contracts. Always, therefore, calculate the confidence limits of these statistics using the methods described in Section 3.2.5. The benefits of following the procedures in the Project Management Manual (Environment Agency 1996a, 1996b), become apparent when problems arise. It is recommended that one or more technical experts, who could be biologists from other Areas or Regions, provide the Project Executive (the senior manager, usually the budget holder, responsible for signing-off projects) with an independent evaluation of the technical quality of any product, particularly if the Project Manager is concerned about its quality. Such a person (or persons) are termed the Quality Review Panel in the Project Management Manual. Unfortunately, the Project Management Manual does not give advice on what to do if the quality of the product is wanting.

The key to resolving problems of poor quality is to state the acceptable quality level clearly in the contract, the means by which that quality is to be measured, and the consequences of it not being achieved. Specify a similar quality to that expected of an Agency laboratory. Do not specify a poorer quality than the Agency's quality target (Section 3.2.4).

# 4 EQUIPMENT SPECIFICATIONS

See BT001.

## 4.1 Recording forms for the AQC and audit procedures

AQC Sample Record Sheets and AQC Cusum Record Forms will be needed to run the AQC, see Figure 4.1 and Figure 4.2.

AQC Sample Record Sheets are for the AQC inspectors to record the results of their inspections.

Cusum Record Forms are to enable the quality controllers to determine the control state of the primary analyses, based on the results of AQC inspection.

Net Gains Record Sheets (Figure 4.3) are for recording the dates of primary analyses covered by each AQC sample and for determining net gains from AQC inspections.

Audit Data Sheets (Figure 4.4) will be needed for the audit. They provide the auditors with information from the primary analysis and the AQC inspection, so that they can undertake both the primary audit and AQC-audit.

**AQC Sample Record Sheet**

**Part A** to be completed by the Quality Controller before inspection

| AQC sample code |
|---|

**Part B** to be completed by the AQC Inspector

| AQC inspector name | AQC inspector code |
|---|---|
| Inspection date | |
| Errors (Gains, losses, probable causes including misidentifications) | |
| State of sample (e.g. amount of detritus) | |
| Number of gains in sample | |

**Part C** to be completed by Quality Controller after inspection

| Original sample code |
|---|
| Sampling date |
| Watercourse |
| Site name |
| Primary analyst's code |
| Date of primary analysis |

Version 1.1 29 August 1996 JM-B

**Figure 4.1 AQC sample record sheet.**

**AQC Cusum Record Form**

| Laboratory: | | | | | | |
|---|---|---|---|---|---|---|
| Reference Value, R = | | | | Decision Interval, D = | | |
| AQC sample code | Inspection date | Gains T | Score T - R | Cusum of Score | State | Action |
| | | | | | | |

Version 1.2 23 April 1997 JM-B

**Figure 4.2 AQC cusum record form.**

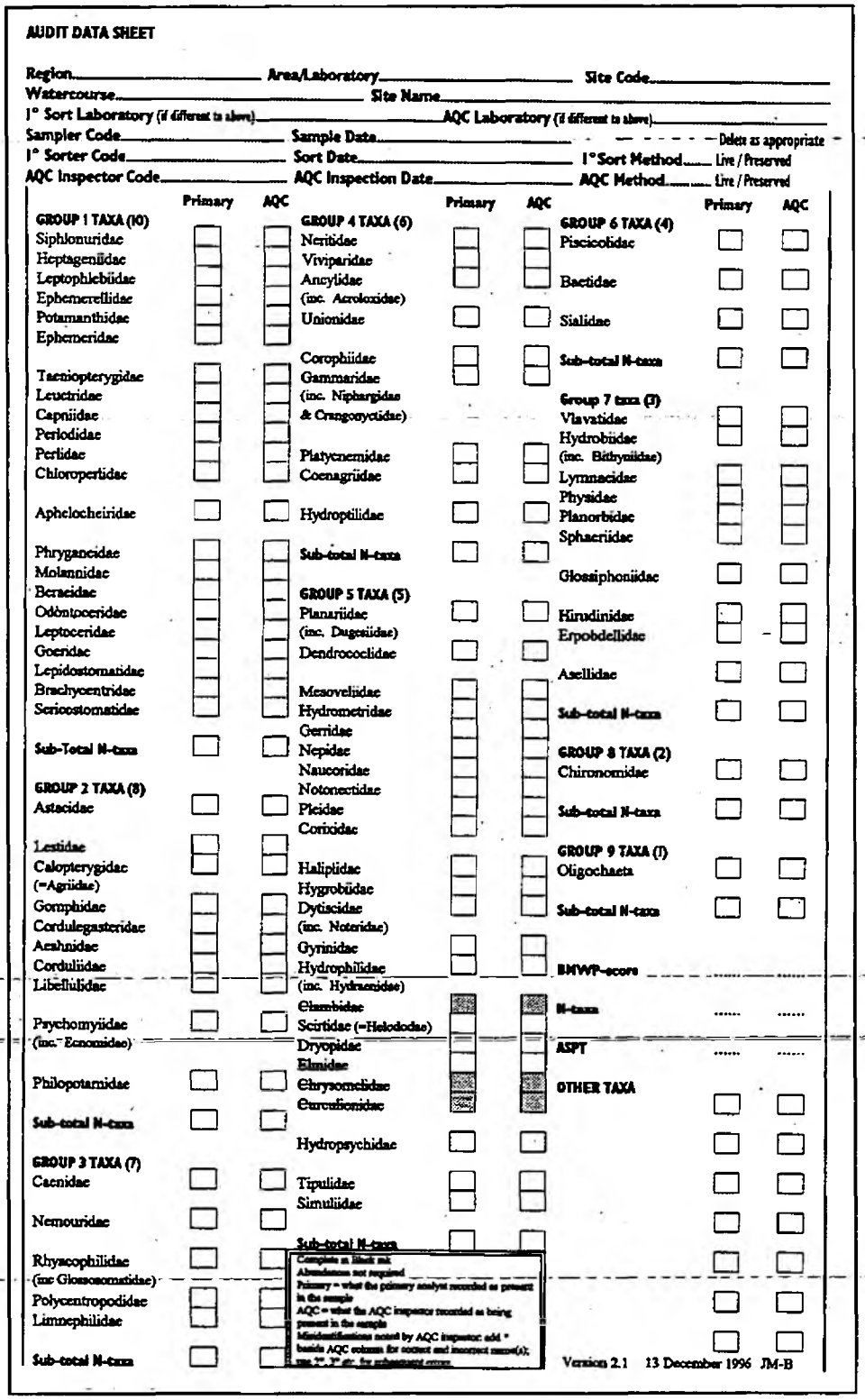
**Net Gains Record Sheet**

Laboratory:

| Primary sample code | Sample date | Primary analyst code | Survey code | AQC sample code | Dates of primary analyses represented by AQC sample | | Net gains observed by AQC inspection | Net gains in AQC inspection observed by AQC-audit | Net gains in primary data |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | From | To | | | |
| | | | | | | | | | |

Version 1.1 3 August 1999 JM-B

**Figure 4.3 Net gains record sheet**



**AUDIT DATA SHEET**

Region..... Area/Laboratory..... Site Code.....
Watercourse..... Site Name.....
1° Sort Laboratory (if different to above)..... AQC Laboratory (if different to above).....
Sampler Code..... Sample Date..... — — — — — Delete as appropriate
1° Sorter Code..... Sort Date..... 1° Sort Method..... Live / Preserved
AQC Inspector Code..... AQC Inspection Date..... AQC Method..... Live / Preserved

| | Primary | AQC |
|---|---|---|
| **GROUP 1 TAXA (10)** | | |
| Siphlonuridae | | |
| Heptageniidae | | |
| Leptophlebiidae | | |
| Ephemerellidae | | |
| Potamanthidae | | |
| Ephemeridae | | |
| Taeniopterygidae | | |
| Leuctridae | | |
| Capniidae | | |
| Perlodidae | | |
| Perlidae | | |
| Chloroperlidae | | |
| Aphelocheiridae | | |
| Phryganeidae | | |
| Molannidae | | |
| Beraeidae | | |
| Odontoceridae | | |
| Leptoceridae | | |
| Goeridae | | |
| Lepidostomatidae | | |
| Brachycentridae | | |
| Sericostomatidae | | |
| **Sub-Total N-taxa** | | |
| **GROUP 2 TAXA (8)** | | |
| Astacidae | | |
| Lestidae | | |
| Calopterygidae (=Agriidae) | | |
| Gomphidae | | |
| Cordulegasteridae | | |
| Aeshnidae | | |
| Corduliidae | | |
| Libellulidae | | |
| Psychomyiidae (inc. Ecnomidae) | | |
| Philopotamidae | | |
| **Sub-total N-taxa** | | |
| **GROUP 3 TAXA (7)** | | |
| Caenidae | | |
| Nemouridae | | |
| Rhyacophilidae (inc Glossosomatidae) | | |
| Polycentropodidae | | |
| Limnephilidae | | |
| **Sub-total N-taxa** | | |

| | Primary | AQC |
|---|---|---|
| **GROUP 4 TAXA (6)** | | |
| Neritidae | | |
| Viviparidae | | |
| Ancylidae (inc. Acroloxidae) | | |
| Unionidae | | |
| Corophiidae | | |
| Gammaridae (inc. Niphargidae & Crangonyctidae) | | |
| Platycnemidae | | |
| Coenagriidae | | |
| Hydroptilidae | | |
| **Sub-total N-taxa** | | |
| **GROUP 5 TAXA (5)** | | |
| Planariidae (inc. Dugesiidae) | | |
| Dendrocoelidae | | |
| Mesovelidae | | |
| Hydrometridae | | |
| Gerridae | | |
| Nepidae | | |
| Naucoridae | | |
| Notonectidae | | |
| Pleidae | | |
| Corixidae | | |
| Haliplidae | | |
| Hygrobiidae | | |
| Dytiscidae (inc. Noteridae) | | |
| Gyrinidae | | |
| Hydrophilidae (inc. Hydraenidae) | | |
| Clambidae | | |
| Scirtidae (=Helodidae) | | |
| Dryopidae | | |
| Elmidae | | |
| Chrysomelidae | | |
| Curculionidae | | |
| Hydropsychidae | | |
| Tipulidae | | |
| Simuliidae | | |
| **Sub-total N-taxa** | | |

| | Primary | AQC |
|---|---|---|
| **GROUP 6 TAXA (4)** | | |
| Piscicolidae | | |
| Bactidae | | |
| Sialidae | | |
| **Sub-total N-taxa** | | |
| **Group 7 taxa (3)** | | |
| Viviparidae | | |
| Hydrobiidae (inc. Bithyniidae) | | |
| Lymnaeidae | | |
| Physidae | | |
| Planorbidae | | |
| Sphaeriidae | | |
| Glossiphoniidae | | |
| Hirudinidae | | |
| Erpobdellidae | | |
| Asellidae | | |
| **Sub-total N-taxa** | | |
| **GROUP 8 TAXA (2)** | | |
| Chironomidae | | |
| **Sub-total N-taxa** | | |
| **GROUP 9 TAXA (1)** | | |
| Oligochaeta | | |
| **Sub-total N-taxa** | | |
| **BMWP-score** | ..... | ..... |
| **N-taxa** | ..... | ..... |
| **ASPT** | ..... | ..... |
| **OTHER TAXA** | | |

Complete in Black ink
Abundances not required
Primary = what the primary analyst recorded as present in the sample
AQC = what the AQC inspector recorded as being present in the sample
Misidentifications noted by AQC inspector: add * beside AQC column for correct and incorrect taxon(a); use 2°, 3° etc. for subsequent errors

Version 2.1 13 December 1996 JM-B

**Figure 4.4 Audit data sheet**

# 5 GLOSSARY

Definitions are provided for words in italics.

**accept state**, this occurs in the *AQC* scheme when the number of gains in *AQC samples* is less than *R*, and the scheme is not in a *defer state* or *alarm state*; the *cusum score* remains at zero, and no action is necessary.

**alarm state**, this occurs in the *AQC* scheme when the *cusum score* exceeds *D*, and ends when action has been taken to remedy the poor quality of the *primary analyses*, and the cusum score has been reset to zero.

**AQC, analytical quality control**, procedures to control errors in laboratory analyses within specified limits; formal *AQC procedures* for this method only cover *sorting* and *identification*.

**AQC-audit**, the *audit* of the quality of the *AQC inspection*.

**AQC inspector**, a person re-analysing *AQC samples* for *AQC*.

**AQC inspection**, the process of re-analysing *AQC samples* for *AQC*.

**AQC parameters**, *D* and *R*; the values of D and R define a particular *AQC scheme*.

**AQC procedure**, the general methodology used for *AQC*, cf. *AQC scheme*.

**AQC sample**, a sample from the primary samples, used to provide information about the primary analysis for *AQC*; also termed control sample, for instance in van Dijk (1994b).

**AQC sample record sheet**, the recording sheet used by the *AQC inspector* for the results of their inspection (see Figure 4.1).

**AQC scheme**, a member of the family of AQC schemes that make-up an *AQC procedure*; an AQC scheme is a particular manifestation of the AQC procedure with a given set of *AQC parameters*.

**AQL, acceptable quality level**, the limiting *process average* (i.e. the worst value of the underlying average quality) that is still acceptable for *AQC* purposes; if the average quality is at or better than this limit, the process does not require corrective action; for this procedure AQL = 2.0 gains.

**$AQL_A$, adjusted acceptable quality level**, the *AQL* less the average number of *gains* found in the *AQC-audit*.

**$AQL_w$, Working acceptable quality level**, The *AQL* used for the practical working of the *AQC procedure*, and used to determine the value of the *AQC parameters* to define the *AQC scheme*; $AQL_w$ is $AQL_A$ to the nearest 0.25 taxa.

**Area**, each *Region* of the *Environment Agency* is split into smaller geographical Areas for the purposes of undertaking its operational work.

**ARL, average run length**, the average number of *AQC samples* between alarms; these may be genuine alarms or false alarms; ARL diminishes as the *process average* deteriorates (i.e. the worse the performance, the sooner it will be detected by the *AQC scheme*); for this *AQC* scheme the target ARL = 100 samples; the target ARL is defined as that achieved when the process average = *AQL*, but usually differs from the true ARL at this process average because of rounding errors.

**audit**, an independent measurement of the quality of the analysis of samples, the analysis may be the *primary analysis*, the *AQC inspection*, or both

**auditing**, the process of re-analysing *audit samples* for the *audit*.

**audit sample**, a sample from the *AQC samples* (or *primary samples)*, used to provide information about the *AQC inspection* and/or the *primary analysis* for the *audit*.

**auditor**, a person re-analysing *audit samples* for the *audit*.

**biotic index**, an index, usually of water or environmental quality, based on biological information.

**BMWP-score, Biological Monitoring Working Party score**, a *biotic index* of ecological quality; based on numerical values assigned to each *BMWP-scoring taxon* which represent their tolerance to organic pollution; the BMWP-score of a site is the sum of the values of each taxon in a sample collected from it, and it is therefore based on both the tolerance of the taxa to organic pollution and the taxonomic richness.

**BMWP-score system**, the *BMWP-score* and its two component biotic indices: the Average Score per Taxon (*ASPT*) and the number of BMWP-scoring taxa (*N-taxa*).

**BMWP-scoring taxon**, a *taxon* used for the *BMWP-score*; with the exception of the class Oligochaeta, they are all families as defined in Maitland (1977).

**cl**, confidence limit(s).

**control sample**, *AQC sample*.

**corrective action**, action taken to bring the *process average* back to withing the *AQL* and to ensure that it remains there; corrective action includes scrapping poor quality data, re-analysing poorly analysed samples, and re-training biologists.

**current process average**, the *process average* whilst the *AQC* is in the current quality state: when the current quality is in the *accept state* or the *defer state*, the current process average is based on all *AQC samples* analysed whilst in the current state; when in an *alarm state*, it is based on all samples analysed whilst in the defer state that preceded the alarm state, i.e. in the *terminating sequence*.

**cusum**, cumulative sum; for the *AQC* the cusum in question is the sum of successive differences of the observed results from a fixed reference value *R*, i.e. the cusum of the *score*.

**cusum record**, a data sheet used by the laboratory showing the ongoing record of the quality achieved by the laboratory as measured by the *AQC*; it is updated by the *quality controller* after each *AQC sample* has been inspected (see Figure 4.2).

**D, decision interval**, the cumulative number of gains in excess of *R* which will trigger an alarm.

**defer state**, when an *AQC sample* exceeds the *AQL*, the *AQC scheme* is in the defer state and the *score* and its *cusum* are recorded; the defer state ends either when the cusum equals or exceeds *D* and the *alarm state* is triggered, or it reaches either zero or a negative number, and the *accept state* is regained.

**ecological quality**, good ecological quality exists where the environment supports its natural biota; ecological quality is affected by many factors, including water quality, flow regime, habitat degradation, and biological influences.

**Environment Agency**, the statutory regulatory agency in England and Wales responsible for the environmental protection since 1st April 1996.

**EQI, Environmental Quality Index**, (also known as Observed : Expected ratio, O/E ratio, formerly termed Ecological Quality Index); a *biotic index* expressed as a proportion of the value of the same index that would be expected under conditions of good ecological quality, predicted by *RIVPACS*.

**error**, a general term covering *gains* and *losses*, cf. *sampling error*.

**fixative**, a substance which makes biological material more resistant to disintegration by toughening connective tissue and muscle; fixatives are used as a pre-treatment before preservation; *formalin* is the most commonly used fixative for biological material; some fixatives are also *preservatives*, though not all preservatives are fixatives, see *IMS*.

**formalin**, a 40% aqueous solution of formaldehyde (which is also known as methanal); used as a *fixative* and *preservative* in dilute solutions (generally 4% formaldehyde which is equivalent to 10% formalin).

**GQA, General Quality Assessment**, surveillance undertaken by the Environment Agency to determine changes in environmental quality, as opposed to monitoring compliance with specific legal standards.

**gain**, a *BMWP-scoring taxon*, found in a sample by the *AQC inspector* or *auditor*, which was not recorded as present by the analyst whose quality is being inspected.

**IFE, Institute of Freshwater Ecology**, an institute of the Natural Environment Research Council specialising in the biology and chemistry of freshwaters.

**IMS, industrial methylated spirit**, ethanol with 5% methanol; it is a good *preservative* for biological samples, but a poor *fixative*; usually used in a 70% aqueous solution, sometimes with the addition of 5% glycerol to prevent dehydration of specimens if the container should leak or the IMS evaporate.

**identification**, determining the identity of the *taxa* in a sample.

**loss**, a *BMWP-scoring taxon* recorded as present by the analyst whose quality is being inspected, but which was not found in the sample by the *AQC inspector* or *auditor*.

**lotic**, pertaining to running waters.

**main analyst**, the person who undertakes the bulk of the analysis of a sample and is responsible for its quality.

**macro-invertebrate**, an invertebrate animal large enough to be seen without magnification; often defined as an animal retained on a 500 µm aperture sieve, though for this procedure it is an animal captured by a net of approximately 1 mm mesh.

**MS**, manuscript.

**national audit manager**, the person managing the national audit contract with the auditors.

**National Rivers Authority (NRA)**, the statutory regulatory agency in England and Wales responsible for the environmental protection of controlled waters from 1st September 1989 to 31st March 1996: one of the precursors of the *Environment Agency*.

**net gains**, the number of gains, minus the number of losses; termed bias in RIVPACS error modules.

**omission**, a *BMWP-scoring taxon* which was recorded as present by the *primary analyst* or *AQC inspector*, and which the *auditor* found in the sample but not in the vial; omissions are recorded in the *audit* only.

**Petri dish**, a shallow transparent dish, usually about 10 cm diameter and 1.5 cm deep, used during *sorting* and *identification*.

**preservative**, a substance that protects biological material from decomposition; *formalin* or *IMS* are used to preserve freshwater macro-invertebrate samples; cf. *fixative*; some preservatives are also fixatives.

**primary analysis**, the main analysis of the sample, i.e. the *sorting* and *identification*, which produces the data from which information about the environment is obtained.

**primary analyst**, the person undertaking the *primary analysis* of a sample.

**primary audit**, *audit* of the quality of the *primary analysis*.

**primary data**, data obtained from the *primary analysis*, from which information about the environment is obtained: the provision of primary data is the reason for collecting the sample from the environment.

**primary sample**, a sample from the environment used to provide information about the whole environment (cf. *AQC sample* and *audit sample*). All primary samples which are *RIVPACS* compatible and analysed to the level required for the *BMWP-score system* must be subject to *AQC* and *audit*, by having a chance of being selected for inspection as an *audit sample* or *AQC sample*.

**process average**, the quality of the *primary analysis* during a given period, usually a calendar year; see also *current process average*; when estimated from the *AQC inspections*, the quality of the AQC inspection must be taken into account.

**quadrule**, summing in quadrule is done by summing the squares of the values to be summed, and then taking the square root: the general formula is $\sqrt{(a^2 + b^2 + ... + i^2)}$.

**quality assurance**, procedures to quantify and control quality.

**quality controller**, person responsible for administering the *AQC* or *audit* schemes, including the selection of *AQC samples* and *audit samples*, delivery of samples to *AQC inspectors* or *auditors*, maintenance of AQC records, and paperwork relevant to the schemes.

**R, reference value**, the number of *gains* that triggers the *defer state* in the *AQC* scheme.

**Region**, the geographical areas into which the Environment Agency divides England and Wales, for administrative purposes; there are eight Regions; see also *Area*.

**RIVPACS, River Invertebrate Prediction and Classification System**, a computer system developed by *IFE* for classifying *macro-invertebrate* samples from watercourses on an existing national macro-invertebrate community classification (**RIVPACS classification**), and for predicting the macro-invertebrate fauna that any site should support under natural conditions, based on a limited number of environmental measurements, (**RIVPACS prediction**); RIVPACS is also used to predict the values of *biotic indices* that would be expected under natural conditions; these indices are used to determine *EQIs* and to allocate sites to ecological quality classes based on their EQIs; it is suitable for permanently flowing freshwaters in Britain and Northern Ireland; the current version is RIVPACS III.

**sampling area,** the area at a sampling site from which the samples are actually collected, cf. *survey area*.

**sampling error**, inaccuracies and imprecision in statistics that are based on samples.

**score**, the number of *gains* in excess of *R* when the *AQC* scheme is in the *defer state*.

**singleton**, a taxon whose presence in a sample is restricted to a single specimen.

**sorting,** searching for the appropriate *macro-invertebrate taxa* amongst other material in a sample, and where necessary, removing representatives of each for *identification*; this procedure includes placing representatives of each identified taxon into a vial for *quality assurance*; estimating the abundances of each taxon is also considered to be a part of sorting. Sorting is described in more detail in BT001.

**summing in quadrule**, see *quadrule*.

**survey area,** a length of watercourse encompassing the *sampling area* and extending either seven stream widths or 50 m either side of it; it should have the same physical characteristics (and therefore the same *macro-invertebrate* habitats) as the sampling area.

**taxon,** pl. **taxa,** a particular type of organism, irrespective the taxonomic level at which it is defined.

**terminating sequence**, *AQC samples* analysed whilst the *AQC* scheme is in a *defer state* preceding an *alarm state*.

**voucher specimen**, a specimen retained as evidence of the presence and identity of a taxon.

# 6 REFERENCES

Environment Agency (1996a) *The appraisal and management of projects in the Environment Agency.* Procedural Manual 14, Version 1. Bristol: Environment Agency.

Environment Agency (1996b) *Project management and the scheme of delegation - A summary guide.* Bristol: Environment Agency.

Environment Agency (1996c, draft) *Procedure for collecting and analysing river macro-invertebrate samples for GQA surveys.* Quality Management Systems for Environmental Monitoring: Biological Techniques, BT002. Bristol: The Environment Agency.

Environment Agency (1999) *Procedure for collecting and analysing macro-invertebrate samples.* Quality Management Systems for Environmental Monitoring: Biological Techniques, BT001. Version 2.0. Bristol: The Environment Agency.

Furse, M.T., R.T. Clarke, J.M. Winder, K.L. Symes, J.H. Blackburn, N.J. Grieve and R.J.M. Gunn (1995) *Biological assessment methods: controlling the quality of biological data Package 1 - The variability of data used for assessing the biological condition of rivers.* NRA R&D Note 412. Wareham: Institute of Freshwater Ecology.

Kinley, R.D. and J.C. Ellis (1991) *The application of statistical quality control methods to macroinvertebrate sampling.* Report No. CO2905. Medmenham: Water Research Centre (for NRA Thames Region).

Maitland, P.S. (1977) *A coded checklist of animals occurring in freshwater in the British Isles.* Edinburgh, Institute of Terrestrial Ecology.

National Rivers Authority (1990) *RIVPACS field sampling. Pond-net sampling.* Video recording. Birmingham: Spectrum Communications.

van Dijk, P. (1994a) *Biological assessment methods.* NRA R&D Project Record 504/6/S. Bristol: National Rivers Authority.

van Dijk, P. (1994b) *Analytical quality control for macroinvertebrate enumeration.* NRA R&D Note 331. Bristol: National Rivers Authority.

## APPENDIX A CONFIDENCE LIMITS FOR THE MEAN OF A POISSON DISTRIBUTION

This table is based on the theory presented in Biometrika Tables for Statisticians, Volume 1, edited by E.S. Pearson and H.O. Hartley, 1966, and was compiled by Julian Ellis of WRc.

Note that 'gains' in the table can be substituted by losses, net gains, or other measures of error in which you are interested.

| Total No. of gains | Observed 95% confidence limits for λ lower | upper | Total No. of gains | Observed 95% confidence limits for λ lower | upper | Total No. of gains | Observed 95% confidence limits for λ lower | upper |
|---|---|---|---|---|---|---|---|---|
| 0 | 0.00 | 3.69 | 31 | 21.06 | 44.00 | 62 | 47.53 | 79.49 |
| 1 | 0.03 | 5.57 | 32 | 21.88 | 45.18 | 63 | 48.41 | 80.60 |
| 2 | 0.24 | 7.22 | 33 | 22.71 | 46.35 | 64 | 49.29 | 81.73 |
| 3 | 0.62 | 8.77 | 34 | 23.55 | 47.52 | 65 | 50.17 | 82.83 |
| 4 | 1.09 | 10.24 | 35 | 24.38 | 48.68 | 66 | 51.05 | 83.97 |
| 5 | 1.62 | 11.67 | 36 | 25.21 | 49.83 | 67 | 51.92 | 85.09 |
| 6 | 2.20 | 13.06 | 37 | 26.06 | 50.99 | 68 | 52.80 | 86.20 |
| 7 | 2.81 | 14.43 | 38 | 26.89 | 52.15 | 69 | 53.68 | 87.33 |
| 8 | 3.45 | 15.76 | 39 | 27.73 | 53.30 | 70 | 54.56 | 88.42 |
| 9 | 4.12 | 17.09 | 40 | 28.58 | 54.47 | 71 | 55.46 | 89.56 |
| 10 | 4.80 | 18.38 | 41 | 29.42 | 55.63 | 72 | 56.33 | 90.69 |
| 11 | 5.49 | 19.68 | 42 | 30.28 | 56.78 | 73 | 57.21 | 91.77 |
| 12 | 6.20 | 20.96 | 43 | 31.12 | 57.92 | 74 | 58.11 | 92.90 |
| 13 | 6.92 | 22.23 | 44 | 31.98 | 59.06 | 75 | 59.00 | 94.03 |
| 14 | 7.65 | 23.48 | 45 | 32.82 | 60.21 | 76 | 59.88 | 95.11 |
| 15 | 8.40 | 24.74 | 46 | 33.68 | 61.37 | 77 | 60.76 | 96.24 |
| 16 | 9.14 | 25.98 | 47 | 34.53 | 62.50 | 78 | 61.67 | 97.36 |
| 17 | 9.90 | 27.23 | 48 | 35.40 | 63.63 | 79 | 62.53 | 98.44 |
| 18 | 10.67 | 28.44 | 49 | 36.25 | 64.79 | 80 | 63.44 | 99.57 |
| 19 | 11.44 | 29.66 | 50 | 37.12 | 65.92 | 81 | 64.31 | 100.69 |
| 20 | 12.22 | 30.90 | 51 | 37.98 | 67.06 | 82 | 65.23 | 101.79 |
| 21 | 13.00 | 32.10 | 52 | 38.83 | 68.20 | 83 | 66.11 | 102.88 |
| 22 | 13.79 | 33.31 | 53 | 39.70 | 69.32 | 84 | 67.00 | 104.00 |
| 23 | 14.57 | 34.52 | 54 | 40.56 | 70.47 | 85 | 67.91 | 105.10 |
| 24 | 15.37 | 35.71 | 55 | 41.44 | 71.59 | 86 | 68.79 | 106.20 |
| 25 | 16.18 | 36.91 | 56 | 42.31 | 72.74 | 87 | 69.67 | 107.31 |
| 26 | 16.98 | 38.10 | 57 | 43.17 | 73.85 | 88 | 70.58 | 108.43 |
| 27 | 17.79 | 39.28 | 58 | 44.04 | 74.98 | 89 | 71.47 | 109.52 |
| 28 | 18.60 | 40.47 | 59 | 44.92 | 76.10 | 90 | 72.38 | 110.61 |
| 29 | 19.42 | 41.65 | 60 | 45.80 | 77.25 | 91 | 73.28 | 111.73 |
| 30 | 20.24 | 42.84 | 61 | 46.65 | 78.35 | 92 | 74.17 | 112.84 |

| Total No. of gains | Observed 95% confidence limits for λ lower | upper |
|---|---|---|
| 93 | 75.07 | 113.93 |
| 94 | 75.97 | 115.02 |
| 95 | 76.87 | 116.13 |
| 96 | 77.77 | 117.24 |
| 97 | 78.68 | 118.33 |
| 98 | 79.56 | 119.41 |
| 99 | 80.47 | 120.52 |
| 100 | 81.38 | 121.63 |
| 101 | 82.26 | 122.74 |
| 102 | 83.18 | 123.82 |
| 103 | 84.07 | 124.90 |
| 104 | 84.99 | 126.01 |
| 105 | 85.88 | 127.11 |
| 106 | 86.78 | 128.22 |
| 107 | 87.70 | 129.30 |
| 108 | 88.60 | 130.38 |
| 109 | 89.50 | 131.48 |
| 110 | 90.40 | 132.58 |
| 111 | 91.30 | 133.69 |
| 112 | 92.23 | 134.76 |
| 113 | 93.14 | 135.84 |
| 114 | 94.02 | 136.94 |
| 115 | 94.93 | 138.04 |
| 116 | 95.84 | 139.14 |
| 117 | 96.75 | 140.24 |
| 118 | 97.67 | 141.31 |
| 119 | 98.58 | 142.38 |
| 120 | 99.50 | 143.48 |
| 121 | 100.38 | 144.58 |
| 122 | 101.30 | 145.68 |
| 123 | 102.22 | 146.77 |
| 124 | 103.15 | 147.84 |
| 125 | 104.04 | 148.91 |
| 126 | 104.96 | 150.01 |
| 127 | 105.89 | 151.10 |
| 128 | 106.79 | 152.20 |
| 129 | 107.68 | 153.29 |
| 130 | 108.61 | 154.38 |
| 131 | 109.54 | 155.45 |
| 132 | 110.44 | 156.52 |
| 133 | 111.35 | 157.61 |
| 134 | 112.28 | 158.70 |
| 135 | 113.19 | 159.79 |
| 136 | 114.09 | 160.88 |
| 137 | 115.03 | 161.98 |
| 138 | 115.94 | 163.04 |
| 139 | 116.84 | 164.13 |
| 140 | 117.79 | 165.19 |
| 141 | 118.70 | 166.28 |
| 142 | 119.61 | 167.37 |
| 143 | 120.52 | 168.46 |
| 144 | 121.43 | 169.55 |
| 145 | 122.35 | 170.64 |
| 146 | 123.30 | 171.69 |
| 147 | 124.18 | 172.78 |
| 148 | 125.13 | 173.84 |
| 149 | 126.05 | 174.93 |
| 150 | 126.97 | 176.01 |
| 151 | 127.89 | 177.10 |
| 152 | 128.81 | 178.19 |
| 153 | 129.73 | 179.27 |
| 154 | 130.62 | 180.33 |
| 155 | 131.54 | 181.41 |
| 156 | 132.47 | 182.47 |
| 157 | 133.39 | 183.55 |
| 158 | 134.32 | 184.64 |
| 159 | 135.25 | 185.72 |
| 160 | 136.18 | 186.81 |
| 161 | 137.11 | 187.89 |
| 162 | 138.00 | 188.97 |
| 163 | 138.93 | 190.06 |
| 164 | 139.86 | 191.11 |
| 165 | 140.79 | 192.19 |
| 166 | 141.69 | 193.24 |
| 167 | 142.64 | 194.32 |
| 168 | 143.56 | 195.40 |
| 169 | 144.50 | 196.49 |
| 170 | 145.39 | 197.57 |
| 171 | 146.33 | 198.65 |
| 172 | 147.27 | 199.73 |
| 173 | 148.17 | 200.81 |
| 174 | 149.11 | 201.86 |
| 175 | 150.05 | 202.94 |
| 176 | 150.95 | 204.02 |
| 177 | 151.88 | 205.06 |
| 178 | 152.80 | 206.14 |
| 179 | 153.74 | 207.22 |
| 180 | 154.65 | 208.30 |
| 181 | 155.59 | 209.38 |
| 182 | 156.50 | 210.46 |
| 183 | 157.45 | 211.54 |
| 184 | 158.35 | 212.61 |
| 185 | 159.30 | 213.66 |
| 186 | 160.23 | 214.73 |
| 187 | 161.16 | 215.81 |
| 188 | 162.07 | 216.86 |
| 189 | 162.98 | 217.93 |
| 190 | 163.93 | 219.01 |
| 191 | 164.87 | 220.08 |
| 192 | 165.80 | 221.16 |
| 193 | 166.71 | 222.24 |
| 194 | 167.67 | 223.31 |
| 195 | 168.58 | 224.39 |
| 196 | 169.54 | 225.46 |
| 197 | 170.45 | 226.54 |
| 198 | 171.37 | 227.58 |
| 199 | 172.31 | 228.65 |
| 200 | 173.25 | 229.73 |

## APPENDIX B SUMMARY OF QUALITY CONTROLLER'S DUTIES

Estimate the number samples to be analysed in the year at the beginning of the year and regularly thereafter, and whenever the planned number of analyses changes. If necessary, adjust the batch size for the audit to maintain regular intervals between audit samples. (3.2.6)

▼

Select the AQC sample from the batch of 10 primary samples as soon as the primary analyses of that batch are completed (or earlier) and record the earliest and latest date of primary analyses covered by the batch. (3.2.6)

▶ As soon as the AQC sample has been selected, re-label the AQC sample with its AQC code, record the primary sample data associated with that code and prepare the AQC sample data sheet. (3.3.4)
Pass the AQC sample and its AQC sample data sheet to the AQC inspector for inspection.

▼

Select the audit sample from the batch of AQC samples and record the earliest and latest date of primary analyses covered by the batch. (3.2.6)

On receipt of each AQC inspection result:
- ❖ complete Part C of the AQC sample data sheet. (3.3.4)
- ❖ update the cusum record to determine the control state of analysis. (3.3.7)
- ❖ update the net gains record. (3.3.11)

▼

Complete the audit data sheet for each audit sample. (3.4.2)

▼

Dispatch the audit samples to the auditors in batches of five, when the control state is accept. (3.4.3)

▼

On receipt of each batch of AQC-audit results, recalculate y, recalculate $AQL_A$, and redetermine $AQL_W$. If $AQL_W$ changes, recalculate R and D, record them on the cusum record form, and recalculate the score, cusum and control state for all AQC samples covered by the batch represented by the audit sample. (3.3.5)

▶ If the control state changes, inform the laboratory manager. If the defer state is entered, remind the lab manager that samples will need to be stored until that state has passed.

▼

Re-set the cusum to zero after action has been taken following an alarm. (3.3.7)

▼

Store copies of all audit results sheets carefully. (3.4.5)

If samples are scrapped or re-analysed, reselect AQC and audit samples. (3.2.6)

## APPENDIX C CHANGES TO THIS DOCUMENT

This section outlines changes between Versions 0.3 and 1.0.

Note that * indicates no more than a typographical or grammatical correction.

**Overall structure**
A new section, Section 3.3.6 has been introduced, to separate information that was necessary to operate the AQC procedure from additional information that enables you to understand how the AQC procedure works. This information was previously in Section 3.3.5. Likewise, Table 3.2 was previously incorporated in Table 3.1.

**Details page**
Added

**Glossary**
The following terms added: cl

**Section 1.1 paragraph 1**
* General Quality Assessment capitalized.

**Section 1.1, paragraph 6**
Sentence added about the National Audit Manager.

**Section 1.2, paragraph 4**
References for BT001 and BT002 given. Note added that Section numbers in references to BT001 relate to Version 2.

**Section 2.1**
Title altered.

**Section 3.1, paragraph 2**
Regional Biologist named as the Regional staff member with overall responsibility for the audit.

**Section 3.1 paragraph 4**
* Appendix B cited.

**Section 3.2.1 paragraph 1**
"Two or three specimens" replaces "up to three specimens".

**Section 3.2.2, paragraph 1**
References to Section numbers in BT001 corrected.

**Section 3.2.2, paragraph 2**
Reference to section about warning labels in BT001 corrected to Section 5.10.2.

**Section 3.2.3**
Reference to procedures for identification in BT001 corrected to Section 3.10.4.

**Section 3.2.4 paragraph I**
* note added about the target being measured over the year.

**Section 3.2.5 paragraphs 8 and 9**
Formerly one paragraph. Both re-phrased to improve clarity.

**Section 3.2.5 paragraph II**
Re-phrased to improve clarity.

**Section 3.2.6, paragraph 7**
Re-worded with instruction that start and end date of AQC sample batches must be recorded on the net gains record sheet.

**Section 3.3.3, paragraph I**
"in the samples are detected and identified correctly" replaces "are identified".

**Section 3.3.3, paragraph 3**
*

**Section 3.3.4, paragraph 2**
New paragraph with additional instruction, to ensure anonymity of AQC samples.

**Section 3.3.4, paragraph 3, first sentence.**
"... and either replace the labels or re-pot the sample (see Section 3.3.2)" added.

**Section 3.3.4, paragraph 3, sentence 4**
"or primary analysts" added; "before inspecting them" deleted from end of this sentence.

**Section 3.3.5**
Minor alterations to most paragraphs to improve the clarity of the explanations, but no changes to method. No reference to the term $AQL_w$.

**Section 3.3.6**
New section, extracted from Section 3.3.5, with minor alterations to improve the clarity of the explanations. The term $AQL_w$ is restricted to Table 3.2.

**Section 3.3.7, tile**
New title.

**Section 3.3.7, paragraph 1**
*

**Section 3.7.7, paragraph 10**
minor alteration to clarify that cusum is reset to zero following corrective action *following an alarm.*

**Section 3.3.8**
*cl used as abbreviation for confidence limit(s) throughout.

**Section 3.3.8, title**
New title.

**Section 3.3.8, paragraph 1**
*

**Section 3.3.8, paragraph 3**
*

**Section 3.3.8, paragraph 4**
*

**Section 3.3.11, paragraph 2**
New paragraph, explaining when the net gains recorded on the net gains record sheet is useful..

**Section 3.3.11, paragraph 3**
New paragraph, explaining how to complete the Net Gains Record Sheet and calculate the net gains in primary data.

**Section 3.3.11, paragraph 5**
*

**Section 3.3.12, paragraph 3**
*

**Section 3.4, paragraph 1**
* Note added that the audit is to be based on samples analysed for AQC by the end of February and the shortfall made up with primary samples. This was agreed by Biology Technical Group at their meeting in March 1999.

**Section 3.4.1, paragraph 1**
Note added that codes improve anonymity in annual audit reports.

**Section 3.4.1, paragraph 2**
New paragraph, about national audit manager maintaining record of the codes, and that all codes must be confirmed with them to ensure that all codes issued are unique.

**Section 3.4.3**
Reference to Section in BT001 corrected.

**Section 3.4.3, paragraph 2**
*

**Section 3.4.5**
Paragraph about the need for Regional Biologists to send audit data sheets to the National Biology Database deleted.

**Section 3.4.5, paragraph 1**
The national audit manager (John Murray-Bligh) replaces Roger Sweeting.
Instruction added for Regional Biologists to send copies of the audit results sheets to Areas (and analysing laboratory if different).

**Section 3.4.5, paragraph 4**
Split from paragraph 3. Note about Roger Sweeting receiving a full set of reports erroneous and deleted. Instruction for national audit manager to obtain IC codes for the report and to disseminate them to Biology Technical Group and to Areas added.

**Section 3.4.8, paragraph 2**
Advice added about how to avoid problems of contractors knowing which samples are to be audited.

**Section 3.6**
New section.

**Section 4.1**
Title altered.

**Section 4.1, paragraph 4**
New paragraph stating the role of the Net Gains Record Sheet.

**Section 5**
Slight amendment to the definitions for AQC parameters, BMWP-score, IFE , primary analysis, RIVPACS, singleton, sorting, taxon.
Entry for national audit manager added.

**Section 6**
References re-formatted to comply with "Guidance for production of R&D outputs, Version 1.1"

**Section 6**
Reference to Furse *et al.*, 1995 corrected.

**Appendix B**
New Appendix added.

**Appendix C**
Renumbered: formerly Appendix B.

**Figure 3.1**
*. Errors corrected: Sample 2 not retained. Sample 5 rather than sample 6 retained as it is unrealistic for primary data from AQC sample representing a scrapped or re-worked batch to be retained.

**Figure 3.5**
Minor alteration to figure and legend: communication centre replaces "control room".

**Table 3.1**
Maximum $AQL_A$ set to 2.

**Figure 4.3**
New figure (column headings re-phrased for clarity).

**Figure 4.4**
* New figure.