



NMBAQC

NE Atlantic Marine Biological Analytical Quality Control Scheme

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Benthic Invertebrate Component Own Sample Exercise Protocol

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1. Objective

The Own Sample exercise is a full audit; results are used to assess the suitability of data from a particular laboratory and project for the purposes of comparability with data from other sources. The exercise assigns pass/fail criteria (see [Benthic Invertebrate Component Scheme Standards, 2010](#)) and labs are required to conduct remedial action upon remaining replicates (detailed below) from failing samples. The objective of the Own Sample exercise is to ensure consistency of extraction and identification of biota from samples. The Own Sample audits are undertaken and reported by the scheme component's contractor (currently APEM Ltd.), or the scheme's approved external contractor. Any significant issues raised are reviewed by the component contract manager, on behalf of the NMBAQC committee.

All participants should be aware that if they choose to submit Competent Monitoring Authority (CMA) samples as part of their Own Sample submission, the results of the audits will be shared with the CMA owner of those samples.

The UK CMAs include the Environment Agency (EA), Cefas, Department of Agriculture, Environment and Rural Affairs, Northern Ireland (DAERA), Agri-food and Biosciences Institute, Northern Ireland (AFBI), Scottish Environment Protection Agency (SEPA), Marine Scotland, Natural England, Scottish Natural Heritage (SNH) and Natural Resources Wales (NRW).

2. Protocol

Initially, the contractor will send a **Data Request Form** to request a matrix of suitable completed sample data and samples for which residue material is available, from each laboratory. Own Sample participants must supply (on the **Data Submission Form**) their previous year's CSEMP/WFD/MCZ/MPA data matrices, where relevant, for Own Sample selection. If a laboratory has not been involved in CSEMP or WFD monitoring, then they should submit data for as many of their annual benthic samples as possible. A minimum of 20 samples is required for selection from each laboratory. **If fewer than 20 samples are available, please contact the auditor to discuss sample availability and alternative options. Samples proposed for Own Sample selection must not have been processed with prior knowledge of their Own Sample submission, i.e. laboratories must not pre-determine which samples will be provided for audit ahead of primary analysis. Samples must also not be 'checked' or altered once selected for audit; erroneous observations during sample submission preparation can be noted but the audit process will compare against the original primary data set.**

All data should be sent electronically to nmbaqc@apemltd.co.uk. The auditor randomly selects three samples from these data for examination. For large project datasets, the selection of samples for OS submission may follow a stratified random selection procedure, where cluster groups are isolated from the primary data set and a random selection of three cluster groups is followed by random sample selection within each group. This approach enables the audit process to cover a range of cluster groups (where present) and greatly assists the assignment of any potential remedial actions. The Own Sample selection will be communicated to the participating laboratory and a submission pack will be sent electronically.

Samples are reanalysed by the auditor following the guidelines of the NMBAQC Processing Requirements Protocol (PRP) and the NMBAQC Taxonomic Discrimination Protocol (TDP) ([Worsfold, Hall & O'Reilly \(Ed.\), 2010](#)).

Interim reports compare extraction efficiency, identification accuracy, enumeration accuracy and (where applicable) biomass estimates. Participating laboratory (Original Data or OD) vs. contractor (Audit Data or AD) data sets for each sample are compared using the Bray-Curtis similarity index (untransformed).

2.1 Pass / fail Criteria

Sample flagging scoring criteria are based upon the Bray Curtis Similarity Index (BCSI) as follows:

- BCSI scores of 100% achieve a PASS-EXCELLENT.
- BCSI scores of 95 to <100% achieve a PASS-GOOD;
- BCSI scores of 90 to <95% achieve a PASS – ACCEPTABLE;
- BCSI scores of 85 to <90% achieve a FAIL – POOR;
- BCSI scores of 0 to <85% achieve a FAIL – BAD.

2.2 Automatic Own Sample 'Fail'

An Own Sample will be subject to an automatic 'fail' if:

- selected samples are not submitted;
- residues are not submitted (such as where they have been discarded or lost);
- taxon pots have not been supplied (contact the contractor to discuss any individual non-supplied elements).

3. Preparation

- the laboratory should send **all** parts of each sample, in **70% Industrial Denatured Alcohol** (IDA) in sealed pots; certain taxa may be dried (e.g. encrusting Bryozoa);
- residues should be appropriate to the sieve mesh size stated in the Data Submission Form (i.e. if the sieve mesh size stated is 0.5 mm then sample residue of that mesh size should be submitted);
- residue pots should be labelled with the laboratory code and the Own Sample number;
- all extracted biota from the sample should be divided into separate sealed containers for each recorded taxon (occasional exceptions will be accepted – e.g., where a taxon is firmly attached to another) and labelled using the label sheet provided;
- electronic **Sample Detail Sheets** are provided to each laboratory. These must be completed for each Own Sample and submitted electronically. The forms record processing requirements, to ensure that the audit is comparable. Biomass (blotted wet-weight, 0.0001g) data by taxon should be submitted for audit, if available.

All samples and specimens are returned to the participating laboratory upon completion of the audit. To reduce transport costs and possible problems with preservative leakage, it is preferable that sorted sample residues be discarded rather than returned (following participants review of interim results). Participants should indicate (via the Sample Detail sheets) if they require sorted residues to be returned.

4. Interim Reports

Interim reports will be prepared for each participant. They include three summary tables with the following information:

- sample details, for the three submitted samples;
- comparisons of numbers of taxa and individuals;
- pass/fail flags and Bray-Curtis similarity indices.

Each submitted Own Sample will also be reported. Each sample's submission form will be included, followed by audit details, in table format. Each OS Interim Results table includes sample details, followed by sample data. The columns to the left contain the participant's original data (OD), with identification, abundance and (if appropriate) biomass. The adjacent columns include the equivalent

audit data (AD), provided by the contractor; to the right, there are comments on any changes and the results of the World Register of Marine Species (WoRMS) nomenclature check for taxon names. A Residue Resort table, below the original data, details additional material found in the sediment. Audit data and comments are colour coded. Revised text colour coding and comment standardization are detailed in the policy review report ([Worsfold, 2017](#)), and summarized below:

- **blue text** indicates full agreement in identification, enumeration and biomass;
- **green text** indicates a change which does not affect the audit. This may include an increase or decrease in taxonomic resolution, a recording policy difference, a spelling or nomenclature change, or a note on specimens that were not found (which are removed from the OD and AD);
- **red text** indicates an error with audit consequences. This would include identification errors, count errors and missed individuals or taxa; new taxa (found by APEM but missed from the original data) are indicated in the Residue Resort table.

Each Interim Results table also includes an ‘audit summary’, with the sample flag, and a ‘further action’ box. This details the remedial action that would be **required** in order for a failing sample to pass the audit, as well as **optional** actions that would improve the data.

4.1 Scoring policy

A review of past and current formats for describing differences between original and audit data is now available ([Worsfold, 2017](#)), which includes a preliminary standard format for future NMBAQC exercises. There remains a need for a clearly defined, repeatable scoring policy for audit samples. A preliminary version is provided below; we would expect this to be edited in future, with input from participants.

The following differences are considered errors, which will be marked in **red text** and influence audit scores and pass / fail flags.

Enumeration

Count differences contribute to statistical flags and missed individuals (from residue or from within taxon pots) add to count differences. Where the NMBAQC Scheme’s Processing Requirements Protocol ([Worsfold & Hall, Ed. O’Reilly, 2010](#)) states that a taxon should be enumerated, records of

'Present' are treated as count errors. Where a non-countable taxon has been missed or ignored (and the PRP requires it to be recorded), the audit record of 'Present' will be scored as 1.

Identification

A simple identification error involves a record of a taxon that the auditor considers to be a different taxon. Where a specimen pot contains a mixture of species (where one was recorded), a taxonomic error will be scored unless a clear processing oversight is evident, e.g. a single brittlestar in a polychaete taxon vial (recorded as a 'processing error').

An increase or decrease in taxonomic resolution would not normally constitute an error. However, over-cautious identifications and the use of multiple identifiers for a single taxon cause significant problems with the combination and comparison of data sets. It is also evident that some laboratories use, for example, 'juvenile' or 'damaged' categories as a 'catch-all' for some taxa. From Scheme year 2016/2017 (Year 23), over cautious identifications (defined) are scored as errors in the following circumstances:

- Where the TDP (Taxonomic Discrimination Protocol) specifies a required taxonomic level for a particular taxon, an error would be scored. Currently, the TDP is not developed but we hope that progress will be made, with input from participants;
- where the contractor has dropped a taxon to a higher taxonomic level, but can provide evidence that it was not the species recorded by the participant.

The following differences are considered policy differences, which will be marked in **green text** with no influence on audit scores or pass / fail flags. Some examples are listed below; they would be without audit impact where there is no specified NMBAQC Scheme policy for a particular example.

- juvenile size classifications, sex or growth stage assignments;
- taxonomic resolution differences; for example, the scheme contractor may identify some taxa to genus/species which the participating lab prefers to leave at a higher taxonomic level, or vice versa;
- recording presence of some taxa which a participating lab may choose to count, or vice versa;

- addition of aggregate or c.f. to a taxon name;
- identification of un-named species, with an identifier (e.g. 'type A').

4.2 Audit queries

Participants may question the results of audits if they believe mistakes have been made by the contractor. Any queries must be directed to the contractor in the first instance. Typically, the contractor will then request return of any disputed specimens for another check. If the auditors believe that the audit identification should be retained, they will provide further explanation. The procedure for further action following receipt of an Interim Own Sample Report is provided in Appendix I.

5. Remedial Action

When samples fail a QC audit, remedial action will be recommended to ensure the quality of data from the project or laboratory. Details of required or optional remedial action will be detailed under 'Further Action' on the Interim Results sheet for each sample. If an action is listed as 'required', it must be satisfactorily completed before the sample and project can have an 'amended to remedial pass' flag. Optional remedial action recommendations are suggestions for the participating laboratory to review in-house policy procedures, such as the assignment of juvenile to specimens, identification policies for certain taxa or extraction procedures in certain sediment types. Examples of remedial action required in different circumstances are described below.

5.1 Identification errors

Where an Own Sample fails an audit due to errors with identification, these errors should be checked and updated throughout the whole project. The data should be updated, sent to the NMBAQC Benthic Invertebrate component contractor for checking and then reissued to the ultimate client.

5.2 Extraction errors

Where an Own Sample fails an audit due to extraction efficiency, associated samples should be subject to further extraction checks. Definition of associated samples is provided below in section 5.4.

5.3 Biomass

In Own Sample audits, the total biomass estimation has a 20% margin for error. Failures are noted and reported (see Column 22 of Table one in the Own Sample Annual Reports) but do not form part of the overall sample pass/fail flag. APEM has previously highlighted some obvious differences in the individual Own Sample reports, such as where it appeared that either the material had not been weighed or that a transcription error may have occurred. The first step by the participant should be to investigate the variances and correct these data where appropriate. This is optional in terms of the Scheme's Own Sample remedial actions.

5.4 Repeat audits following remedial action

If a laboratory has had only one sample audited from a project of fewer than ten samples and it failed the audit, then all samples from the project are considered associated and should be re-checked. For CSEMP sites, the five replicate samples at each site will be deemed associated samples and remedial actions will be limited to each site, unless the audit report shows fundamental sample processing errors that would extend beyond site specific errors. For CSEMP 'sites' with dispersed (stratified) sampling, then the five (or more) samples collected over the CSEMP stratum are considered associated. Similarly for EU Water Framework Directive (WFD) sites where sampling is dispersed over a defined water body, then all the samples from that water body may be regarded as associated.

If a laboratory had multiple samples from the same project audited as part of the Own Sample process and only one sample failed, or if only one sample from a large (more than 10 samples) project was audited, and it failed the audit then:

- the whole dataset is run through Primer using untransformed Bray Curtis analysis;
- the results are then plotted using a cluster analysis (including the SIMPROF routine);
- all samples within the same cluster (as defined by SIMPROF) as the failing sample should be subject to re-sort remedial action. Where the SIMPROF routine shows >20 samples that are statistically similar to the failed sample, or statistically similar associated samples including Own Sample 'Pass' sample(s), a 20-30% similarity will be applied to limit the extent of the proposed remedial actions.
- if the sample's audit report shows fundamental sample processing errors that would extend beyond cluster group specific errors, wider remedial actions may be necessary.

More than one fail within a project would result in the above process for identifying associated samples being repeated for each failed sample.

5.5 Remedial Action Completion

In order for all remedial action to be signed off, the following procedures should be followed:

- Where a failure is due to extraction, one sample from the associated samples should be submitted for re-analysis by the NMBAQC Benthic Invertebrate component contractor. This will be subject to a re-analysis fee; repeat extraction failures will require repeat auditing until processing targets are achieved.
- Where a failure is due to identification, the updated data set should be submitted to the NMBAQC Benthic Invertebrate component contractor for checking.

Once the remedial action has been completed by the participant and reviewed/evaluated and agreed by the NMBAQC Benthic Invertebrate Contractor then the relevant Own Samples can receive a 'Pass - Remedial Action' flag. Thereafter the Statement of Performance, Own Sample Annual Report and Benthic Invertebrate Component Annual Report records, if not already issued, will be edited accordingly.

6. Timescales

Species / abundance data matrices and sample summary sheets should be sent to the auditor by **31st July**; selected Own Samples must be supplied to the auditor by **31st August** of each year.

All reporting should be completed before **31st March** of the following year.

If a participant requires an extension to the timescale, they should contact nmbaqc@apemltd.co.uk as soon as possible.

Any queries regarding Own Sample audits provided in the Interim reports must be submitted to the auditor by 31st May of the year following submission of data. No action can be taken on queries submitted after this date; it is the responsibility of each participant to deal with any issues that arise as soon as possible after receipt of the Own Sample Interim Report.

6.1 Remedial Action

All queries regarding Remedial Action should be submitted to nmbaqc@apemltd.co.uk within one month of issue of Interim Own Sample reports. For the process of further actions following receipt of Own Sample Interim Reports, see Appendix I.

It is expected that all Remedial Action be undertaken within two calendar months of issue of Interim Own Sample reports. Beyond this date, the Own Sample Annual Report, the Benthic Invertebrate Component Annual Report and the participants Statement of Performance cannot be updated and reissued.

Participants are reminded that the Scheme year ends on 31st March annually and therefore every effort should be made to complete remedial actions before the production of the annual report and Statement of Performance documents. After this date, there would be an additional charge for remedial action appeals or queries that relate to past years' Scheme components. Outstanding remedial actions are detailed and tracked in the annual reports.

APPENDIX I

Own Sample Interim Report Review and Remedial Action Processes



NMBQC

NE Atlantic Marine Biological Analytical Quality Control Scheme

Own Sample Interim Report Review and Remedial
Action Processes

Benthic Invertebrate Component

June 2016

Own Sample Interim Report Review Process

Own Sample Interim Report issued to participating laboratory

Participating laboratory reviews the interim report and returned samples (taxa and residue re-sort material)

Key:
NMBAQC Scheme representative action
NMBAQC Scheme participant action

Do you have any issues/questions to raise regarding the report?

Yes

no

Data from interim report will be presented in the OS summary report, annual report (anonymous) and shown on statement of performance sheet
All participants are encouraged to update their master data with audit data and notify other data users

Provide the NMBAQC auditor with detailed queries within a reasonable timeframe
Supply specimens for reanalysis by the auditor if requested

Auditor feedback on queries
This may include a revision of the Own Sample Interim Report

Have your issues/questions been resolved?

No

yes

Data from interim report will be presented in the OS summary report, annual report (anonymous) and shown on statement of performance sheet
All participants are encouraged to update their master data with audit data and notify other data users

Provide the NMBAQC auditor with detailed queries within a reasonable timeframe
Supply specimens for reanalysis by the auditor if requested

Where specimen identification is contested, these may be sent to a third party or recognised expert for comment
Auditor feedback on queries
This may include a revision of the Own Sample Interim Report

Have your issues/questions been resolved?

No

yes

Data from interim report will be presented in the OS summary report, annual report (anonymous) and shown on statement of performance sheet
All participants are encouraged to update their master data with audit data and notify other data users

Do you wish to escalate your issues?

Yes

no

Continue discussions with the NMBAQC auditor until satisfied with the outcome
Note that the NMBAQC auditor may recommend that the issues are escalated to the Contract Manager or NMBAQC Committee, if at an impasse

Request that the NMBAQC auditor passes queries to the Contract Manager for comment

Contract Manager provides a response to the participating laboratory

Have your issues/questions been resolved?

No

yes

Data from interim report will be presented in the OS summary report, annual report (anonymous) and shown on statement of performance sheet
All participants are encouraged to update their master data with audit data and notify other data users

Notify the NMBAQC auditor and Contract Manager that your issues have not been resolved and table the detail for discussion by the NMBAQC Committee via your Committee Representative
[Up to date Committee Representatives and contact details can be found on the NMBAQC website](#)
The NMBAQC Committee will review the details and provide a final ruling

Data from interim report will be presented in the OS summary report, annual report (anonymous) and shown on statement of performance sheet
All participants are encouraged to update their master data with audit data and notify other data users

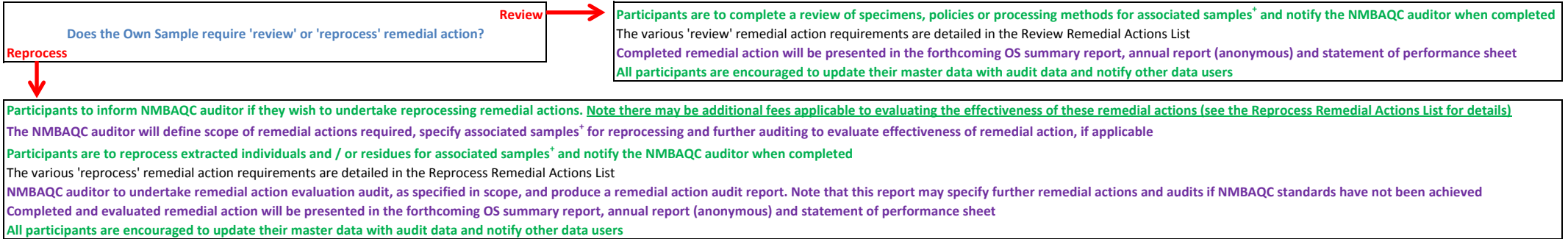
Own Sample Remedial Action Process

Own Sample Interim Report issued (following Interim Report Review Process) with details of recommended/suggested remedial action

[The NMBAQC scheme's Own Sample standards and pass/fail criteria are available on the scheme's website](#)

'FAIL' sample flags will remain until the remedial action process has been completed and evaluated

Key:
NMBAQC Scheme representative action
NMBAQC Scheme participant action



The processing laboratory can raise and escalate any issues/concerns throughout the remedial action process

If not resolved, the participating laboratory can request that the NMBAQC auditor passes their queries to the Contract Manager for comment

If still not resolved, the participating laboratory can notify the NMBAQC auditor and Contract Manager that the queries have not been resolved and table the detail for discussion by the NMBAQC Committee via your Committee Representative

[Up to date Committee Representatives and contact details can be found on the NMBAQC website](#)

The NMBAQC Committee will review the details and provide a final ruling

⁺ Associated samples will be defined by the NMBAQC auditor with assistance from the participating laboratory (i.e. reviewing full data set and sample variables). Remedial actions must not be undertaken without agreement of the scope required.

Remedial action requirements can include reprocessing all remaining samples in project/survey or an isolated batch of samples based upon sample type, habitat or analyst.

'Review' Remedial Actions List

'Review' remedial actions are completed and evaluated to attain 'Pass' sample flags for failing Own Samples. These remedial actions are mandatory for CMA data. The 'review' category is activated when an audit sample scores less than 90% Bray Curtis Similarity and the respective extraction (taxa/individuals) and taxonomic error values are between 5 and 10% or >10% and involving 2 or less units (taxa/individuals).

Remedial Action	Error Category	Requirement	Evaluation
Review extraction (individuals)	Individual missed in residue	Review methods/protocols for extraction, possible errors include: floating & blasting methods, petri dish searching methods, tray extraction procedures, quality assurance mechanisms, analyst capability/training	Receipt of written (email) confirmation of details and completion of review actions from participating laboratory. Sample flag changed to 'Pass - Remedial Action'
Review extraction (taxa)	Taxa missed in residue	Review methods/protocols for extraction, possible errors include: floating & blasting methods, petri dish searching methods, tray extraction procedures, quality assurance mechanisms, analyst capability/training, extraction policy	Receipt of written (email) confirmation of details and completion of review actions from participating laboratory. Sample flag changed to 'Pass - Remedial Action'
Review taxonomic errors	Taxonomic errors in extracted biota	Review methods/protocols for identification, possible errors include: literature, reference collection, staff training/contractor, quality assurance mechanisms, transcription error	Receipt of written (email) confirmation of details and completion of review actions from participating laboratory. Sample flag changed to 'Pass - Remedial Action'
Review enumeration methods	Count variance	Review methods/protocols for enumeration, possible errors include: mechanical counter malfunction, biomass loss/damage, handling care, 'countable' recording policy, <i>in situ</i> approximation, transcription error	Receipt of written (email) confirmation of details and completion of review actions from participating laboratory. Sample flag changed to 'Pass - Remedial Action'

'FAIL' sample flags will remain until the remedial action process has been completed and evaluated

'Reprocess' Remedial Actions List

'Reprocess' remedial actions are completed and evaluated to attain 'Pass' sample flags for failing Own Samples. These remedial actions are mandatory for CMA data. The 'reprocess' category is activated when an audit sample scores less than 90% Bray Curtis Similarity and the respective extraction (taxa/individuals) and taxonomic error values are between >10% and involving 2 or more units (taxa/individuals).

Remedial Action	Error Category	Requirement	Evaluation
Reprocess residues and taxonomic errors for associated samples ⁺	Extraction and identification	Reprocess associated samples ⁺ - resort residues and extracted biota for all associated samples ⁺ processed by a particular analyst or isolated by cluster analysis of the original data (to be defined by NMBAQC auditor). Provide the NMBAQC auditor with details of resorted residues and an updated data matrix for the full sample batch. A further sample will be randomly selected to evaluate the effectiveness of remedial actions (a separate purchase order will be required to commission additional auditing)	Receipt of revised data matrix following completion of remedial actions defined by the NMBAQC auditor. Successful audit of additional sample randomly selected by the NMBAQC auditor (additional fees apply @ 100% Own Sample rates). Sample flag changed to 'Pass - Remedial Action'. Note that failing additional audit samples will require further remedial actions.
Reprocess residues for associated samples ⁺	Individuals missed in residue	Reprocess associated samples ⁺ - resort residues for all associated samples ⁺ processed by a particular analyst or isolated by cluster analysis of the original data (to be defined by NMBAQC auditor). Provide the NMBAQC auditor with details of resorted residues and an updated data matrix for the full sample batch. A further sample residue will be randomly selected to evaluate the effectiveness of remedial actions (a separate purchase order will be required to commission additional auditing)	Receipt of revised data matrix following completion of remedial actions defined by the NMBAQC auditor. Successful audit of additional sample residue randomly selected by the NMBAQC auditor (additional fees apply @ 30% of the standard Own Sample rates). Sample flag changed to 'Pass - Remedial Action'. Note that failing additional audit samples will require further remedial actions.
Reprocess residues for associated samples ⁺	Taxa missed in residue	Reprocess associated samples ⁺ - resort residues for all associated samples ⁺ processed by a particular analyst or isolated by cluster analysis of the original data (to be defined by NMBAQC auditor). Provide the NMBAQC auditor with details of resorted residues and an updated data matrix for the full sample batch. A further sample residue will be randomly selected to evaluate the effectiveness of remedial actions (a separate purchase order will be required to commission additional auditing)	Receipt of revised data matrix following completion of remedial actions defined by the NMBAQC auditor. Successful audit of additional sample residue randomly selected by the NMBAQC auditor (additional fees apply @ 30% of the standard Own Sample rates). Sample flag changed to 'Pass - Remedial Action'. Note that failing additional audit samples will require further remedial actions.
Reprocess taxonomic errors for associated samples ⁺	Taxonomic errors in extracted biota	Reprocess associated samples ⁺ - reanalyse biota associated with taxonomic errors across full data set. Provide the NMBAQC auditor with a revised data matrix as evidence of completion of remedial actions and submit further specimens for identification review if requested to do so	Receipt of revised data matrix following completion of remedial actions defined by the NMBAQC auditor. Successful identification audit of any additional material requested by the NMBAQC auditor. Sample flag changed to 'Pass - Remedial Action'. Note that failing additional audit specimen identification will require further remedial actions.
Reprocess enumeration for associated samples ⁺	Count variance	Reprocess associated samples ⁺ - re-enumerate extracted biota associated with count variance errors across full data set. Provide the NMBAQC auditor with a revised data matrix as evidence of completion of remedial actions and submit further taxon vials for enumeration review if requested to do so.	Receipt of revised data matrix following completion of remedial actions defined by the NMBAQC auditor. Successful enumeration audit of any additional vials requested by the NMBAQC auditor. Sample flag changed to 'Pass - Remedial Action'. Note that failing additional audit enumeration will require further remedial actions.

Participants to inform NMBAQC auditor if they wish to undertake reprocessing remedial actions. [Note there may be additional fees applicable to evaluating the effectiveness of these remedial actions \(contact the NMBAQC auditor for current audit rates\)](#)

The NMBAQC auditor will define scope of remedial actions required, specify associated samples⁺ for reprocessing and further auditing to evaluate effectiveness of remedial action, if applicable

'FAIL' sample flags will remain until the remedial action process has been completed and evaluated

⁺ Associated samples will be defined by the NMBAQC auditor with assistance from the participating laboratory (i.e. reviewing full data set and sample variables). Remedial actions must not be undertaken without agreement of the scope required. Remedial action requirements can include reprocessing all remaining samples in project/survey or an isolated batch of samples based upon sample type, habitat or analyst.