PURPOSE AND PRINCIPLE

Method validation is about providing objective evidence that a method is suitable for a particular task. Method validation provides documented objective evidence that the test method measures what it is intended to measure with an acceptable level of performance.

This procedure describes the steps to be taken for the validation of chemical tests.

PERSONNEL

The work detailed in this procedure may be carried out by any suitably trained member of the laboratory staff on the authority of the relevant Section Leader. Guidance regarding the implementation of work covered by this procedure is available from a Laboratory Quality Scientist or a relevant Senior Scientist.

SAFETY

This procedure has no direct safety implications. Safety implications associated with carrying out a specific task are detailed in the relevant work procedure or test method.

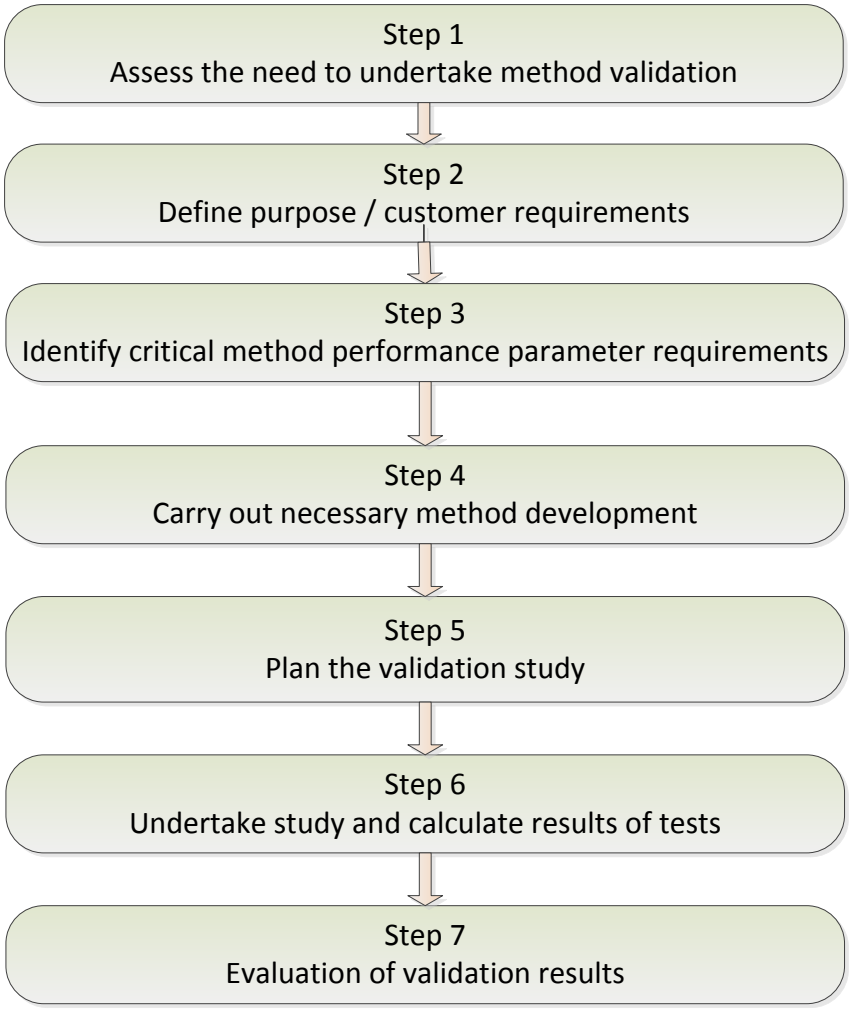
ENVIRONMENTAL CONTROL

Environmental controls detailed in the relevant work procedures or test methods will be applied where necessary.

CONTENTS

PROCEDURE

There are seven general steps for method validation:



Each of these steps will be discussed in detail through this procedure.

It is implicit in the validation process that the studies to determine method performance parameters are carried out using equipment that is within specification, working correctly and adequately calibrated. Likewise the operator carrying out the studies must be competent in the field of work under study and have sufficient knowledge related to the work to be able to make appropriate decisions from the observations made as the study progresses.

It is accepted for some procedures SEPA will follow MCERTS protocols or European Standards. In such situations validation requirements are likely to be fully specified as part of the MCERTS document or European Standard. For some field techniques e.g. use of sondes, landfill gas monitoring, etc. direct measurements are made therefore all that is required is verification that the instruments perform to manufacturers specifications or better. Comprehensive validation is generally not required but certain tests still need to be conducted. An example for validation for use of sondes is shown in Appendix B.

1 STEP 1 – ASSESS THE NEED TO UNDERTAKE METHOD VALIDATION

1.1 Assess whether method validation is required.

Method validation will always be required for a new method to be put into operation. For a change to a current method, some level of method validation will be required unless the change is considered insignificant (for example, a change in the supplier of a chemical reagent).

The following is a list of all circumstances which would require method validation to be carried out:

- Development of a new method
- Changes to an existing method
    - Introduction of a new determinand to an existing method
    - Introduction of a new stable matrix to an existing method
    - Introduction of a new stable matrix to an existing method
    - Replacement of a critical part of instrumentation e.g. detector
    - Significant change to the range of a method
    - Direct replacement of a significant piece of test equipment in test method
    - Changes to a sample preparation process
- Relocation of existing test equipment
- Transfer of method to a second laboratory
- Reassessment of method detection limit as part of a relevant validation review programme
- Reassessment of limit of quantitation as follow-up to a relevant validation review programme

NOTE: A change in operator is dealt with via the training and competency procedure. As long as the operator has been trained in the procedure and there is verification that performance is of the required standard then no further validation exercise is required.

2 STEP 2 – DEFINE PURPOSE / CUSTOMER REQUIREMENTS

2.1 Before any validation work is started, consideration must be given to whether or not the validation will result in a change to the fixed or flexible scope of accredited tests. The request for the change must be justified and approved on the Science Accreditation Change Record (BF-213).

Full details of the Change to Accreditation Procedure are given in BP-213.

2.2 Ensure there is a clear understanding of who will be using the results and what the results from the test method will be used for (i.e. the monitoring purpose).

2.3 Establish which matrices should be applied to the method.

2.4 Establish any relevant consent limits applicable to samples which would be submitted for method test analysis.

2.5 Establish any relevant Environmental Quality Standards or other standards which would be applicable to samples which would be submitted for method test analysis.

2.6 Establish likely concentration ranges of samples which would be submitted for method test analysis.

3 STEP 3 – IDENTIFY CRITICAL METHOD PERFORMANCE PARAMETER REQUIREMENTS

Sections 3.1 to 3.3 are given as a hierarchy of steps to follow. No further action is required in Step 3 once method performance requirements are established in a section.

3.1 Refer to ES-CHEM-G-002 (Analytical Performance Criteria for Chemical Parameters) to determine required performance parameters and their required values. If performance parameter requirements are not stated, proceed to Step 3.2.

3.2 For marine analysis, refer to the Clean Seas Environment Monitoring Programme (CSEMP) Green Book to determine required performance parameters and their required values. If performance parameter requirements are not stated, proceed to Step 3.3.

3.3 In the absence of pre-defined performance requirements, advice must be sought from the customer as to acceptable performance targets to attempt to achieve.

Consideration must be given to whether or not proposed performance targets could cause problems with how the measurement data will be interpreted and used. On the other hand, too much caution may result in excessively challenging performance targets which would require unnecessary laboratory resource to achieve.

3.3.1 For guidance, the following table may be used to help steer advice sought from customers:

	RSD %	Bias %	Target Accuracy (2xRSD)+Bias	Recovery %
Inorganics - aqueous	5	10	20	N/A
Inorganics - leachate	7.5	15	30	N/A
Inorganics – sediment, soils and waste solids	10	20	40	N/A
Inorganics – field measurements	10	20	40	N/A
Inorganics – air ambient and stack	10	20	40	N/A
Inorganics - biota	20	40	80	N/A
Organics - aqueous	12.5	25	50	60-120
Organics - leachate	18.75	37.5	75	50-130
Organics – sediment, soils and waste solids	25	50	100	50-130
Organics – air ambient and stack	25	50	100	50-130
Organics - biota	50	100	200	50-130
Metals - aqueous	7.5	15	30	85-115*
Metals - leachate	11.25	22.5	45	N/A
Metals – sediment, soils and waste solids	15	30	60	N/A
Metals – air ambient and stack	15	30	60	N/A
Metals - biota	30	60	120	N/A

*Only applicable to methods which require recovery correction of sample results, otherwise N/A

3.3.2 For guidance, the following table may be used to help steer performance targets for method detection limits:

	Determinand Where EQS Exists	Determinand Where No EQS Exists
Surface; Ground and Saline Waters	PHS; PS; SP – 1/10th EQS	Surface and Ground Waters – customer agreed target Saline Waters – CSEMP Green Book target
Treated Sewage and Trade Effluents	PHS – 1/10th EQS PS; SP – 2 x EQS	CAR license – 1/10th consent limit Non-CAR license – customer agreed target
Other Matrices	WFD matrices - PHS; PS; SP – 1/10th EQS Other matrices – customer agreed target	Customer agreed target

PHS = Priority Hazardous Substance; PS = Priority Substance; SP = Specific Pollutant

4 STEP 4 – CARRY OUT NECESSARY METHOD DEVELOPMENT

4.1 Appropriate records for method development must be kept.

4.1.1 Method development plans should be recorded within a dedicated laboratory notebook and/or electronically recorded. These records should include an initial draft of the proposed method to be used.

4.1.2 If the proposed method is significantly new in comparison to existing methods, records should also include hazard identification and risk assessment following procedure HSMS/2.3.1.

4.1.3 If the proposed method is significantly new in comparison to existing methods, a COSHH Assessment Form (HSMS/3.3.4) should be completed prior to undertaking any new method development work.

4.2 Appropriate considerations must be made when designing the method approach to be developed.

4.2.1 Method development will involve optimisation of the whole method process (sampling, sample preparation and instrument analysis where applicable). This optimisation should be carried out with regard to the performance parameter requirements to encompass the following:

- Selectivity and specificity
- Precision
- Bias
- Recovery – where applicable
- Accuracy
- Detection capability
- Linearity and working range
- Ruggedness
- Potential sources of uncertainty

4.2.2 Method development should be carried out in order to deliver a method which is fit-for-purpose. Consideration should also be given to any imminent future requirements which are likely to be requested. This may arise through expected legislation changes.

4.2.3 Method development should be carried out with full consideration of health and safety, environmental impacts and resource implications. Consideration should include the following:

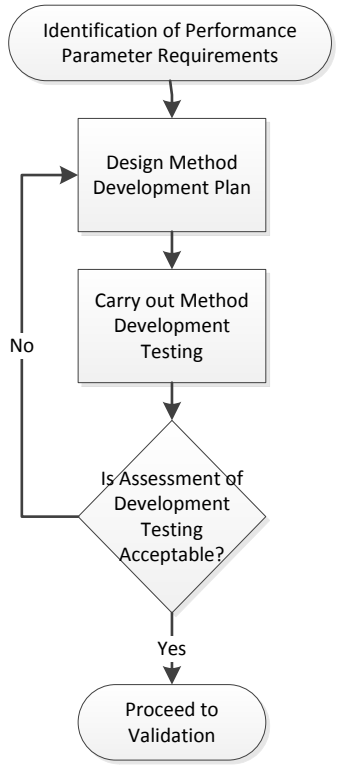
- Hazards
- Precautions required to control risks
- Possible alternatives to minimise hazards and risks
- Expected levels of waste produced by method
- Expected levels of consumables and reagents required by method
- Expected costs of equipment, consumables and reagents required by method
- Possible alternatives to minimise levels of consumables and reagents required, waste produced and energy consumption
- Possible alternatives to minimise staff resource required by method
    - Combination of method steps
    - Merging of method with other existing methods

4.2.4 During method development, if unspiked test matrices are found to contain positive component levels then further specificity checks may be required. This is particularly relevant to organics analysis. If information is available on typical environmental levels of the component of interest, then this should be used in the assessment of method component specificity.

Consideration must be given to the specificity of the technique in use (e.g. peak shape, multi-dimension detector specificity) and further tests involving multiple matrix sources, multiple instrument columns and/or alternative analytical techniques may be required to help ensure method component specificity.

4.3 When initial method development steps and instrument set-up have been established, it is prudent to carry out further preliminary testing to obtain an indication of expected method performance. Many of the method performance parameters associated with method validation can be evaluated approximately during method development.

The following diagram details the process that can be followed prior to method validation to gain confidence in the method set-up before committing the significant resource required to complete method validation.



NOTE: It is not essential to carry out the following preliminary method development steps if there is sufficient confidence that the proposed method should meet the required performance parameter requirements during method validation.

4.3.1 Where multiple instruments / laboratories have to implement the same analysis, only one instrument / laboratory requires full development. A lead chemist under the guidance of a project manager should be appointed to provide a coordinated approach.

4.3.2 Preliminary Stage 1

This consists of a verification that the procedure is capable of meeting the performance requirements short term. It will provide an indication of precision, bias and/or recovery from a controlled matrix (for example, interference-free water). In this way, the basic method can be tested without effects from real matrix.

6 or 11 replicates of the following test types can be included in Stage 1:

Direct Methods

- Blank
- Instrument Performance Standard equivalent to 10% of expected method range
- Instrument Performance Standard equivalent to 90% of expected method range

Pre-Treatment Methods

- Process Blank
- Process Check Standard equivalent to 10% of expected method range
- Process Check Standard equivalent to 90% of expected method range

Definitions and purposes of test types are given in **APPENDIX A**.

NOTE: If the resource effort required to prepare and analyse the above test replicates is considered significant, it is permissible to prepare and analyse two replicates only of the Blank or Process Blank test type.

NOTE: 10% and 90% level test types are not absolute required levels and in some situations it may be more practicable to spike to alternative levels (e.g. 10-30% and 50-90%). For example, if method performance is expected to return a large precision then the spiking level must be chosen to ensure there is no risk of delivery of final results over or below the method range. Also, it should be ensured that these test types are set at a level appropriate to the actual method range (for example, a method with a range of 8 to 100 mg/l would have a 10% test type not at 10 mg/l but at 17 mg/l).

Ideally these replicates should be analysed randomly in one batch of analysis where possible. However, this may not always be possible due to batch size capacity and it is this circumstance it must be ensured that all replicates for each test type are analysed within a single batch.

Results should be entered onto spreadsheet ES-VALID-S-009 and indicative performance compared to performance targets. Where results indicate that the required performance is capable of being achieved then the second stage can be started. If required performance has not been achieved then further method development may be required to improve method performance and this stage should be repeated.

Any accepted deviations from target performance should be commented upon and justified before proceeding.

See APPENDIX C on details on how to use spreadsheet ES-VALID-S-009.

4.3.3 Preliminary Stage 2

This consists of verification that the procedure is capable of meeting performance requirements short term. It will provide an indication of precision, bias and/or recovery from a real matrix for which the method is intended to be used. In this way, the method can be tested with effects from real matrix.

6 or 11 replicates of the following test types can be included in Stage 2:

Direct and Pre-treatment Methods:

- Unspiked Sample Matrix
- Spiked Sample Matrix – spiked between 50 and 90% of method range

Or

- Certified Reference Material (CRM)

Definitions and purposes of test types are given in APPENDIX A.

NOTE: If the resource effort required to prepare and analyse the above test replicates is considered significant, it is permissible to prepare and analyse two replicates only of the unspiked sample matrix test type. It is not permissible to reduce the number of replicates of the spiked sample matrix test type.

Ideally these replicates should be analysed randomly in one batch of analysis where possible. However, this may not always be possible due to batch size capacity and it is this circumstance it must be ensured that all replicates for each test type are analysed within a single batch.

Results should be entered onto spreadsheet ES-VALID-S-009 and indicative performance compared to performance targets. Where results indicate that the required performance is capable of being achieved then the validation study can be started. If required performance has not been achieved then further method development may be required to improve method performance and this stage should be repeated.

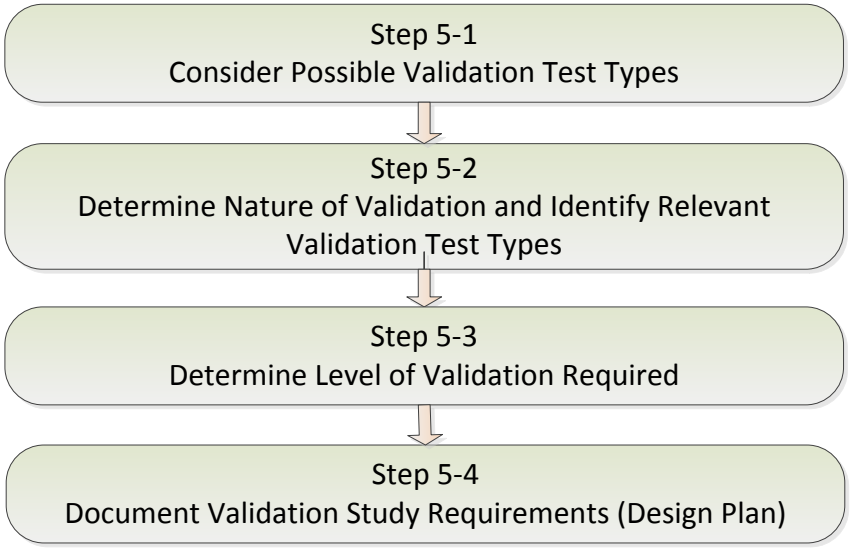
Any accepted deviations from target performance should be commented upon and justified before proceeding.

See APPENDIX C on details on how to use spreadsheet ES-VALID-S-009.

NOTE: Stage 2 testing above is only required for a single matrix during method development. If a method is intended for multiple matrices, and it is expected that several matrices behave similarly enough to warrant them to be treated as a single matrix group, then this will require demonstration during method validation.

5 STEP 5 – PLAN THE VALIDATION STUDY

There are four general sub-steps for planning the validation study:



5.1 Step 5-1 – Consider Possible Validation Test Types

The validation will require one, some or all of the following test types depending on the reason for the validation exercise.

5.1.1 For Direct Methods:

- Blank
- Instrument Performance Standard equivalent to 10% of expected method range
- Instrument Performance Standard equivalent to 90% of expected method range
- Certified Reference Material (CRM) or
  - Unspiked Sample Matrix
  - Spiked Sample Matrix – spiked between 50 to 90% of method range
- Method Detection Limit test types – see Section 5.2.9
- Limit of Quantitation test types– for WFD parameters only - see Section 5.2.10

Definitions and purposes of test types are given in APPENDIX A.

NOTE: 10% and 90% level test types are not absolute required levels and in some situations it may be more practicable to spike to alternative levels (e.g. 10-30% and 50-90%). For example, if method performance is expected to return a large precision then the spiking level must be chosen to ensure there is no risk of delivery of final results over or below the method range.

5.1.2 For Pre-Treatment Methods:

- Process Blank
- Process Check Standard equivalent to 10% of expected method range
- Process Check Standard equivalent to 90% of expected method range
- Certified Reference Material (CRM) or
  - Unspiked Sample Matrix
  - Spiked Sample Matrix – spiked between 50 to 90% of method range
- Method Detection Limit test types – see Section 5.2.9
- Limit of Quantitation test types– for WFD parameters only - see Section 5.2.10

NOTE: 10% and 90% level test types are not absolute required levels and in some situations it may be more practicable to spike to alternative levels (e.g. 10-30% and 50-90%). For example, if method performance is expected to return a large precision then the spiking level must be chosen to ensure there is no risk of delivery of final results over or below the method range. Also, it should be ensured that these test types are set at a level appropriate to the actual method range (for example, a method with a range of 8 to 100 mg/l would have a 10% test type not at 10 mg/l but at 17 mg/l).

Definitions and purposes of test types are given in APPENDIX A.

5.1.3 For matrix test types, consideration must be given to the UKAS categorisation for aqueous matrices. UKAS have classified water matrices relevant to SEPA into the following broad matrix categories:

- Ground Water
- Surface Water
- Land Leachate
- Prepared Leachate
- Untreated Sewage
- Treated Sewage
- Trade Effluent
- Saline Water

If a method is intended to be used for multiple matrices which fall into a single matrix category above, then these should be treated as a single matrix.

Although there is no UKAS matrix categorisation for non-aqueous matrices, these should be considered in a similar manner.

5.2 Step 5-2 – Determine Nature of Validation and Identify Relevant Validation Test Types

Although a number of circumstances will require the need for validation (see Section 1), the validation test types required will depend on the circumstance in hand. The items in the list of circumstances in Section 1.1 are considered in turn.

NOTE 1: Limit of Quantitation test type is only required for WFD parameters. See Section 5.2.10 for details on the selection of appropriate test types to use to determine Limit of Quantitation performance.

NOTE 2: CRMs should be used where they are available, in appropriate matrix and at appropriate levels. If a suitable CRM for all required determinands and matrices is available, it is not necessary to run Unspiked Sample Matrix and Spiked Sample Matrix test types.

NOTE 3: Method Detection Limit test type requirements is dependent on the nature of the method and the availability of blank matrix. See Section 5.2.9 for details on the selection of appropriate test types to use to determine method detection limits.

5.2.1 *Development of a new method:*

- Direct methods would require <u>all</u> test types listed in Section 5.1.1
- Pre-treatment methods would require <u>all</u> test types listed in Section 5.1.2

NOTE: If a suitable CRM for all required determinands and matrices is available, it is not necessary to run Unspiked Sample Matrix and Spiked Sample Matrix test types.

5.2.2 *Changes to an existing method – Introduction of a new determinand to an existing method:*

- Direct methods would require <u>all</u> test types listed in Section 5.1.1
- Pre-treatment methods would require <u>all</u> test types listed in Section 5.1.2

NOTE: If a suitable CRM for all required determinands and matrices is available, it is not necessary to run Unspiked Sample Matrix and Spiked Sample Matrix test types.

5.2.3 *Changes to an existing method – Introduction of a new stable matrix to an existing method:*

The scope of the validation exercise should consider the range of matrices to which the method will be applied. Tests must be carried out on samples representative of the matrix or matrices.

When combining matrices within one test method, care must be taken to ensure that the method achieves the minimum standard of performance required. Before combining matrices, consideration must be given to the physical properties of the matrices (e.g. turbidity) and how these may affect the final sample result. In general it would be assumed that 'dirty' matrices such as effluents would not be combined with 'clean' matrices such as rivers unless a suitable justification is given to do so.

Direct and pre-treatment methods would require a <u>minimum</u> of the following test types:

- Certified Reference Material (CRM) or
- Unspiked Sample Matrix
- Spiked Sample Matrix – spiked between 50 to 90% of method range

If the new matrix cannot be demonstrated to be similar to an already existing method matrix, then the following test types would also be required:

- Certified Reference Material (CRM) or
- Unspiked Sample Matrix
- Spiked Sample Matrix – spiked between 50 to 90% of method range
- Method Detection Limit test types – see Section 5.2.9
- Limit of Quantitation test types – for WFD parameters only - see Section 5.2.10

5.2.4 *Changes to an existing method – Replacement of critical part of instrumentation (e.g. detector):*

It is possible that replacement of a critical part of instrumentation may affect overall instrument performance.

However, any change in performance will be assessed in terms of significance through on-going statistical control charts and any change in the method ability to achieve the method detection limit will be considered by assessment of the instrument detection limit.

5.2.4.1 Instrument detection limit will be assessed as follows for key performance determinands only (as identified by the method System Suitability QC measure):

- Methods which are capable of returning numeric values for levels below the instrument detection limit (i.e. negative values) would require the following test types:
  - Blank

- Methods which are not capable of returning numeric values for levels below the instrument detection limit would require the following test types:
  - Instrument Performance Standard - spiked at approximately 2 to 5 times estimated LOD

5.2.4.2 Instrument detection limits for the key performance determinands would then be directly compared to previous instrument detection limits. This will be by direct comparison with previous method detection limit for direct methods or by back calculation to obtain previous expected instrument detection limits for pre-treatment methods.

5.2.4.3 If estimated method detection limits for the key performance determinands demonstrate a significant deterioration in performance as a result of replacement of a critical part of instrumentation (a deterioration greater than 30% is considered significant) then consideration should be given to reassessing method detection limits for all method determinands (Section 5.2.9).

5.2.4.4 If on-going statistical control charts demonstrate a significant deterioration in performance as a result of replacement of a critical part of instrumentation then consideration should be given to reassessing method performance according to use of a new piece of critical instrumentation (Section 5.2.6)

5.2.5 *Changes to an existing method – Significant change to the range of method:*

If the upper end of the method range has been extended then the 90% method range level will require validation. If the lower end of the method range has been extended then the 10% method range level will require validation.

- Direct methods would require the following test types:
  - Blank

  and
  - Instrument Performance Standard equivalent to 10% of expected method range

  or
  - Instrument Performance Standard equivalent to 90% of expected method range

- Pre-treatment methods would require the following test types:
  - Process Blank

  and
  - Process Check Standard equivalent to 10% of expected method range

  or
  - Process Check Standard equivalent to 90% of expected method range

If the lower end of the method range has been extended so that the detection limit requires reassessment then Section 5.2.9 should be followed.

5.2.6 *Changes to an existing method – Direct replacement of a significant piece of test equipment in test method:*

It is expected that direct replacement of a significant piece of test equipment will affect overall method performance. As long as the replacement equipment does not have a significantly different mode of operation, it can be assumed that:

- Matrix effects towards the new test equipment will be equivalent to matrix effects towards the old test equipment.
- For direct methods, the Instrument Performance Standard test types can be considered as equivalent to real matrix samples, as demonstrated by original validation data.
- For pre-treatment methods, the Process Check Standard test types can be considered as equivalent to real matrix samples, as demonstrated by original validation data.

- Direct methods would require the following test types:
  - Blank
  - Instrument Performance Standard equivalent to 10% of expected method range
  - Instrument Performance Standard equivalent to 90% of expected method range
  - Method Detection Limit test types – see Section 5.2.9
  - Limit of Quantitation test types – for WFD parameters only - see Section 5.2.10

- Pre-treatment methods would require the following test types:
  - Process Blank
  - Process Check Standard equivalent to 10% of expected method range
  - Process Check Standard equivalent to 90% of expected method range
  - Method Detection Limit test types – see Section 5.2.9
  - Limit of Quantitation test types – for WFD parameters only - see Section 5.2.10

NOTE: Detection limit reassessment should be carried out following steps in Section 5.2.9.

5.2.7 *Relocation of existing test equipment:*

In the absence of a UKAS agreed laboratory move validation protocol, the following validation is required:

- Direct methods would require the following test types:
  - Blank
  - Instrument Performance Standard equivalent to 10% of expected method range
  - Instrument Performance Standard equivalent to 90% of expected method range
  - Method Detection Limit test types – see Section 5.2.9

- Limit of Quantitation test types – for WFD parameters only - see Section 5.2.10

- Pre-treatment methods would require the following test types:
  - Process Blank
  - Process Check Standard equivalent to 10% of expected method range
  - Process Check Standard equivalent to 90% of expected method range
  - Method Detection Limit test types – see Section 5.2.9
  - Limit of Quantitation test types – for WFD parameters only - see Section 5.2.10

NOTE: Detection limit reassessment should be carried out following steps in Section 5.2.9. Limit of Quantitation assessment should be carried out following steps in Section 5.2.10.

5.2.8 *Transfer of method to second laboratory:*

- Direct methods would require the following test types:
  - Blank
  - Instrument Performance Standard equivalent to 10% of expected method range
  - Instrument Performance Standard equivalent to 90% of expected method range
  - Method Detection Limit test types – see Section 5.2.9
  - Limit of Quantitation test types – for WFD parameters only - see Section 5.2.10

- Pre-treatment methods would require the following test types:
  - Process Blank
  - Process Check Standard equivalent to 10% of expected method range
  - Process Check Standard equivalent to 90% of expected method range
  - Method Detection Limit test types – see Section 5.2.9
  - Limit of Quantitation test types – for WFD parameters only - see Section 5.2.10

NOTE: Detection limit reassessment should be carried out following steps in Section 5.2.9. Limit of Quantitation assessment should be carried out following steps in Section 5.2.10.

5.2.9 *Reassessment of method detection limit as part of a relevant validation review programme:*

Although method sensitivity is monitored via a routine method System Suitability QC checks, actual detection limit determination may be compromised over time due to instrument deterioration. Method detection limits must be re-evaluated every six years as a minimum.

NOTE: a group of matrices may be represented by a single matrix. For aqueous samples, for example, normal practice would be to use a representative matrix for ‘cleaner’ matrices (e.g. river, ground water, marine water) and a representative matrix for ‘dirty’ matrices (e.g. discharges).

In addition, for other validation circumstances requiring detection limit determination (5.2.1 to 5.2.8) the following steps should also be applied.

5.2.9.1 For methods which are capable of returning numeric values for levels below the instrument detection limit (i.e. negative values), the method detection limit is ideally determined using a blank real sample matrix which contains no determinand.

- Direct and pre-treatment methods would require the following test types:
  - Unspiked Sample Matrix - blank

5.2.9.2 For methods which are not capable of returning numeric values for levels below the instrument detection limit (e.g. chromatographic methods), there should be a measurable amount of the determinand of interest. The method detection limit is ideally determined by using blank real sample matrix which contains no determinand which has then been spiked to return an instrument response which is approximately 2 to 5 times the actual instrument detection limit.

Alternatively, a real matrix sample may be used with sufficiently low level of determinand naturally present. There would not be a requirement to spike this matrix sample.

- Direct and pre-treatment methods would require the following test types:
  - Spiked Sample Matrix – spiked to approximately 2 to 5 times estimated MDL

  or
  - Unspiked Sample Matrix –sufficiently natural low level

NOTE: The lower the returned instrument response then the lower the determined method detection limit is likely to be.

5.2.9.3 When it is not possible to obtain a sample which has no (or sufficiently low) determinand levels to satisfy steps 5.2.9.1 or 5.2.9.2 above, sample matrix with significant determinand levels may be used but there is a significant risk of over-estimation of the detection limit. If the performance target remains met then this risk is allowable.

5.2.9.4 When determinand levels are of a magnitude to risk over-estimation of detection limit and so is expected to not meet the performance target, it is acceptable to determine a detection limit based on the difference between a spiked and an unspiked real sample matrix. The unspiked real sample matrix determinand level must be sufficiently low to allow effective spiking of a low concentration spike.

- Direct and pre-treatment methods would require the following test types:
  - Unspiked Sample Matrix –
  - Spiked Sample Matrix – spiked with approximately 2 to 5 times estimated MDL

5.2.9.5 For some method matrices, it may not be possible to source a sample which has a determinand level sufficiently low enough to allow further spiking within the method range. This is particularly true for sediment samples which have naturally high levels of organics which does not facilitate further spiking of low concentration spikes.

When determinand levels are not sufficiently low to allow spiking as required in step 5.2.9.4 above, it is permissible to determine method detection limit using a blank or process blank test type. Caution must be used to determine detection limits in this way since matrix effects are not accounted for.

- Methods which are capable of returning numeric values for levels below the instrument detection limit (i.e. negative values) would require the following test types:
  - Direct methods would require the following test types:
    - Blank
  - Pre-treatment methods would require the following test types:
    - Process Blank

- Methods which are not capable of returning numeric values for levels below the instrument detection limit would require the following test types:
  - Direct methods would require the following test types:
    - Instrument Performance Standard - spiked at approximately 2 to 5 times estimated instrument LOD
  - Pre-treatment methods would require the following test types:
    - Process Check Standard - spiked at approximately 2 to 5 times estimated MDL

5.2.9.6 Method detection limit would then be calculated by accounting for method calculation factors and recovery factors.

5.2.9.7 If reassessment of detection limit results in a change to the reported method detection limit, it may be necessary to re-evaluate the method limit of quantitation (LOQ). See Section 5.2.10 for details on how to determine LOQ.

5.2.10 *Reassessment of limit of quantitation as follow-up to a relevant validation review programme:*

Validation requirements for limit of quantitation (LOQ) determination are applicable to WFD reportable parameters only. The target level of limit of quantitation is 30% of the relevant EQS.

As detection limit determination may be compromised over time due to instrument deterioration, limit of quantitation may also be compromised in the same way. Limit of quantitation levels and the corresponding performance characteristics must be reassessed following any re-evaluation of method detection limits.

In addition, for other validation circumstances requiring limit of quantitation determination (5.2.1 to 5.2.8) the following steps should also be applied.

5.2.10.1 The limit of quantitation is ideally determined using a blank real sample matrix which contains no determinand.

- Direct and pre-treatment methods would require the following test types:
  - Unspiked Sample Matrix - blank
  - Spiked Sample Matrix – spiked at a level corresponding to expected LOQ level

5.2.10.2 If it is not possible to source a matrix which contains no determinand, then a matrix with low levels of determinand may be used and this must be spiked appropriately to make the final concentration of determinand in the sample matrix correspond to the required level.

5.2.10.3 For some method matrices, it may not be possible to source a sample which has a determinand level sufficiently low enough to allow further spiking to the required LOQ level. When determinand levels are not sufficiently low to allow spiking as required in step 5.2.10.1 – 5.2.10.2 above, it is permissible to determine limit of quantitation using a blank or process blank test type. Caution must be used to determine LOQ levels in this way since matrix effects are in no way accounted for.

- Direct methods would require the following test types:
  - Instrument Performance Standard - spiked at a level corresponding to expected LOQ level

- Pre-treatment methods would require the following test types:
  - Process Check Standard - spiked at a level corresponding to expected LOQ level

5.2.10.4 The concentration level to spike the sample matrix will be dependent on the general performance requirements for the method, the routine QC level performance and the achieved method detection limit. The following table details the minimum level for limit of quantitation to be applied.

Routine Method Statistical QC Level	Routine Method Statistical QC Performance	LOQ Level Determination
QC < 10 x MDL	Actual method QC RSD < 50% target RSD	LOQ = MDL x 2.15
QC < 10 x MDL	50% target RSD < Actual method QC RSD < 100% target RSD	LOQ = MDL x 3
QC < 10 x MDL	Actual method QC RSD > 100% target RSD	LOQ = MDL x 4
QC > 10 x MDL	Actual method QC RSD < 100% target RSD	LOQ = MDL x 3
QC > 10 x MDL	Actual method QC RSD > 100% target RSD	LOQ = MDL x 4

For methods which are routinely running, the QC performance should be taken from the routine method statistical control charts.

For methods which have been newly validated, the QC performance should be taken from the validation test type which most closely resembles the intended on-going method statistical QC measure.

NOTE: the above table details the minimum multiple to apply to the method detection limit to set the limit of quantitation level. If the method detection limit is not sufficiently low then the limit of quantitation target of 30% of the EQS will not be met.

5.2.10.5 Before the limit of quantitation can be determined, performance targets for limit of quantitation must be satisfied. The target performance for LOQ is in the following table:

Target Method RSD %	Target LOQ RSD%	Target LOQ Bias %
5	≤ 10	≤ 20
7.5	≤ 15	≤ 30
12.5	≤ 25	≤ 50
30	≤ 60	≤ 120
50	≤ 100	≤ 200

5.2.10.6 If the performance targets in 5.2.10.5 are not satisfied then the validation must be repeated at a higher multiple of the method detection limit until the performance targets are met.

5.2.10.7 Unlike the determination of MDL it is permissible to use single batches of the correct matrix type when determining LOQ.

5.2.11 *Assessment of pre-preparation stage repeatability:*

Some analyses will require a pre-preparation stage to produce a number of pre-prepared samples to then progress analyses using further analytical procedures. It is critical that such a pre-preparation stage demonstrates the required repeatability to ensure representative pre-

prepared samples are taken forward for subsequent analyses. The following validation is required:

5.2.11.1 For follow-on analyses for which any spiking process has potential to affect overall integrity of sample matrix being tested for repeatability (for example, sample leachability of metals could be affected by a change to the sample pH caused by the spiking process):

Direct and pre-treatment methods would require the following test types:

- Unspiked Sample Matrix

Only components present at prevalent concentrations can be used in the overall assessment.

5.2.11.2 For follow-on analyses for which any spiking process does not have potential to affect overall integrity of sample matrix being tested for repeatability:

Direct and pre-treatment methods would require the following test types:

- Spiked Sample Matrix

5.3 Step 5-3 – Determine Level of Validation Required

5.3.1 Considerations to be made are presented below:

- Is a comprehensive data set of method performance required to establish method performance for the first time?
- Have method changes been carried out to improve method performance?
- Does method validation already exist and the aim is to demonstrate that method changes can deliver equivalent or better performance to that previously quoted?
- Does method validation already exist and the aim is to demonstrate that individual matrices behave similarly so that they can be considered as a combined group?

5.3.2 *Full Validation Applicability*

In literature, full validation generally includes collaborative studies with different laboratories using the same method on different equipment. SEPA methods are often well established or based on national or international standards and as such these have been previously subject to full validation.

Within SEPA the term “full validation” is taken to mean the evaluation of the long term within-laboratory performance in terms of reproducibility, bias accuracy and recovery. In line with literature definition, the limit of detection of the method is evaluated in terms of within-batch performance. The term within-batch is described in more detail in Appendix D, however it specifically does not refer to performance within a *single* batch.

Please note that other publications may use this term differently.

Full validation is <u>always</u> required in the following circumstances:

- Development of a new method
- Change to an existing method – Introduction of a new determinand to an existing method

- Reassessment of method detection limit as part of a relevant validation review programme

If previous method performance is not to be retained and new performance characteristics are sought then full validation would be required in the following circumstances:

- Change to an existing method – Introduction of a new stable sample matrix to an existing method
- Change to an existing method – Significant change to the range of a method
- Change to an existing method – Direct replacement of a significant piece of test equipment in test method
- Change to an existing method – Relocation of existing test equipment
- Change to an existing method – Transfer of method to second laboratory

5.3.3 *Full Level Design*

5.3.3.1 Full validation would normally consist of sets of duplicates or quadruplicates of the validation test types outlined in Sections 5.1 and 5.2.

For example, a 11 x 2 validation study would comprise of 11 separate batches with each batch consisting of all required validation test types analysed in duplicate.

5.3.3.2 The validation study must provide adequate validation testing to provide an assessment of method performance without entailing an unmanageable amount of work or excessive and unsustainable costs. Figure 2 exemplifies this.

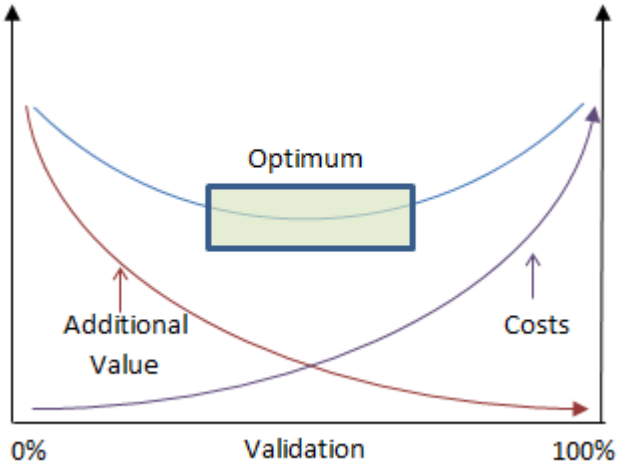


Figure 2

The selection and validation of methods needs to be considered in the context of 'cost-benefit' and 'fitness-for-purpose' criteria. This will help establish a validation plan and assess the effort required. The purpose of undertaking the validation exercise must be clear so that a suitable exercise is developed.

In order to ensure that business outputs are not compromised by the commitment of excessive resource towards validation testing, the following risk table should be used to ascertain

acceptable minimum validation level to be carried out whilst ensuring an appropriate amount of rigour to ensure the method is suitable for its intended purpose. This will vary according to the importance of any decisions that will be based on the measurement results.

The following table makes use of estimated method standard analysis times (SATs). For a new method, the SAT may be estimated from information on existing SEPA methods. The more complex the method, the higher the SAT will be and it can be assumed that the method will be more costly not just in terms of staff resource, but also in terms of cost of equipment, reagents and consumables.

Type of Method Samples	Method Resource Low (SAT ≤0.1)	Method Resource Medium (0.1 <SAT ≤1.0)	Method Resource High (SAT >1.0)
Formal	11 x 2 or 6 x 4	11 x 2 or 6 x 4	6 x 2
Regulatory	11 x 2 or 6 x 4	11 x 2 or 6 x 4	6 x 2
Non-Regulatory	11 x 2 or 6 x 4	6 x 2	6 x 2

The above table satisfies the NS30 criterion of batch size x number of batches ≥ 10 for each proposed minimum validation level. Most validation levels satisfy the NS30 recommendation of batch size x number of batches ≥ 20 unless business needs and/or costs do not facilitate this recommendation.

5.3.4 *Full Validation Batch Requirements*

5.3.4.1 A validation study of sets of duplicates or quadruplicates of the validation test types would be analysed as separate batches preferably on different days but as a minimum, the method instrument must be allowed to return to 'ground state' between batches. Instrumentation must be returned to its ground state between each exercise to avoid falsely low precision being obtained in the analysis. Failure to heed this may potentially lead to falsely optimistic performance data and lead to 'tighter' warning and action limits on control charts and thus an increased number of control chart failures.

5.3.4.2 All test type samples within a batch should be analysed in random order in each instrument run.

5.3.4.3 If all required validation test type sets cannot be accommodated in a single batch then multiple batches should be carried out ensuring all replicates of a single test type are included in a single batch.

5.3.5 *Full Validation for Determination of Instrument Detection Limit*

Instrument detection limit must be determined using within-batch performance data measured across multiple batches. This should be carried out with a minimum of 10 degrees of freedom. This can be done by:

- Running 11 x 2 method instrument limit validation test types and extracting the within-batch performance component during evaluation

5.3.6 *Full Validation for Determination of Method Detection Limit*

- Method detection limit must be determined using within-batch performance data, as defined in NS30. This should be carried out with a minimum of 10 degrees of freedom using real matrix where possible. This can be done by:

- Running method detection limit validation test types as part of the duplicate or quadruplicate test sets in an 11 x 2 or a 6 x 4 validation study respectively and extracting the within-batch performance component during evaluation.

The preference will be made on the basis of resource implications and allowable batch size.

5.3.7 *Full Validation for Determination of Limit of Quantitation*

Determination of limit of quantitation (LOQ) is a function of method detection limit and so must be determined using within-batch performance data. This should be carried out with a minimum of 10 degrees of freedom using real matrix where possible. This can be done by either:

- Running LOQ validation test types as part of the duplicate or quadruplicate test sets in an 11 x 2 or a 6 x 4 validation study respectively and extracting the within-batch performance component during evaluation.

The preference will be made on the basis of resource implications and allowable batch size.

5.3.8 *Mini-Validation Applicability*

Mini-validation is carried out to demonstrate that performance is equivalent or better to the performance characteristics currently being quoted for a method. A method may already perform as fit-for-purpose and meet all performance targets. If this is the case then any method changes would not be made in an effort to improve performance characteristics but would be for some other purpose.

If previous method performance is to be retained and new performance characteristics are not sought then a mini-validation is sufficient for the following circumstances:

- Change to an existing method – Introduction of a new stable sample matrix to an existing method
- Change to an existing method – Significant change to the range of method
- Change to an existing method – Direct replacement of a significant piece of test equipment in test method
- Change to an existing method – Relocation of existing test equipment
- Change to an existing method – Transfer of method to second laboratory

5.3.9 *Mini-Validation Level Design*

5.3.9.1 Introduction of a new stable matrix to an existing method –

It is permissible to group separate matrices together if validation demonstrates similar performance. This is achieved by performing a within-batch validation test of the new method matrix.

For example, a 1 x 11 validation study would comprise of one separate batch consisting of 11 replicates of the required validation test types.

The following table makes use of established method standard analysis times (SATs). The more complex the method, the higher the SAT will be and it can be assumed that the method will be more costly not just in terms of staff resource, but also in terms of cost of equipment, reagents and consumables.

Type of Method Samples	Method Resource Low (SAT ≤0.1)	Method Resource Medium / High (SAT >0.1)
Formal	Unspiked Sample Matrix 1 x 11 Spiked Sample Matrix 1 x 11	Unspiked Sample Matrix 1 x 6 Spiked Sample Matrix 1 x 6
Regulatory	Unspiked Sample Matrix 1 x 11 Spiked Sample Matrix 1 x 11	Unspiked Sample Matrix 1 x 2 Spiked Sample Matrix 1 x 6
Non-Regulatory	Unspiked Sample Matrix 1 x 2 Spiked Sample Matrix 1 x 11	Unspiked Sample Matrix 1 x 2 Spiked Sample Matrix 1 x 6

5.3.9.2 All other applicable mini-validation circumstances –

A validation study comprising of sets of duplicates of the validation test types would be analysed as separate batches preferably on different days but as a minimum the method instrument must be allowed to return to ground state between batches. Instrumentation must be allowed to return to its ground state between each exercise to avoid falsely low precision being obtained in the analysis. Failure to heed this may potentially lead to falsely optimistic performance data and lead to 'tighter' warning and action limits on control charts and thus an increased number of control chart failures.

All test type samples within a batch should be analysed in random order in each instrument run.

If all required validation test type sets cannot be accommodated in a single batch then multiple batches should be carried out ensuring all replicates of a single test type are included in a single batch.

The following table makes use of established method standard analysis times (SATs). The more complex the method, the higher the SAT will be and it can be assumed that the method will be more costly not just in terms of staff resource, but also in terms of cost of equipment, reagents and consumables.

Type of Method Samples	Method Resource Low (SAT ≤0.1)	Method Resource Medium (0.1 <SAT ≤1.0)	Method Resource High (SAT >1.0)
Formal	6 x 2	6 x 2	N/A – full validation 6 x 2 would be carried out
Regulatory	6 x 2	6 x 2	N/A – full validation 6 x 2 would be carried out

Non-Regulatory	6 x 2	N/A – full validation 6 x 2 would be carried out	N/A – full validation 6 x 2 would be carried out

For methods where a full validation comprises of a 6 x 2 validation exercise, it is not appropriate to carry out a mini-validation. Instead, a further full validation of a 6 x 2 exercise should be performed.

5.3.9.3 Assessment of pre-preparation stage repeatability –

A single batch is considered sufficient to determine whether or not repeatability is within the expected typical values. The number of replicates of the single batch should be set with respect to expected SAT as per section 5.3.9.1. Repeatability testing is not concerned with determining all performance characteristics but is solely concerned with demonstrating that the required repeatability is achieved.

Any follow-on analyses would require typical validation of the follow-on procedure to assess actual analysis performance characteristics.

5.4 Step 5-4 – Document Validation Study Requirements (Design Plan)

5.4.1 Regardless of the purpose for undertaking the exercise it is imperative that a validation study design plan has been developed, documented and approved by a lead chemist or senior chemist prior to commencement.

5.4.2 When designing a validation plan it is important to take into account customer requirements. A record should be made of required performance targets, required method matrices and expected sample concentration ranges.

5.4.3 The validation design plan should clearly state the validation test types to be included and the level of validation required for each.

5.4.4 If any mini-validations are to be performed the plan should clearly state the intention of the mini-validation exercise.

5.4.5 An outline of the test method or reference to controlled document should be referenced in the validation design plan.

5.4.6 Some examples are provided in APPENDIX B along with the appropriate templates for the validation exercise.

5.4.7 Review and sign-off of the validation plan must be carried out according to BP-213 (Changes to UKAS Scope of Accreditation).

6 STEP 6 – UNDERTAKE STUDY AND CALCULATION OF TESTS

6.1 It must be ensure that the following points are addressed:

6.1.1 The validation process as far as practicable should cover the whole analytical procedure. The analytical procedure must include any bottles normally used for sampling, any preservation reagent and general equipment used in the process.

6.1.2 It is desirable that the validation exercise is completed by more than one analyst if this is the way real samples are to be analysed, as this will provide the most realistic estimate of the method's true performance. The lowest estimates of within and between-batch variations will be obtained by one analyst undertaking the work. Failure to heed this may potentially lead to falsely optimistic performance data and lead to 'tighter' warning and action limits on control charts and thus an increased number of control chart failures when multiple analysts routinely carry out the method analyses.

6.1.3 The duration of the validation exercise period is a matter of choice and depends on which sources of random error are to be assessed. Ideally, the validation exercise involving between-batch analysis should be carried out in a period of two or three weeks. It is recognised that for complex methods it may take longer to complete the validation exercise.

6.1.4 Samples may be prepared in entirety at the outset of the validation exercise. Alternatively, they may be prepared fresh for each batch of analysis.

If there is a risk of sample instability then this must be recognised and all samples must be prepared fresh for each batch of analysis and these should be treated as 'unstable samples'.

NOTE: sample stability is assessed using procedure ES-CHEM-GEN-P-06.

6.1.5 No changes should be made to the method once the validation exercise has started. If circumstances indicate changes are required then the whole validation exercise should be reassessed.

6.2 Spreadsheet ES-VALID-S-009 must be used for all validation data entry.

6.2.1 The appropriate validation level must be applied for each validation test. Ensure the correct level and validation test types are selected in ES-VALID-S-009.

6.2.2 The validation levels labelled as 'Unstable Sample' are to be used only in the event of validation that has been carried out when samples are inadequately stable to allow tests on one sample to be made over a number of days and the method does not include any sample stabilisation step (see Section 6.1.4).

If it is not practicable to perform a bulk spike of sample matrix to use throughout the validation exercise, it is permissible to spike samples on the day of each validation batch. In this approach validation levels labelled as 'Unstable Sample' should not be used.

6.2.3 Data entry must be entered into the spreadsheet using appropriate units and calculation of results. Entered results should not be rounded but should be displayed to 3 significant figures where possible.

Validation Test Type	Result Entry Format* **
Instrument Performance Standard equivalent to 10% of expected method range	Raw instrument result

Instrument Performance Standard equivalent to 90% of expected method range	Raw instrument result
Blank	Final sample concentration – no recovery correction
Process Blank	Final sample concentration – no recovery correction
Process Check Standard equivalent to 10% of expected method range	Final sample concentration – no recovery correction
Process Check Standard equivalent to 90% of expected method range	Final sample concentration – no recovery correction
Unspiked Sample Matrix	Final sample concentration – no recovery correction
Spiked Sample Matrix – spiked between 50 to 90% of method range	Final sample concentration – no recovery correction
Certified Reference Material (CRM)	Final sample concentration – with recovery correction
Method Detection Limit test types – see Section 5.2.9	Final sample concentration – with recovery correction
Limit of Quantitation test types – for WFD parameters only – see Section 5.2.10	Final sample concentration – with recovery correction

*For Direct Methods all three phrases are identical. i.e.:

Raw instrument result

= Final sample concentration – no recovery correction

= Final sample concentration – with recovery correction

**For Pre-Treatment Non-Recovery Methods two phrases are identical. i.e.:

Final sample concentration – no recovery correction

= Final sample concentration – with recovery correction

6.2.4 Ensure results are entered into spreadsheet ES-VALID-S-009 as the validation exercise progresses. This is critical to identify any issues as they arise and avoid committing the significant staff resource necessary to carrying out validation when the validation exercise can be seen to not be fit-for-purpose.

6.2.5 All relevant information regarding the validation exercise must be recorded alongside the test results.

6.2.6 An interim review of the data must be carried out halfway through the validation exercise according to BP-213 (Changes to UKAS Scope of Accreditation).

7 STEP 7 – EVALUATION OF VALIDATION RESULTS

The method validation critical performance characteristics are calculated using statistical analysis of variance techniques using spreadsheet ES-VALID-S-009.

7.1 Assessment of Validation Exercise Against Required Performance Targets

7.1.1 A comparison of the validation performance against agreed target performance must be assessed. As a general guideline, the acceptability criteria are given in the table below.

Performance Characteristic	Acceptable Level of Performance
Method Detection Limit	≤ Target MDL
Precision (RSD)	≤ Target RSD or ¼ Target MDL* – whichever is the greater
Bias	≤ Target Bias or ½ Target MDL* – whichever is the greater
Recovery	Within Target Range (Section 3.4)

*For methods where the target MDL is not achievable, the acceptable level criterion for precision and bias should be taken as ¼ and ½ Actual MDL respectively.

7.1.2 Assessment of the validation data using spreadsheet ES-VALID-S-009 should be carried out as follows:

7.1.2.1 *Overall Precision Assessment - RSD*

- Overall precision assessment is based on the total relative standard deviation (RSD) of the validation exercise. This is calculated across multiple batches (i.e. 11 x 2, 6 x 4), where the total standard deviation is as defined by NS30 and calculated using the method described in Appendix D.
- Each validation test type will return a single RSD performance.
- Precision assessment is not required at the method detection limit level.
- Precision assessment for limit of quantitation will be addressed separately (see Section 7.1.2.6)
- The final method performance for a determinand will be quoted as the largest RSD performance taken from all the relevant validation test types.
- The final method performance will be compared against the target precision, using an F-test if this is required – see Appendix D.
- A 'Yes/No' return will be given as to whether the method precision is within target performance.
- Where validation levels labelled as 'Unstable Sample' are used, the calculation method follows NS30. This method assumes that the between-batch variation of standards is linearly related to their concentration and is zero in the absence of determinand. This allows the estimation of between-batch variation for unstable unknowns that would otherwise give large between-batch variations. This in then used to estimate the total standard deviation (or overall precision).

7.1.2.2 *ANOVA Assessment*

- The validation data should be assessed using ANOVA. A two-sided F-test is then used to compare the within-batch and between-batch variances (see Appendix D).
- Acceptable outcomes from the F-test are:
  - The within-batch and between-batch variances are not significantly different; and
  - The between-batch variance is significantly greater than the within-batch variance a common outcome in many methods.
- Implicit in both acceptable outcomes for this test is that the precision assessment is also acceptable.

- The only F-test outcome that constitutes a fail is if the within-batch variance is greater than the between-batch variance. This suggests a problem with the method, e.g. for example a consistent within-batch drift.

- Each validation test type will return a single ANOVA assessment.

- ANOVA assessment is not required at the method detection limit level.

- ANOVA assessment is not required at the limit of quantitation level.

- ANOVA assessment criteria must be satisfied for all relevant method validation test types.

7.1.2.3 *Detection Limit Assessment*

- Method detection limit is determined by a multiple (4.65) of the within-batch standard deviation of the validation test type for method detection limit. The definition of within-batch is given in Appendix D.

- Before accepting the calculated method detection limit, it must be ensured that the standard deviation is taken from a data set of 'final' concentration results. i.e. blank corrected and recovery corrected where applicable.

7.1.2.4 *Overall Bias Assessment*

- Overall bias assessment is based on the difference of actual mean of results against the target level.

- Each validation test type will return a single bias performance.

- Bias assessment is not required at the method detection limit level.

- Bias assessment for limit of quantitation will be addressed separately (see Section 7.1.2.6)

- The final method performance for a determinand will be quoted as the largest bias performance taken from all the relevant validation test types.

- A 'Yes/No' return will be given as to whether the method bias is within target performance.

7.1.2.5 *Method Recovery Assessment*

- Overall recovery assessment is identical to bias assessment in Section 7.1.2.4.

- The term 'recovery assessment' is applicable to methods which would routinely carry out recovery correction for the calculation of sample results.

- Overall recovery assessment will be quoted as the largest recovery performance taken from all relevant validation test types which are at a level equivalent or similar to the intended level of the routine Process Check Standard QC measure.

7.1.2.6 *Limit of Quantitation Assessment*

- Precision performance will be assessed for limit of quantitation test type according to the performance targets stated in Section 5.2.10.5.

- Bias performance will be assessed for limit of quantitation test type according to the performance targets stated in Section 5.2.10.5.

7.2 Evaluation of Full Validation Assessment

7.2.1 If the data meets the required performance targets then the test method can be considered fit-for-purpose and can be used for routine use. Real samples can now be analysed.

7.2.2 If data does not meet the required performance targets then <u>one</u> of three approaches must be taken:

7.2.2.1 <u>Approach 1</u>: Action is taken to find the root cause for the failing method performance and this is addressed. The validation exercise must be repeated in it's entirely.

7.2.2.2 <u>Approach 2</u>: It is not considered cost-effective to address the root cause for the failing method performance. No in-house analysis will be performed using the affected method. No further validation exercise is required.

7.2.2.3 <u>Approach 3</u>: It is not considered cost-effective to address the root cause for the failing method performance. The intention is to carry out in-house analysis using the affected method which does not meet all performance targets. Customer acceptance of the failing method performance <u>must</u> be granted analysis of real samples can commence.

NOTE: Where it is apparent that data-points or entire batches of data are out of control leading to failing method performance it is permissible to repeat these tests if it evident that there is a reason for these failing data-points and that they do not reflect actual performance of the method.

7.2.3 The summary report must include any relevant additional information. For example:

- Reason for carrying out validation exercise
- A summary of passing / failing performance targets
- Justifications for acceptance of failing performance targets
- Details of customer acceptance memo – date / granted by / any comments received
- Impact of failing performance targets on sample results
- Sign off – name / position / date

7.3 Evaluation of Mini-Validation Assessment

7.3.1 Even if the data meets the required performance targets, this does not immediately demonstrate that the test method under mini-validation can be considered fit-for-purpose and can be used for routine use.

7.3.2 In order to assess whether or not a mini-validation demonstrates acceptable performance, a direct comparison must be carried out for key performance indicators between the mini-validation data and the original full validation data. These indicators must include precision, bias and detection limit. For methods requiring recovery-correction, it must also include recovery factor.

7.3.2.1 Comparison must be demonstrated in table format to include each method determinand and matrix which had been included in the validation data sets.

7.3.2.2 Any differences which are apparent upon visual inspection of the tabulated comparative results must be critically commented on with regard to their acceptability. No statistical assessment is required.

7.3.2.3 If there are no apparent obvious differences between the performance indicators, then previous full validation performance should continue to be reported for the method.

7.3.2.4 When methods are shared between laboratories, the performance at the second laboratory may be judged to be satisfactory when an assessment has shown no apparent differences in performance which cannot be justified as acceptable.

7.3.2.5 Where a new matrix has been introduced into a method, the performance of the new matrix may be judged to be satisfactory when an assessment has shown no apparent differences in performance against full validation matrix performance which cannot be justified as acceptable.

7.3.2.6 Differences in performance may arise due to an improvement in the overall method performance. In this situation the previous method performance must be quoted rather than the improved performance of the mini-validation.

7.3.3 If data does not meet the required performance targets then one of three approaches must be taken:

7.3.3.1 Approach 1: Action is taken to find the root cause for the failing method performance and if this is found to be due to an anomaly outwith the original method scope then this is addressed (e.g. spiking error by analyst). The mini-validation exercise must be repeated in it's entirely.

7.3.3.2 Approach 2: It is not considered cost-effective to address the root cause for the failing method performance. No in-house analysis will be performed using the mini-validation affected method. No further validation exercise is required. The originally validated method remains unaffected by this approach.

7.3.3.3 Approach 3: Since previous validation performance cannot be quoted, a full validation is carried out to ascertain a new set of performance measures for the method previously submitted to mini-validation. Sections 5 to 7 should be carried out.

7.3.4 The summary report must include any relevant additional information. For example:

- Reason for carrying out validation exercise
- Tabulated comparison of mini-validation performance against original full validation performance
- Justification for any acceptable deviations in the comparison exercise
- Statement of further action required where applicable
- Sign off – name / position / date

7.4 Documentation

7.4.1 All data relating to performance testing must be retained whilst the method is operated within the laboratory and stored centrally to ensure the validation records are readily retrievable and prevented from loss.

7.4.2 Once the validation process is complete and results show that the method is capable of meeting the customer's requirements it is important to review and complete the documented procedure already started from method development stage so that the method can be clearly and unambiguously implemented. Appropriate documentation will help ensure that application of the method from one occasion to the next is consistent.

7.4.3 The performance achieved within the method validation studies should be included in the procedure or can be referenced if this is more appropriate.

7.4.4 Where appropriate, the uncertainty estimate should also be created or updated to include new validation data and is calculated using procedure SS-CHEM-GEN-P-001 Procedure on Estimating Uncertainty Associated with Chemical Tests.

7.4.5 All required NEMS updates must be planned and implemented timeously to ensure reporting of sample results can be carried out as required in the planned timescales.

7.4.6 Collation of documentation into an 'application package' must be collated according to BP-213 (Changes to UKAS Scope of Accreditation).

7.5 On-Going Verification

7.5.1 Suitable controls must be put in place once the method is put into routine use to ensure it continues to meet the performance specifications. SPC 001 Routine Analytical Quality Control in the Laboratory and SPC 002 Use of NWA Quality Analyst for Set Up and Review of Control Charts should be followed to ensure appropriate quality control measures are implemented and maintained.

7.5.2 In the case of WFD–reportable parameters and ISO 17025 accredited parameters, where suitable external proficiency testing schemes are available then these should also be used to measure on-going performance. In the absence of a suitable external scheme, in-house proficiency testing schemes should be considered. SPC 003 Analysis and Reporting of Proficiency Schemes should be followed.

7.5.3 On-going revalidation will require a review of performance every six years. This is required because the expectation is that instrument performance may deteriorate over time and a period of six years is an acceptable time interval to reassess.

7.5.3.1 It is reasonable to assume that method matrix effects will not be affected by variable instrument performance over time. Therefore it is not necessary to include any matrix validation tests during on-going revalidation.

7.5.3.2 Overall method performance is already continually assessed by use of on-going statistical process control. The annual management review ensures annual review of performance. Therefore it is not necessary to include any general method performance validation tests during on-going revalidation.

7.5.3.3 Detection limits must be reassessed following sections 5.2.9 and 5.3.6.

7.5.3.4 For WFD parameters, limit of quantitation must be reassessed following sections 5.2.10 and 5.3.7.

7.5.3.5 If performance is found to vary on revalidation then updates to NEMS reporting, uncertainty assessments, method documentation and method calculation spreadsheets should be updated accordingly.

7.5.4 Other factors such as documentation, training of staff, maintenance and calibration of equipment and audits all have a part to play in ensuring the method continues to meet performance specifications.

8 REFERENCES

8.1 NS30. A Manual on Analytical Quality Control for the Water Industry, R.V. Cheeseman & A.L. Wilson, revised by M.J. Gardner, WRc (1989), ISBN 0 902156 85 3

8.2 Clean Seas Environment Monitoring Programme Green Book Table

8.3 The Fitness for Purpose of Analytical Methods: A Laboratory Guide to Method Validation and Related Topics, EURACHEM. (Second Edition 2014).

8.4 Harmonized Guidelines for Single Laboratory Validation of Methods of Analysis, IUPAC, (2002)

8.5 Harmonised Guidelines for the Use of Recovery Information in Analytical Measurement, IUPAC, (1999)

9 RELATED DOCUMENTS

9.1 SPC 001 Routine Analytical Quality Control in the Laboratory

9.2 SPC 002 Use of NWA Quality Analyst for Set Up and Review of Control Charts

9.3 SPC 003 Analysis and Reporting of Proficiency Schemes

9.4 SS-CHEM-GEN-P-001 Procedure on Estimating Uncertainty Associated with Chemical Tests

9.5 ES-CHEM-G-002 Analytical Performance Criteria for Chemical Parameters

9.6 ES-VALID-S-009 Method Validation for Chemical Tests Workbook Spreadsheet

9.7 HS/SSWCHEM/015 Safe System of Work Procedure – Undertaking Risk Assessment, Method Development and Infrequently Used Methods

9.8 HSMS/2.3.1 Hazard Identification and Risk Assessment Methodology

9.9 HSMS/3.3.4 COSHH Simple Risk Assessment Form

9.10 ES-GEN-P-008 The National Science and Strategy Procedure for Training and Competence

9.11 ES-CHEM-GEN-P-06 Stability of Samples and Prepared Samples / Extracts and Estimation the Maximum Holding Time

9.12 BP-213 Changes to UKAS Scope of Accreditation

10 APPENDIX A
DEFINITIONS AND PURPOSES OF TEST TYPES

A1 Blank

This validation test type is the equivalent of a zero level standard. It will be made using the same ideal matrix which is used to make up routine method QC standards. It will not be made up with real sample matrix. This test type will be analysed in the same way as samples.

This validation test type will be used for direct methods. It will be used to blank correct validation test type results requiring to be reported as final sample concentration.

Where it is not possible to source real matrix with sufficiently absent or low determinand levels, it may also be used determine method detection limit for methods which are able to return numeric results less than zero.

This test type may also be used determine instrument detection limit for methods which are able to return numeric results less than zero. For this purpose, this test type should not undergo any method pre-treatment stages before instrument analysis.

A2 Process Blank

This validation test type is the equivalent of a zero level Process Check Standard. It will be made using the same ideal matrix which is used to make up routine method Process Check standards. It will not be made up with real sample matrix. This test type will be analysed in the same way as samples.

This validation test type will be used for pre-treatment methods. It will be used to blank correct validation test type results requiring to be reported as final sample concentration (with and without recovery correction).

Where it is not possible to source real matrix with sufficiently absent or low determinand levels, it may also be used determine method detection limit for methods which are able to return numeric results less than zero.

A3 Instrument Performance Standard Equivalent to 10% of Expected Method Range

This validation test type is the equivalent of an IPS which had been prepared at 10% of the expected method range. Ideally calibration standard stocks should be used to prepare the test type. It will be made using the same ideal matrix which is used to make up routine method QC standards. It will not be made up with real sample matrix. This test type will be analysed in the same way as samples.

This validation test type will be used for direct methods. It will be used to assess method performance at the lower end of the method range.

A4 Instrument Performance Standard Equivalent to 90% of Expected Method Range

This validation test type is the equivalent of an IPS which had been prepared at 90% of the expected method range. Ideally calibration standard stocks should be used to prepare the test type. It will be made using the same ideal matrix which is used to make up routine method QC standards. It will not be made up with real sample matrix. This test type will be analysed in the same way as samples.

This validation test type will be used for direct methods. It will be used to assess method performance at the upper end of the method range.

A5 Process Check Standard Equivalent to 10% of Expected Method Range

This validation test type is the equivalent of a PCS which had been prepared at 10% of the expected method range. Ideally calibration standard stocks should be used to prepare the test type. It will be made using the same ideal matrix which is used to make up routine method Process Check standards. It will not be made up with real sample matrix. This test type will be analysed in the same way as samples.

This validation test type will be used for pre-treatment methods. It will be used to assess method performance at the lower end of the method range.

A6 Process Check Standard Equivalent to 90% of Expected Method Range

This validation test type is the equivalent of a PCS which had been prepared at 90% of the expected method range. Ideally calibration standard stocks should be used to prepare the test type. It will be made using the same ideal matrix which is used to make up routine method Process Check standards. It will not be made up with real sample matrix. This test type will be analysed in the same way as samples.

This validation test type will be used for pre-treatment methods. It will be used to assess method performance at the upper end of the method range.

A7 Certified Reference Material (CRM)

A certified reference material is a controlled ‘sample’ which has known levels of determinand in a real matrix. This validation test type does not require any preparation and will be analysed in the same way as samples.

This validation test type will be used for both direct and pre-treatment methods. It will be used to assess overall method performance in terms of bias and so should detect any systematic errors that would not be detected by other validation test types.

NOTE: If a CRM is available and covers all of the determinands of interest, it may not be necessary to analyse the validation test types listed in A8 and A9.

A8 Unspiked Sample Matrix

This validation test type is a real sample matrix. The selected sample must be of a suitable matrix to be representative of the matrix group to be analysed by the method. The level of determinand in this sample must be sufficiently low or absent to allow preparation of a further validation test type by spiking (see Section A9). This test type will be analysed in the same way as samples.

This validation test type will be used for both direct and pre-treatment methods. It will be used to blank correct spike sample matrix validation test type results.

A9 Spiked Sample Matrix – Spiked Between 50 to 90% of Method Range

This validation test type is prepared using a real sample matrix. The selected sample must be of a suitable matrix to be representative of the matrix group to be analysed by the method. This test type will be analysed in the same way as samples.

The level of spiking is should ideally result in final determinand levels between 75 and 90% of the method range. It is recognised that this may not always be possible, particularly for multi-component methods. Furthermore, for some methods with a large range, it may be more appropriate to spike to a lower level to demonstrate performance at levels which are more common in real samples.

This validation test type will be used for both direct and pre-treatment methods. It will be used to demonstrate method performance in the presence of matrix effects. It will be used to assess precision, bias and recovery where applicable.

A10 Method Detection Limit Test Types

A10.1 *Unspiked Sample Matrix – Blank*

This test type may only be used determine method detection limit for methods which are able to return numeric results less than zero.

This validation test type is a real sample matrix. The selected sample must be of a suitable matrix to be representative of the matrix group to be analysed by the method. The level of determinand in this sample must be absent. This test type will be analysed in the same way as samples.

A10.2 *Unspiked Sample Matrix –Sufficiently Natural Low Level*

This test type may be used determine method detection limit for methods which are both able and unable to return numeric results less than zero.

This validation test type is a real sample matrix. The selected sample must be of a suitable matrix to be representative of the matrix group to be analysed by the method. The level of determinand in this sample is not absent but is at a level which is low and can be detected. This test type will be analysed in the same way as samples.

NOTE: If the determinand level is not sufficiently low then there is a likely risk of over-estimation of the method detection limit.

A10.3 *Spiked Sample Matrix – Spiked to Approximately 2 to 5 Times Estimated MDL*

This test type may be used determine method detection limit for methods which are both able and unable to return numeric results less than zero.

This validation test type is a real sample matrix. The selected sample must be of a suitable matrix to be representative of the matrix group to be analysed by the method. The level of determinand in this sample may be absent. This test type will be analysed in the same way as samples.

The level of spiking is should ideally result in final determinand levels at approximately 2 to 5 times the estimated method detection limit. Spiking levels must be such that there is no risk of results returned which are 'not detected' since these non-numeric results cannot be used to determine detection limits.

NOTE: If the final determinand level is not sufficiently low then there is a likely risk of over-estimation of the method detection limit.

A10.4 *Spiked Sample Matrix – Spiked with Approximately 2 to 5 Times Estimated MDL*

This test type may be used determine method detection limit for methods which are both able and unable to return numeric results less than zero.

This validation test type is a real sample matrix. The selected sample must be of a suitable matrix to be representative of the matrix group to be analysed by the method. For this test type it is recognised that it cannot be assured that the level of determinand in this sample is absent. This test type will be analysed in the same way as samples.

The level of spiking is should ideally result in final determinand levels at approximately 2 to 5 times the estimated method detection limit but if the original determiand in the unspiked matrix is unknown then spiking to a known level is not possible and so spiking is made with a known level of 2 to 5 times the estimated method detection limit.

This test type would be used in conjunction with an unspiked matrix (Section A8) in order to blank subtract using real matrix to avoid any risk of over-estimation of the method detection limit.

A10.5 *Blank*

This test type may be used determine method detection limit for direct methods which are able to return numeric results less than zero.

This test type is identical to the validation test type described in Section A.1. This validation test type is a not a real sample matrix. This test type will be analysed in the same way as samples.

This test type would be used to determine method detection limit where it is not possible to use real matrix.

A10.6 *Process Blank*

This test type may be used determine method detection limit for pre-treatment methods which are able to return numeric results less than zero.

This test type is identical to the validation test type described in Section A.2. This validation test type is a not a real sample matrix. This test type will be analysed in the same way as samples.

This test type would be used to determine method detection limit where it is not possible to use real matrix.

A10.7 *Instrument Performance Standard - Spiked at Approx 2 to 5 Times Estimated Instrument LOD*

This test type may be used determine method detection limit for direct methods which are both able and unable to return numeric results less than zero.

This test type is similar to that described in Section A.3 but involves spiking to a level of 2 to 5 times the estimated instrument limit of detection. This validation test type is a not a real sample matrix. This test type will be analysed in the same way as samples.

This test type would be used to determine method detection limit where it is not possible to obtain suitable sample matrix with sufficiently low determinand levels to carry out determination of method detection limit.

A10.8 *Process Check Standard - Spiked at Approximately 2 to 5 Times Estimated MDL*

This test type may be used determine method detection limit for pre-treatment methods which are both able and unable to return numeric results less than zero.

This test type is similar to that described in Section A.5 but involves spiking to a level of 2 to 5 times the estimated instrument limit of detection. This validation test type is a not a real sample matrix. This test type will be analysed in the same way as samples.

This test type would be used to determine method detection limit where it is not possible to obtain suitable sample matrix with sufficiently low determinand levels to carry out determination of method detection limit.

A10.9 *Instrument Blank Standard*

This test type may be used determine method detection limit for pre-treatment methods which are able to return numeric results less than zero.

This test type is an instrument standard with no determinand level present. This validation test type is a not a real sample matrix, nor is it an ideal-matrix. This test type will be analysed in the same way as instrument standards.

Final instrument detection limit must then be recalculated to method detection limit accounting for sample concentration factors and recovery factors.

This test type would be used to determine method detection limit where it is not possible to use real or ideal matrix and / or the complexity and challenges of the method does not easily facilitate determination of method detection limit by any previously described approaches without compromising business needs.

A11 Limit of Quantitation Test Types– for WFD parameters only

A11.1 *Unspiked Sample Matrix – Blank*

This test type is identical to the validation test type described in Section A.10.1.

A11.2 *Spiked Sample Matrix – Spiked at a Level Corresponding to Expected LOQ Level*

This test type may be used determine limit of quantitation for all methods.

This test type is similar to the validation test type described in Section A.10.3 but involves spiking to a level corresponding to a predefined multiple of the method detection limit (see Table XX). This test type will be analysed in the same way as samples.

This test type would be used in conjunction with an unspiked matrix (Section A11.1) in order to blank subtract using real matrix to avoid any risk of over-estimation of the limit of quantitation.

A11.3 *Instrument Performance Standard - Spiked at a Level Corresponding to Expected LOQ Level*

This test type may be used determine limit of quantification for direct methods.

This test type is similar to that described in Section A.10.7 but involves spiking to a level corresponding to a predefined multiple of the method detection limit (see Table XX). This validation test type is a not a real sample matrix. This test type will be analysed in the same way as samples.

This test type would be used to determine limit of quantitation where it is not possible to obtain suitable sample matrix with sufficiently low determinand levels to carry out determination of limit of quantitation.

A11.4 *Process Check Standard - Spiked at a Level Corresponding to Expected LOQ Level*

This test type may be used determine limit of quantification for pre-treatment methods.

This test type is similar to that described in Section A.10.8 but involves spiking to a level corresponding to a predefined multiple of the method detection limit (see Table XX). This validation test type is a not a real sample matrix. This test type will be analysed in the same way as samples.

This test type would be used to determine limit of quantitation where it is not possible to obtain suitable sample matrix with sufficiently low determinand levels to carry out determination of limit of quantitation.

A12 Instrument Detection Limit Test Types

A12.1 *Instrument Blank Standard*

This test type may be used determine instrument detection limit for direct and pre-treatment methods which are able to return numeric results less than zero.

This test type is an instrument standard with no determinand level present. This test type is a not a real sample matrix, nor is it an ideal-matrix. This test type will be analysed in the same way as instrument standards.

A12.2 *Instrument Performance Standard - Spiked at Approx 2 to 5 Times Estimated Instrument LOD*

This test type may be used determine instrument detection limit for direct and pre-treatment methods which are both able and unable to return numeric results less than zero.

This test type is similar to that described in Section A.3 but involves spiking to a level of 2 to 5 times the estimated instrument limit of detection. This validation test type is a not a real sample matrix. This test type will be analysed in the same way as standards.

11 APPENDIX B
VALIDATION DESIGN TEMPLATE AND EXAMPLES

VALIDATION DESIGN PLAN TEMPLATE

12 APPENDIX C
Guidance for the use of ES-VALID-S-009

12.1 Introduction

Welcome to the new method validation workbook.

The workbook is designed to be both a robust tool for assessing validation data while also flexible enough to accommodate as many different scenarios as possible, both current and future. Some aspects of the process are mandatory or restricted whereas others are optional or more flexible.

This tool is intended to be used in conjunction with procedure SS-CHEM-GEN-P-003, which details the methodology utilised here.

12.2 Overview

The workbook contains an interface ("userform") for configuring and creating individual worksheets for the assessment of validation data. Sets of sheets are then created dynamically for each task, customised to suit the analyst's requirements. The sheets use a combination of in-cell formulae along with behind the scenes code, applied using control buttons.

The workbook is intended as a template to handle data created during the validation of chemical tests. The data may have to be manipulated or formatted externally before entering into these sheets – depending on the output from the method.

12.3 How to use ES-VALID-S-009 v8

12.4 Overview of the Start Sheet

From the "Start" sheet, a userform is used to configure the layout of the sheets that will be used for entering and assessing validation data. Once configured this will generate 3 sheets with everything you need to perform your validation assessment.

Enter any relevant text information into the "Method Overview", "Personnel Overview "or "Validation Notes" boxes. If you have a list of determinand names they can also be entered into the "Determinand Names" sheet at this stage. See 12.8.1 for details.

Before you can continue you must also complete the "Accreditation and Design Plan Documentation" section. The questions "Has form BF-213 been completed?" and "Has validation design plan been completed?" are mandatory.

To begin configuring the validation sheets, click the "Setup Validation Sheets" button from the "Start" sheet. The userform should appear.

Also on the "Start" sheet are 3 other buttons; "Delete Sheets", "Lock Workbook" and "Unlock Workbook". See sections 12.8.9 and 12.8.10 for more detail on these.

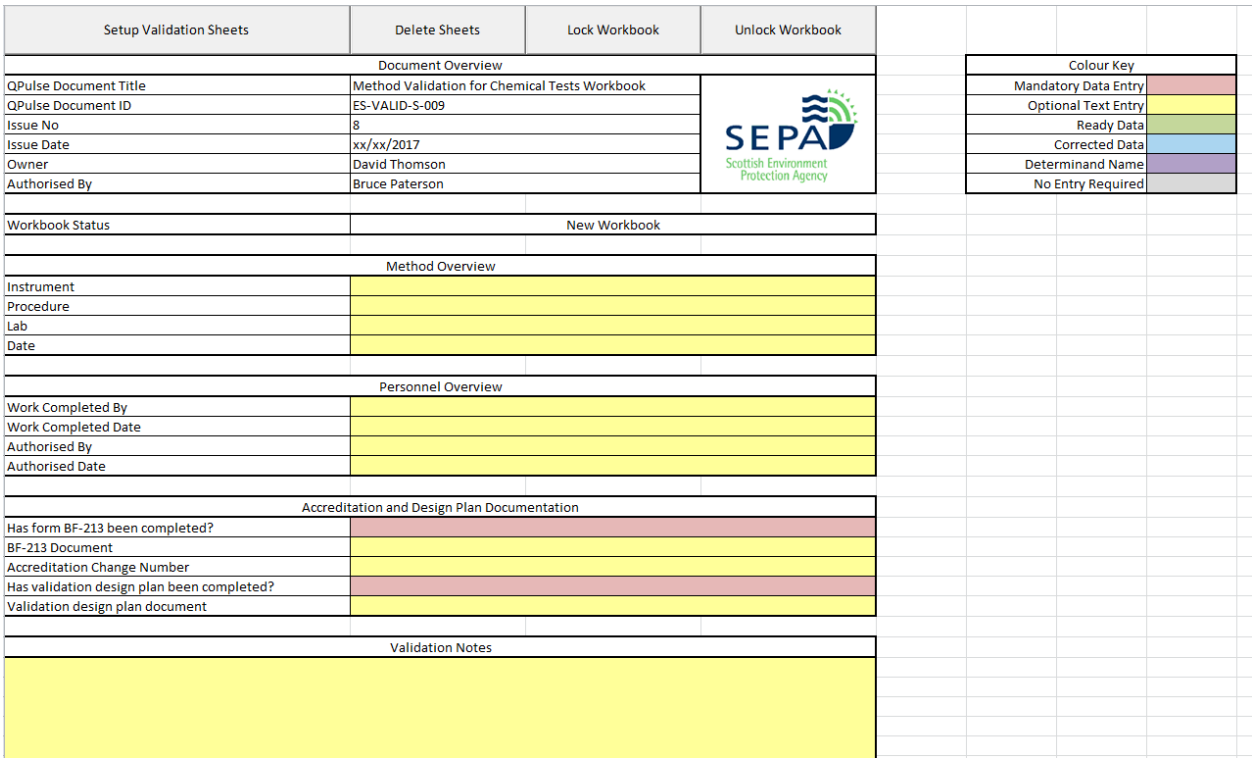
12.5 Overview of the Determinand Names Sheet

This sheet allows the importing of determinand names from a list entered here. This is required if more than 20 are to be used.

12.6 Overview of the Log Sheet

This sheet keeps a record of every action undertaken with the spreadsheet, to what sheets and by whom.

12.7 Overview of the Userform



Setup Validation Sheets	Delete Sheets	Lock Workbook	Unlock Workbook

Document Overview	
QPulse Document Title	Method Validation for Chemical Tests Workbook
QPulse Document ID	ES-VALID-S-009
Issue No	8
Issue Date	xx/xx/2017
Owner	David Thomson
Authorised By	Bruce Paterson

SEPA Scottish Environment Protection Agency

Colour Key	
Mandatory Data Entry	
Optional Text Entry	
Ready Data	
Corrected Data	
Determinand Name	
No Entry Required	

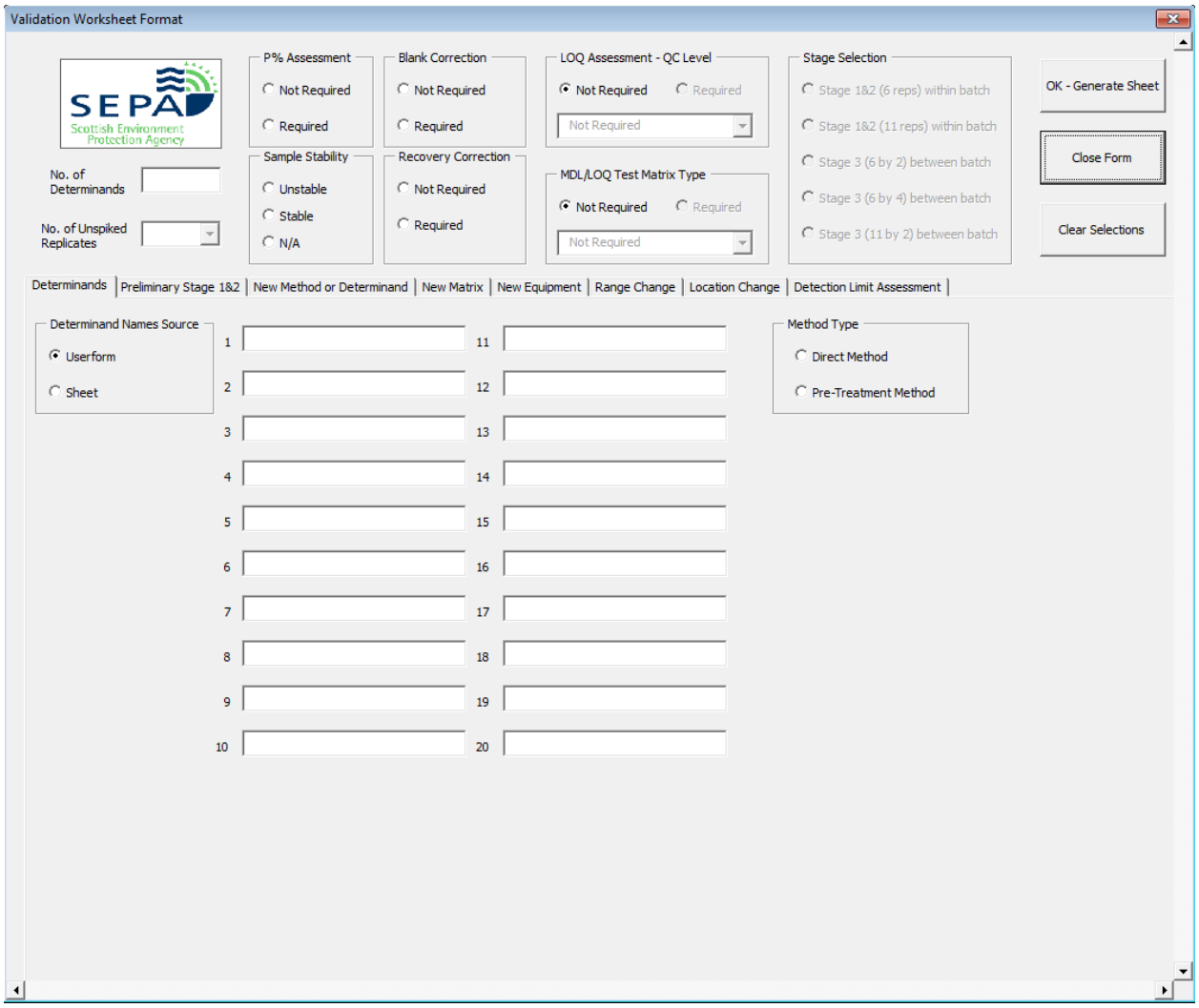
Workbook Status	New Workbook

Method Overview	
Instrument	
Procedure	
Lab	
Date	

Personnel Overview	
Work Completed By	
Work Completed Date	
Authorised By	
Authorised Date	

Accreditation and Design Plan Documentation	
Has form BF-213 been completed?	
BF-213 Document	
Accreditation Change Number	
Has validation design plan been completed?	
Validation design plan document	

Validation Notes



The top part of the form contains a variety of options which reflect aspects of the method being validated and the level of validation required. All selections here are mandatory – if something is not required it must be selected as such. The availability of some selections will change depending on the validation level being undertaken and the test types selected.

The lower part of the form contains a number of pages with options relating to the different validation tasks that may be undertaken (reasons for validation) as well as an area for entering the determinand names and selecting the type of method being validated. Under each validation task is a series of boxes which allows you to select various test types as required – the default test types for each task are shown. If required other test types may be selected.

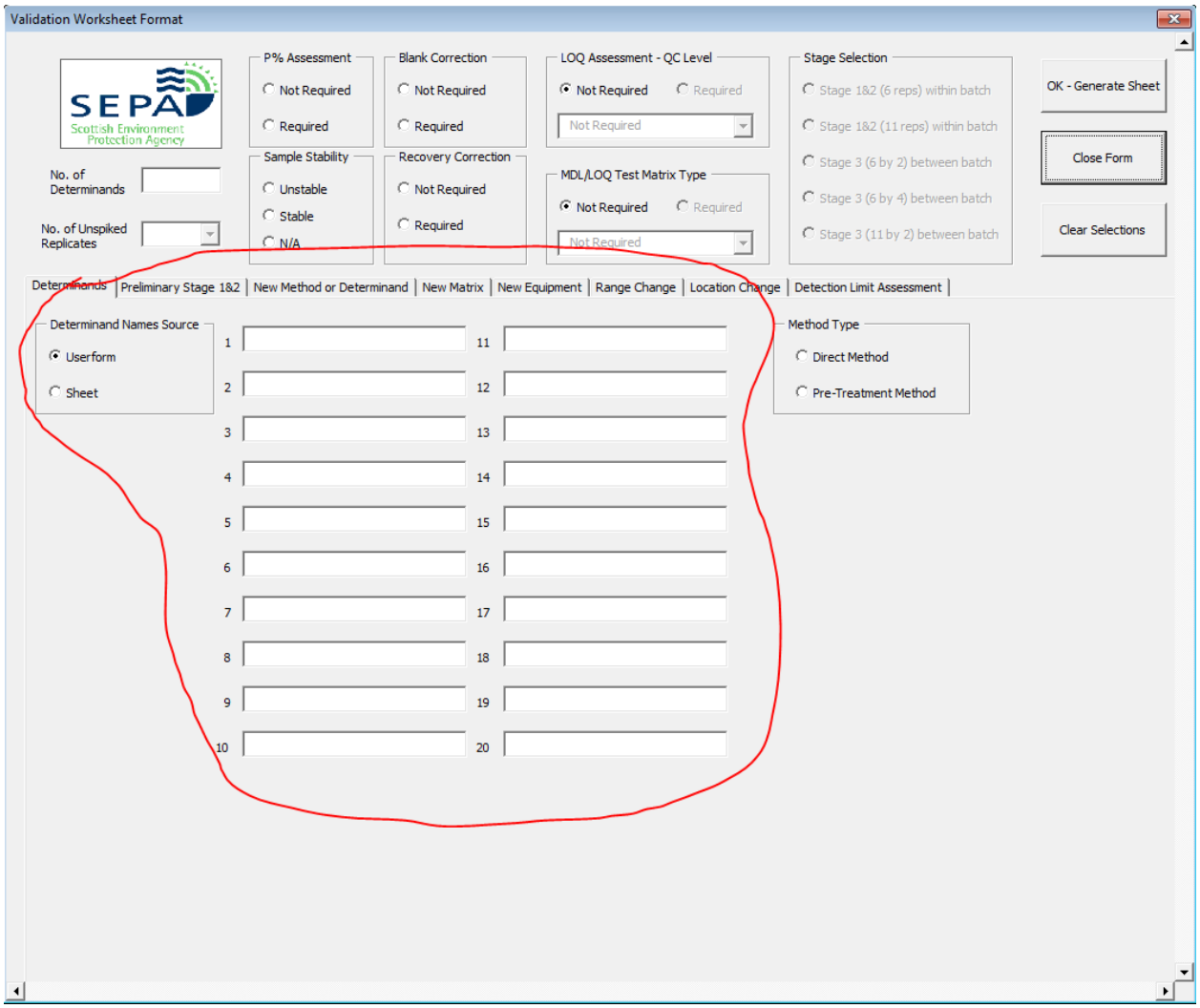
To configure the validations sheets using the userform make the following selections. As the availability of some options in the top part of the form may alter depending on validation stage or task it is best to complete the form in the order described here.

12.8 Configuring the Sheets using the userform

12.8.1 Enter Determinand names

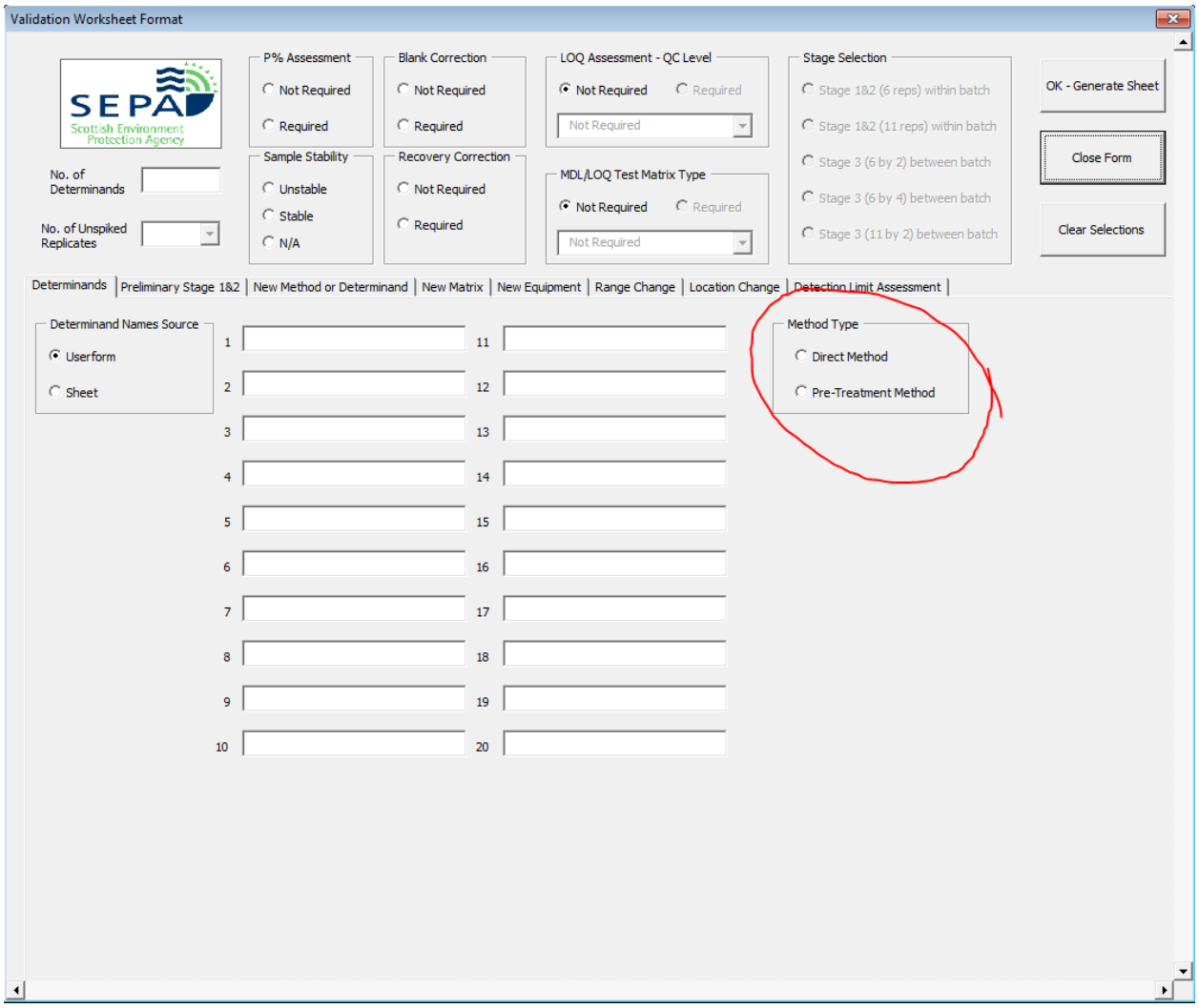
From the first page of the lower part of the form, enter the names of the determinands being validated. If more than 20 are to be assessed (up to 200) then you may select to retrieve the names from the

"Determinand Names" sheet if you have entered them there (this may be preferable for larger analytical suites). It is not essential to enter determinand names at this stage – they may be entered later.



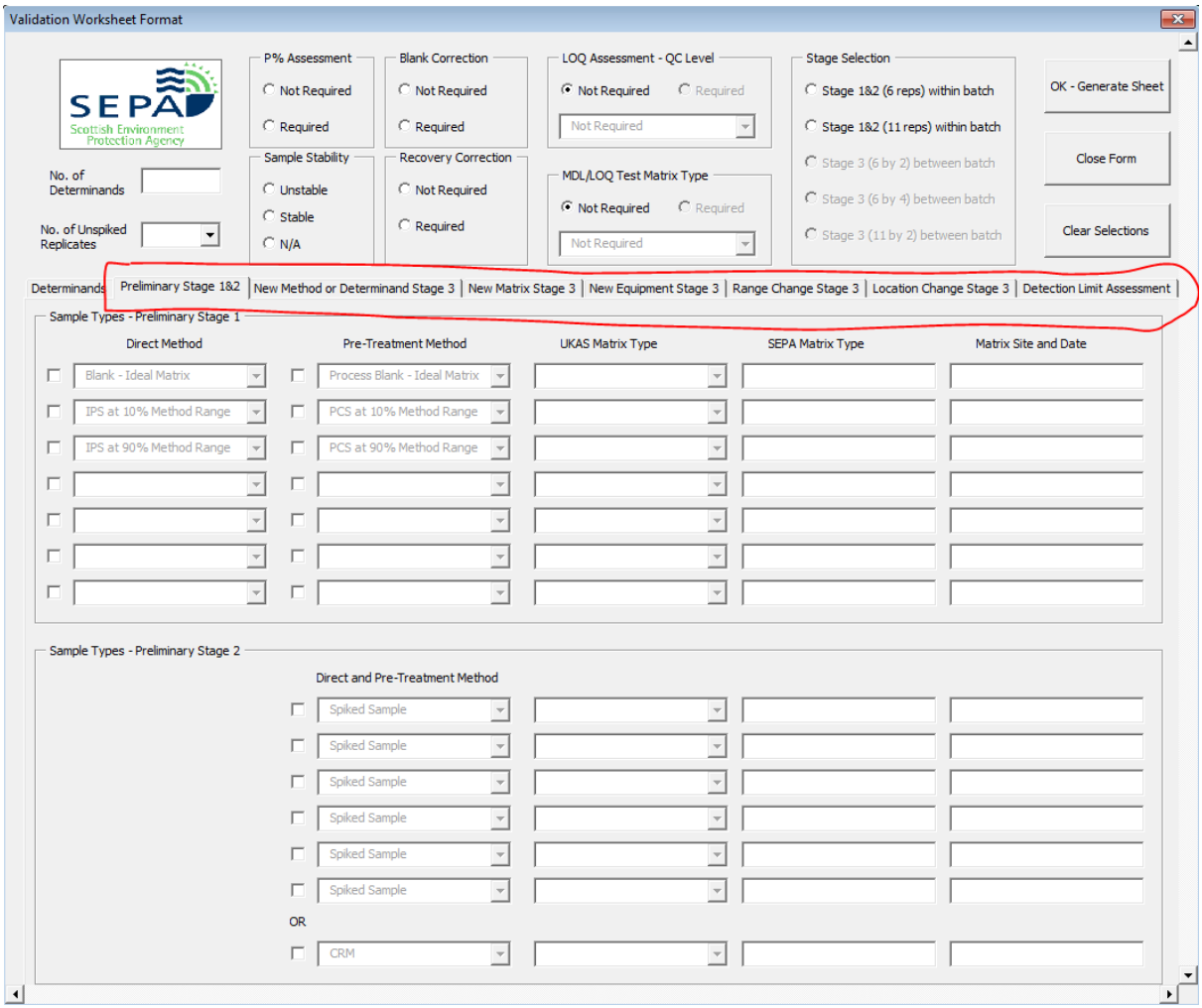
12.8.2 Select Method Type

Still on the Determinands page, select the type of method that is being validated – either Direct or Pre-Treatment



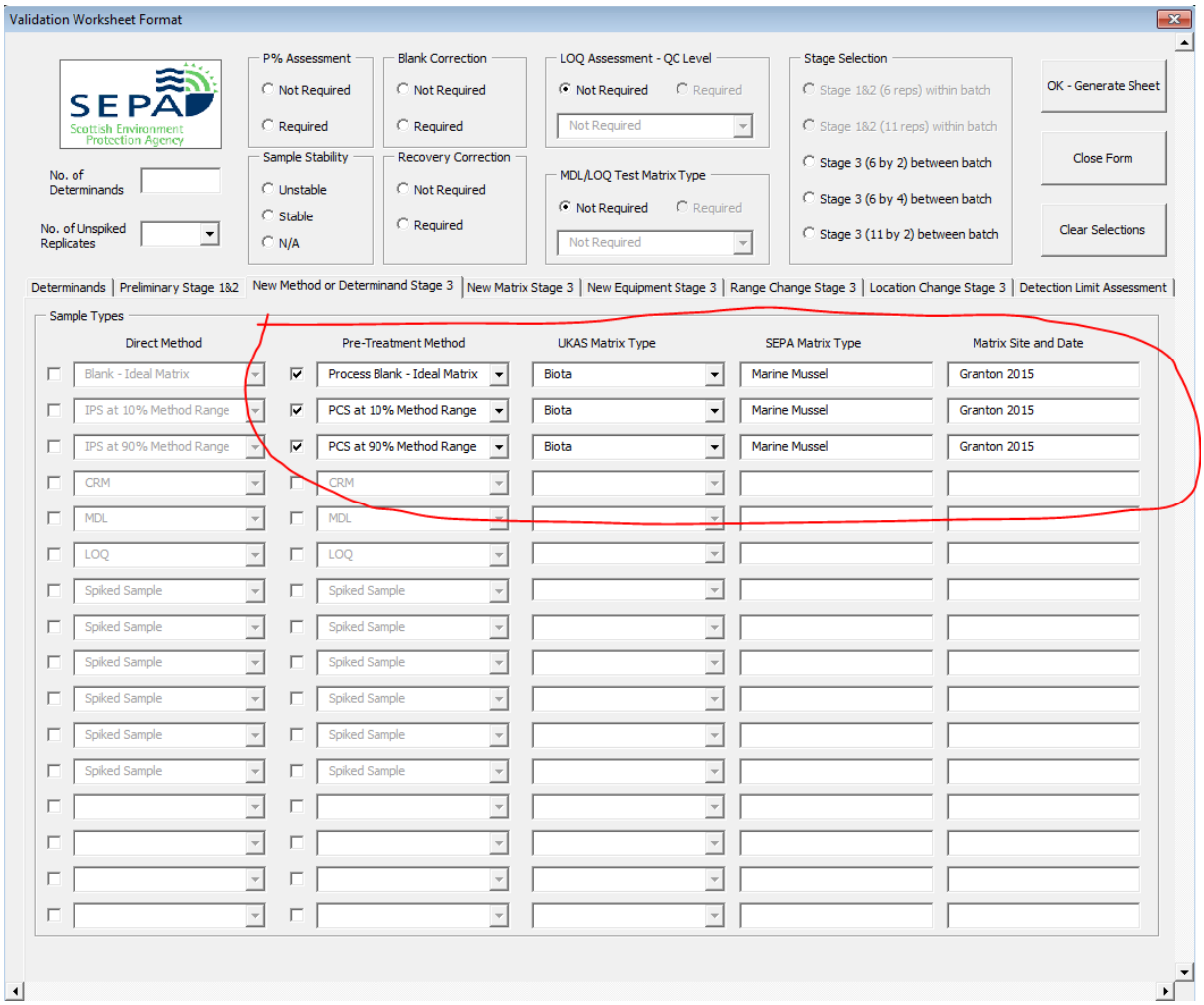
12.8.3 Select validation task

Next, open the page that best represents the reason for validation (e.g. Preliminary Stage 1&2, New Method or Determinand Stage 3, New Matrix Stage 3, New Equipment Stage 3, Range Change Stage 3, Location Change Stage 3, Detection Limit Assessment). Each page contains numerous check boxes representing the available test types for the validation exercise, for direct methods, pre-treatment methods or both. Only one validation task may be completed at a time – any selections made on other pages will be removed when changing pages.



12.8.4 Select Test Types

Select all the required test types. The default set of test types for each validation task are entered initially for guidance. If other test types are required they can also be selected manually. On each of these pages are also text boxes to enter details for the matrices used for each test type. A generic UKAS matrix type may be selected, or details for internal SEPA matrix type can be entered. This optional information may also be entered later if required. Select the required test types and enter matrix data as required.

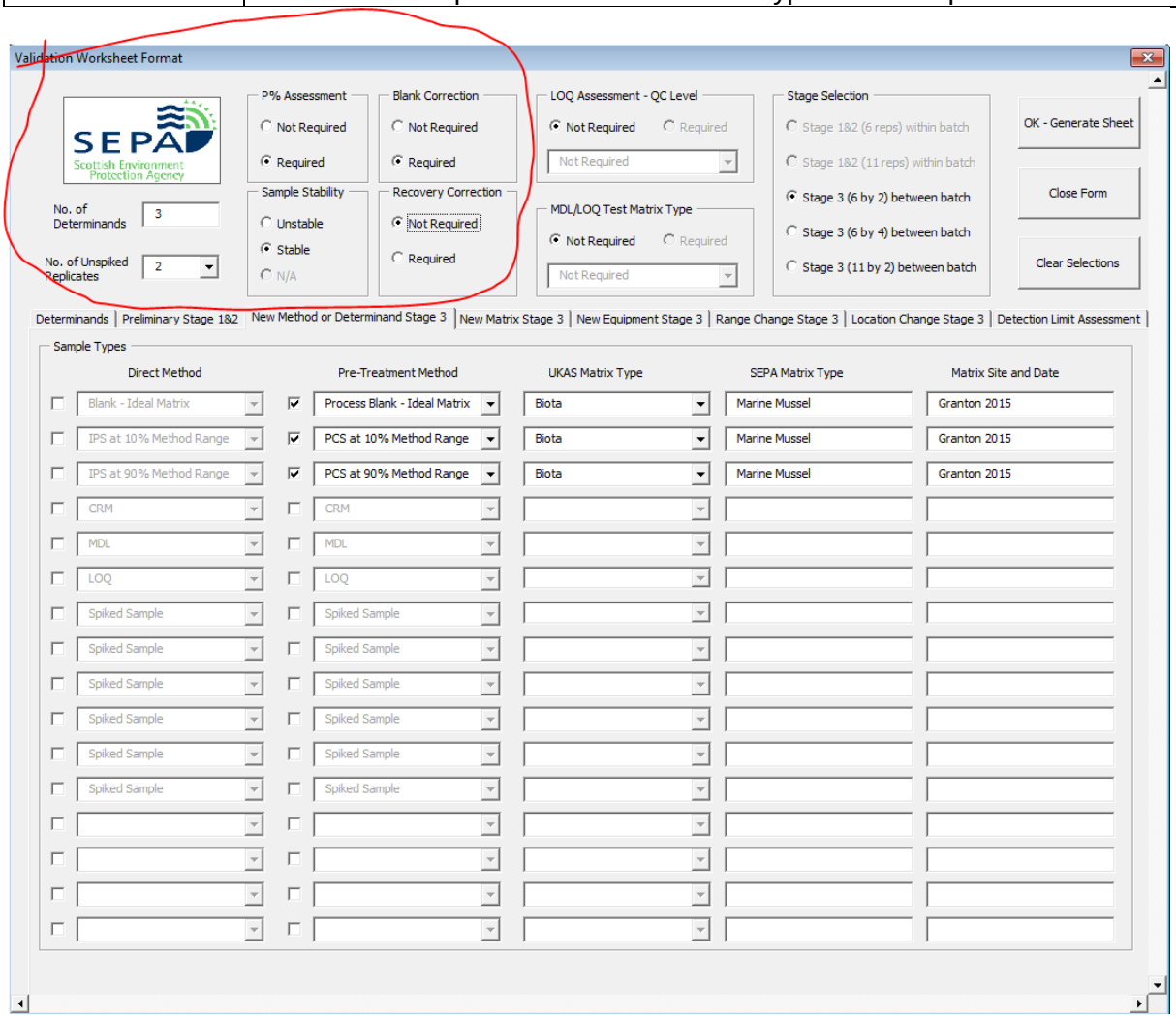


12.8.5 Select Method and Sample Parameters

Now the top part of the userform should reflect all the available selections you can make. Complete all selections.

No. of Determinands	Enter the number of determinands being assessed
No. of Unspiked Replicates	Enter the number of unspiked replicates being assessed (e.g. number of blanks/process blanks, unspiked matrix samples). This depends on the validation stage type.
P% Assessment	select whether assessment against P% criteria is required (this is only applicable for marine methods)
Sample Stability	Select whether samples will be stable for the duration of the validation exercise (this is only applicable to between-batch testing). Enabling this will generate a further set of statistics that accommodate a degradation of baseline concentrations in unspiked matrices. If “Unstable” is selected then 10% and 90% IPS or PCS (as appropriate) test types must be included.
Blank Correction	Select whether the raw sample data will be corrected against the previous raw blank data. A Blank or Process Blank test type will be required.

Recovery Correction	Select if an initial recovery correction of raw sample data will be performed. A recovery factor is calculated from the PCS at 90% values and applied to concurrent samples. A PCS at 90% test type will be required.



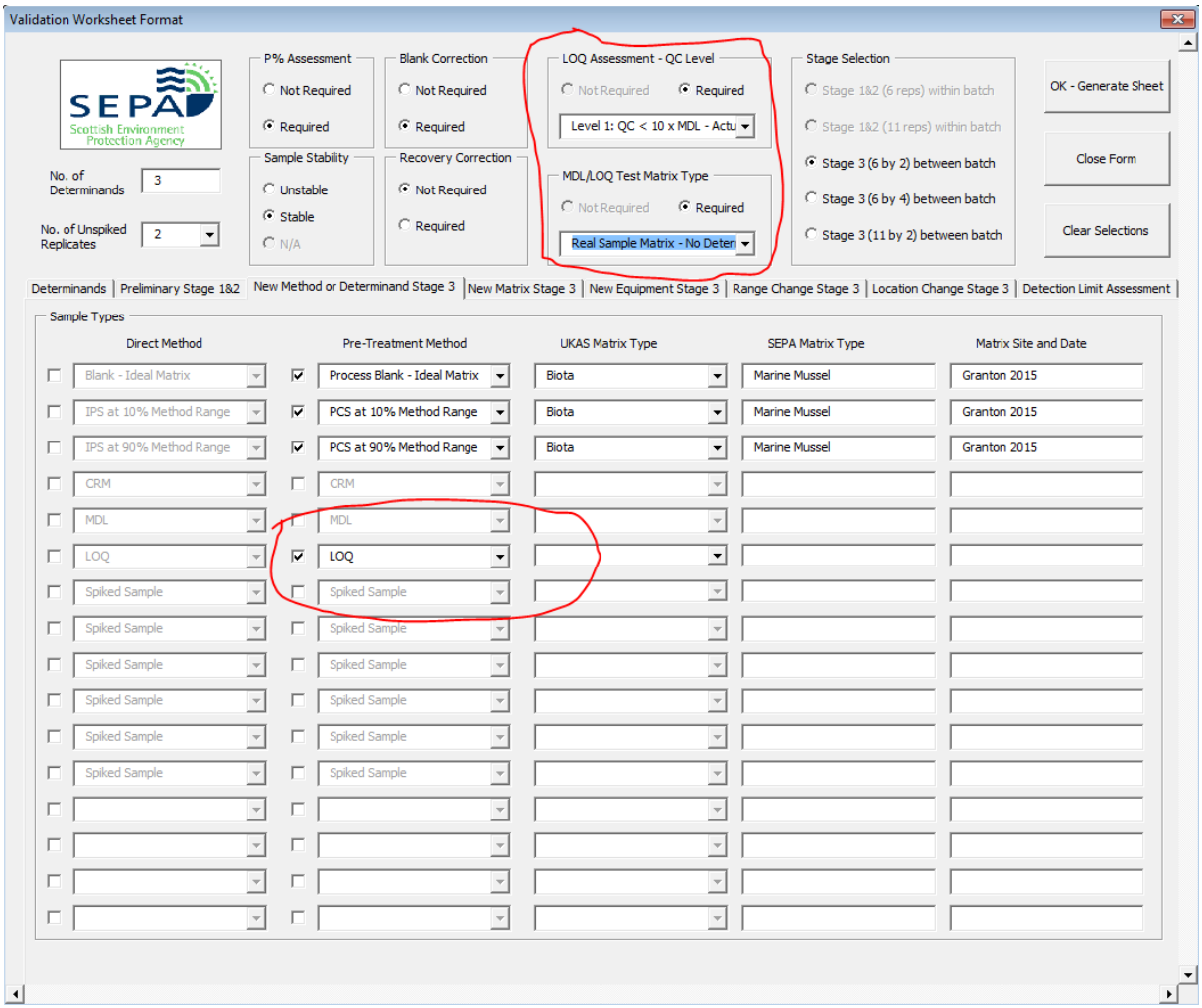
12.8.6 Select LOQ or MDL types

If LOQ or MDL test types are utilised then they must be configured correctly. This is only required when these test types are used. If LOQ or MDL test types are not required then this section can be skipped.

LOQ and MDL cannot be assessed simultaneously so only one or other may be selected at a time.

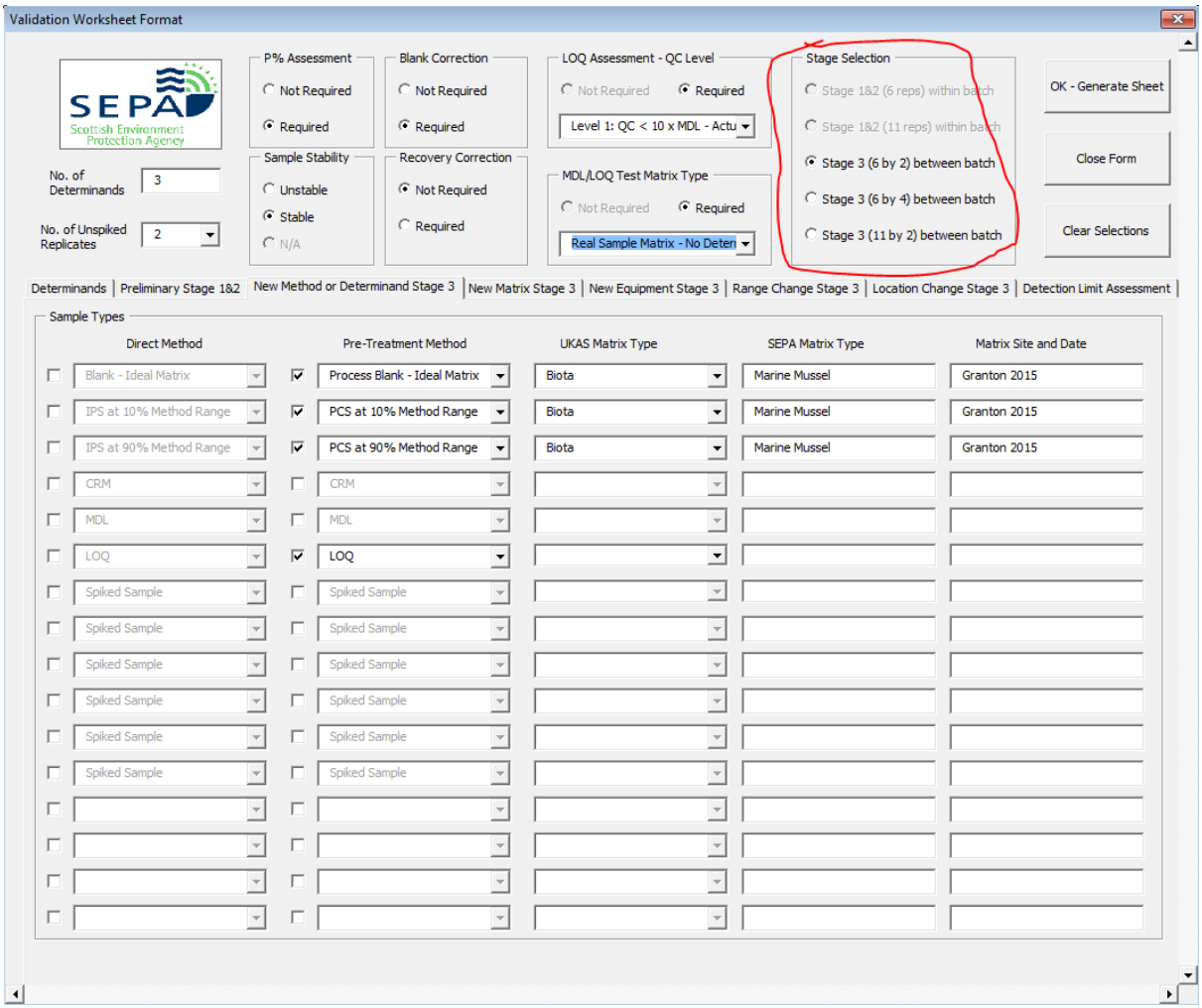
LOQ Assessment – QC Level: this is where the factor for LOQ assessment is selected when calculating LOQ from MDL for LOQ test types. This box is only active when LOQ test types are selected below.

MDL/LOQ Test Matrix Type: this is where the matrix type used for the MDL/LOQ assessment is selected for MDL/LOQ test types. This box is only active when MDL or LOQ test types are selected below.



12.8.7 Select Validation Stage Type

Select the level of validation required here. Selections here may alter the available number of unspiked replicates or whether sample stability may be considered. Available stages also depend on the validation task being undertaken.



12.8.8 Utilise Configuration to Generate Sheets

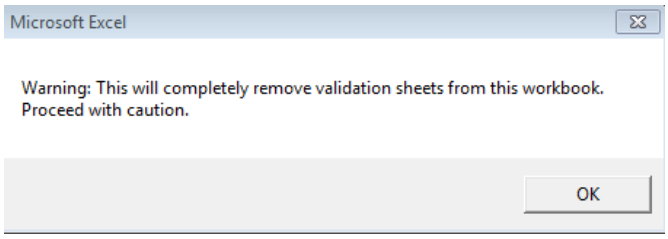
If you wish to close the form but retain your selections press “Close Form”. You can then amend the determinand Names list and return to the form, for example.

If you wish to reset the form to its initial state then press “Clear Selections”. All default values will be returned. All entered text and selections will be lost.

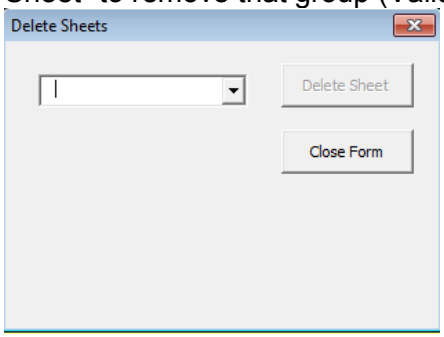
Once all method details and test types are selected, and all other information is entered to your satisfaction, the 3 sheets may be created by pressing the “OK – Generate Sheet” button. Close the form and check that the sheets are correct.

12.8.9 Deleting Sheets

If there are any issues you may delete sets of sheets using the “Delete Sheets” button from the “Start” sheet. A warning will pop up when you click this button.

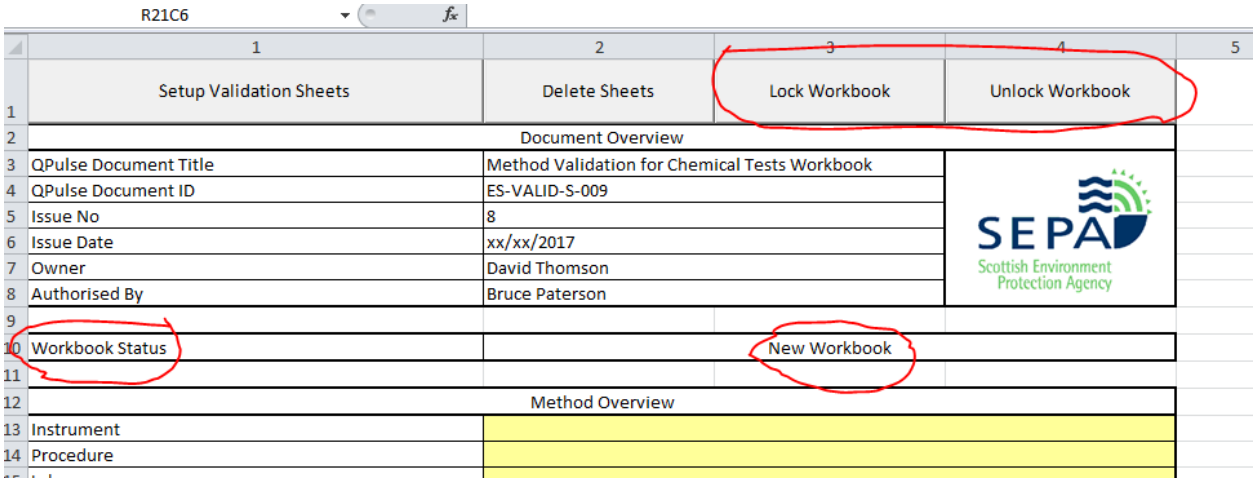


Clicking “OK” opens a small utility which enables you to select the sheet groups that you wish to remove. Select the sheet group you want to remove from the drop down menu and select “Delete Sheet” to remove that group (Validation, Results & Summary will be removed).



When you are finished, or if you do not need to delete any sheets, click “Close Form” to return to the “Start” sheet.

12.8.10 Locking and Unlocking the Workbook



When you are finished using the validation workbook and the validation has been authorised it is recommended that the authoriser “Lock” the workbook by clicking the “Lock Workbook” button. This will disable all buttons on the workbook and protect all of the sheets (including the results and summary sheets which are not normally protected). An entry is recorded in the Log and the “Workbook Status” field on the “Start” sheet will be updated with text to show that the workbook has been locked and by whom. This prevents any accidental modification of results after the authoriser has authorised the sheet.

To enable the workbook again click the “Unlock Workbook” button.

Once the spreadsheet is completed and no other changes will be made the spreadsheet should be made read only.

WARNING: If you delete any sheets the data will be permanently removed. Proceed with caution!

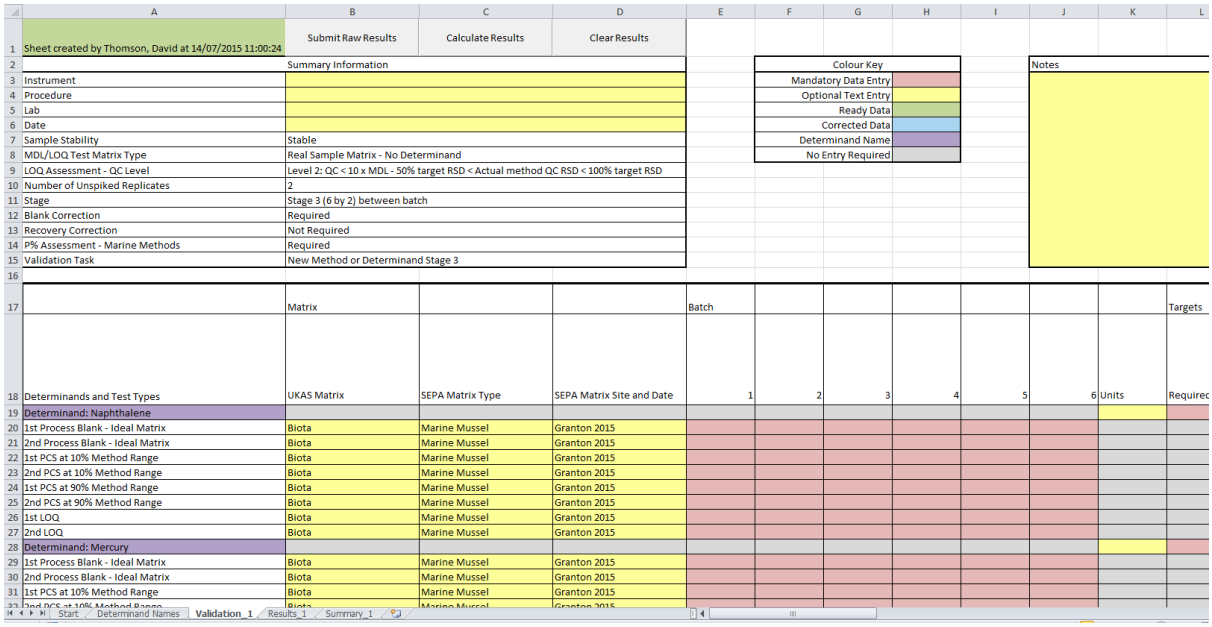
12.9 Overview of Generated Sheets

3 sheets will be generated for each validation exercise; “Validation”, “Results” and “Summary”.

12.9.1 Validation Sheet

“Validation” is where the validation results/statistics are automatically calculated and contains all the controls for the macros. This sheet is protected from any manual modification so that the calculations are secure.

Validation Sheet:

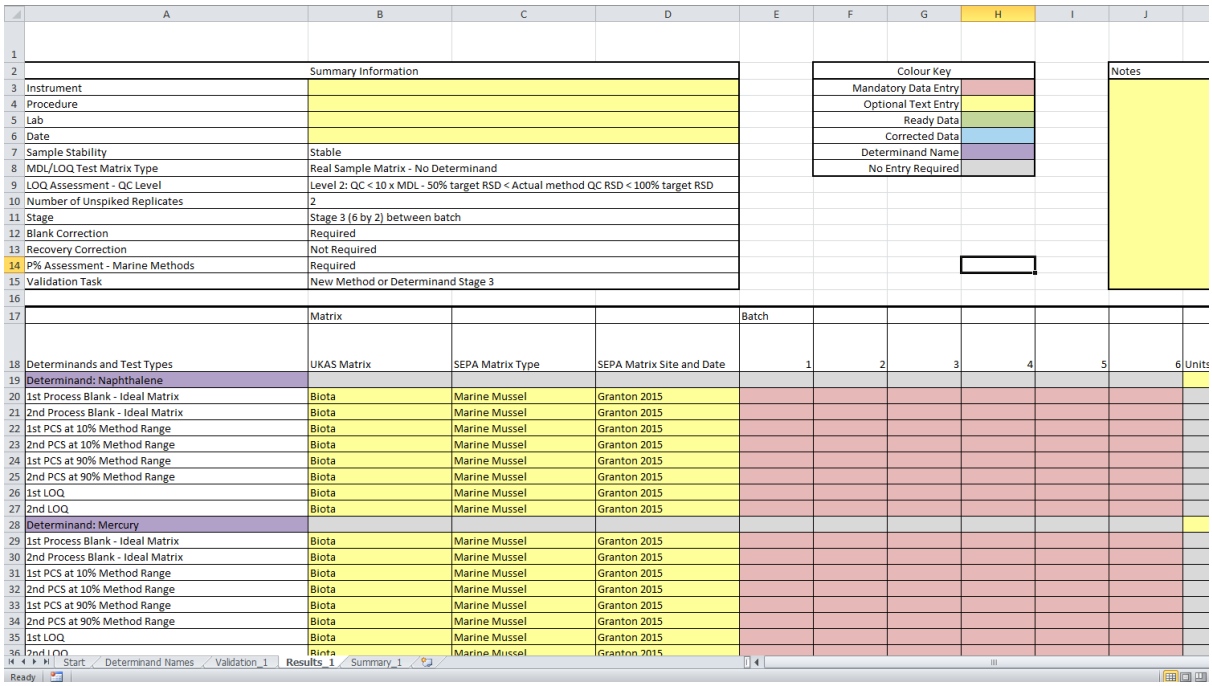


	A	B	C	D	E	F	G	H	I	J	K	L
1	Sheet created by Thomson, David at 14/07/2015 11:00:24	Submit Raw Results	Calculate Results	Clear Results								
2		Summary Information				Colour Key				Notes		
3	Instrument					Mandatory Data Entry						
4	Procedure					Optional Text Entry						
5	Lab					Ready Data						
6	Date					Corrected Data						
7	Sample Stability	Stable				Determinand Name						
8	MDL/LOQ Test Matrix Type	Real Sample Matrix - No Determinand				No Entry Required						
9	LOQ Assessment - QC Level	Level 2: QC < 10 x MDL - 50% target RSD < Actual method QC RSD < 100% target RSD										
10	Number of Unspiked Replicates	2										
11	Stage	Stage 3 (6 by 2) between batch										
12	Blank Correction	Required										
13	Recovery Correction	Not Required										
14	P% Assessment - Marine Methods	Required										
15	Validation Task	New Method or Determinand Stage 3										
16												
17		Matrix			Batch							Targets
18	Determinands and Test Types	UKAS Matrix	SEPA Matrix Type	SEPA Matrix Site and Date	1	2	3	4	5	6	Units	Required
19	Determinand: Naphthalene											
20	1st Process Blank - Ideal Matrix	Biota	Marine Mussel	Granton 2015								
21	2nd Process Blank - Ideal Matrix	Biota	Marine Mussel	Granton 2015								
22	1st PCS at 10% Method Range	Biota	Marine Mussel	Granton 2015								
23	2nd PCS at 10% Method Range	Biota	Marine Mussel	Granton 2015								
24	1st PCS at 90% Method Range	Biota	Marine Mussel	Granton 2015								
25	2nd PCS at 90% Method Range	Biota	Marine Mussel	Granton 2015								
26	1st LOQ	Biota	Marine Mussel	Granton 2015								
27	2nd LOQ	Biota	Marine Mussel	Granton 2015								
28	Determinand: Mercury											
29	1st Process Blank - Ideal Matrix	Biota	Marine Mussel	Granton 2015								
30	2nd Process Blank - Ideal Matrix	Biota	Marine Mussel	Granton 2015								
31	1st PCS at 10% Method Range	Biota	Marine Mussel	Granton 2015								

Start | Determinand Names | Validation_1 | Results_1 | Summary_1

12.9.2 Results Sheet

“Results” is where raw data is entered by the user. The layout of this sheet is similar to the Validation sheet. These raw results are imported into the “Validation” sheet. This sheet is unprotected.



	Summary Information
Instrument	
Procedure	
Lab	
Date	
Sample Stability	Stable
MDL/LOQ Test Matrix Type	Real Sample Matrix - No Determinand
LOQ Assessment - QC Level	Level 2: QC < 10 x MDL - 50% target RSD < Actual method QC RSD < 100% target RSD
Number of Unspiked Replicates	2
Stage	Stage 3 (6 by 2) between batch
Blank Correction	Required
Recovery Correction	Not Required
P% Assessment - Marine Methods	Required
Validation Task	New Method or Determinand Stage 3

Colour Key
Mandatory Data Entry
Optional Text Entry
Ready Data
Corrected Data
Determinand Name
No Entry Required

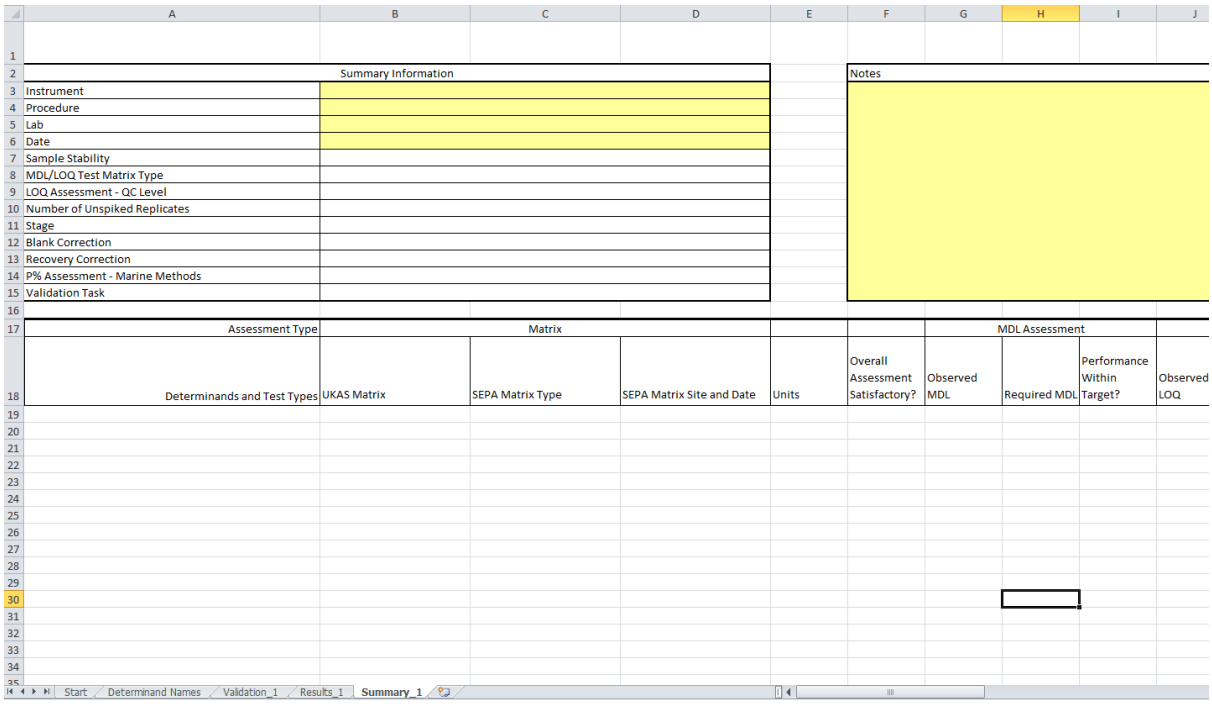
Notes

	Matrix			Batch						
Determinands and Test Types	UKAS Matrix	SEPA Matrix Type	SEPA Matrix Site and Date	1	2	3	4	5	6	Units
Determinand: Naphthalene										
1st Process Blank - Ideal Matrix	Biota	Marine Mussel	Granton 2015							
2nd Process Blank - Ideal Matrix	Biota	Marine Mussel	Granton 2015							
1st PCS at 10% Method Range	Biota	Marine Mussel	Granton 2015							
2nd PCS at 10% Method Range	Biota	Marine Mussel	Granton 2015							
1st PCS at 90% Method Range	Biota	Marine Mussel	Granton 2015							
2nd PCS at 90% Method Range	Biota	Marine Mussel	Granton 2015							
1st LOQ	Biota	Marine Mussel	Granton 2015							
2nd LOQ	Biota	Marine Mussel	Granton 2015							
Determinand: Mercury										
1st Process Blank - Ideal Matrix	Biota	Marine Mussel	Granton 2015							
2nd Process Blank - Ideal Matrix	Biota	Marine Mussel	Granton 2015							
1st PCS at 10% Method Range	Biota	Marine Mussel	Granton 2015							
2nd PCS at 10% Method Range	Biota	Marine Mussel	Granton 2015							
1st PCS at 90% Method Range	Biota	Marine Mussel	Granton 2015							
2nd PCS at 90% Method Range	Biota	Marine Mussel	Granton 2015							
1st LOQ	Biota	Marine Mussel	Granton 2015							
2nd LOQ	Biota	Marine Mussel	Granton 2015							

Start | Determinand Names | Validation_1 | Results_1 | Summary_1 | Ready

12.9.3 Summary Sheet

"Summary" is where a condensed version of the validation results is displayed along with an overall assessment, once final results are calculated (it is initially unpopulated). This sheet is unprotected.



	Summary Information
Instrument	
Procedure	
Lab	
Date	
Sample Stability	
MDL/LOQ Test Matrix Type	
LOQ Assessment - QC Level	
Number of Unspiked Replicates	
Stage	
Blank Correction	
Recovery Correction	
P% Assessment - Marine Methods	
Validation Task	

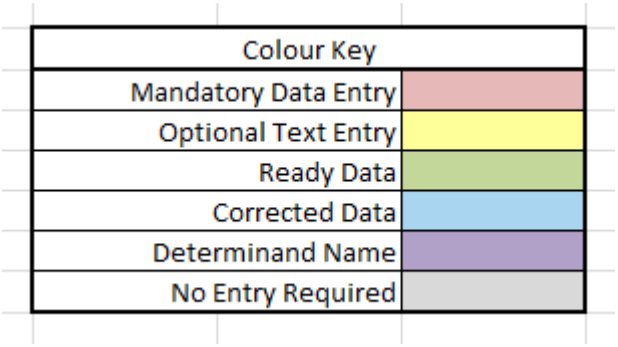
Notes

	Assessment Type	Matrix					MDL Assessment			
Determinands and Test Types	UKAS Matrix	SEPA Matrix Type	SEPA Matrix Site and Date	Units	Overall Assessment Satisfactory?	Observed MDL	Required MDL	Performance Within Target?	Observed LOQ

Start | Determinand Names | Validation_1 | Results_1 | Summary_1

12.9.4 Colour Key

Across each sheet a consistent approach to colour formatting is used, as shown by the colour key on the "Validation" and "Results" sheet. This gives you some guidance as to what is required for each cell in the "Results" sheet or its state in the "Validation" sheet.



Cells are coloured to indicate the following:

Mandatory Data Entry	These require numerical data to be entered (e.g. results for test types or targets). This data is used for calculations.
Optional Text Entry	These are for non-numerical text which may be useful but is not required for any calculations (e.g. matrix info, instrument or method details, notes)
Ready Data	This is applied once required (mandatory) data has been successfully submitted
Corrected Data	This is applied to test results if they have been blank, spike or recovery corrected prior to final statistical assessment
Determinand Name	These are headers for each determinand
No Entry Required	These are not required for any entry (e.g. cells which may be used in other situations but are not required as the sheets are configured currently)

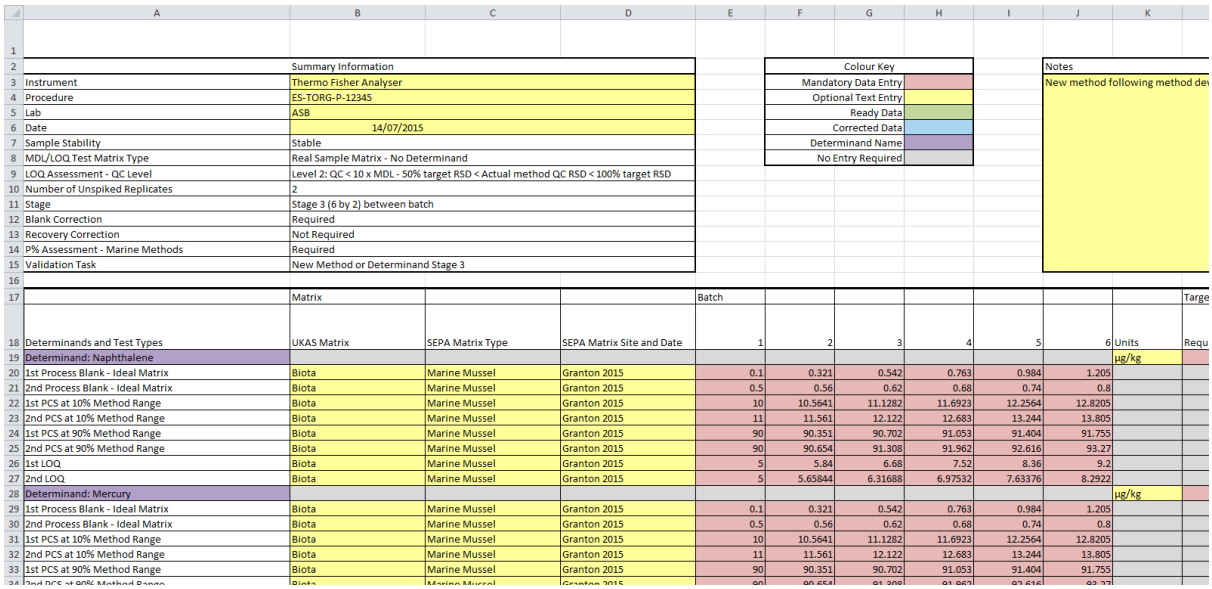
If required the standard colours used in the colour key can be modified by reformatting the colours on the “Start” sheet. Any new colours will then be applied each type new sheets are generated or modified.

12.10 How to Use the Generated Sheets

12.10.1 Enter Results into “Results” Sheet

Raw results from the validation exercise can now be entered onto the “Results” sheet. For each determinand you must enter all analytical results, as well as any required target values. The cells requiring data will be highlighted as “Mandatory Data Entry”. For clarity it may be sensible to only ‘paste values’ to preserve formatting. Test types are grouped by determinand in the first column. Note that for within batch testing the replicates (e.g. 6 or 11) are listed horizontally but for between batch testing the batches are listed horizontally but the replicates (e.g. 2 or 4) are listed vertically. If not previously entered already you can also enter determinand names and matrix details here. You may also add units to the sheet, method or instrument details into the header and notes in the box at the top.

Data entered into “Results” sheet:



	Summary Information
Instrument	Thermo Fisher Analyser
Procedure	ES-TORG-P-12345
Lab	ASB
Date	14/07/2015
Sample Stability	Stable
MDL/LOQ Test Matrix Type	Real Sample Matrix - No Determinand
LOQ Assessment - QC Level	Level 2: QC < 10 x MDL - 50% target RSD < Actual method QC RSD < 100% target RSD
Number of Unspiked Replicates	2
Stage	Stage 3 (6 by 2) between batch
Blank Correction	Required
Recovery Correction	Not Required
P% Assessment - Marine Methods	Required
Validation Task	New Method or Determinand Stage 3

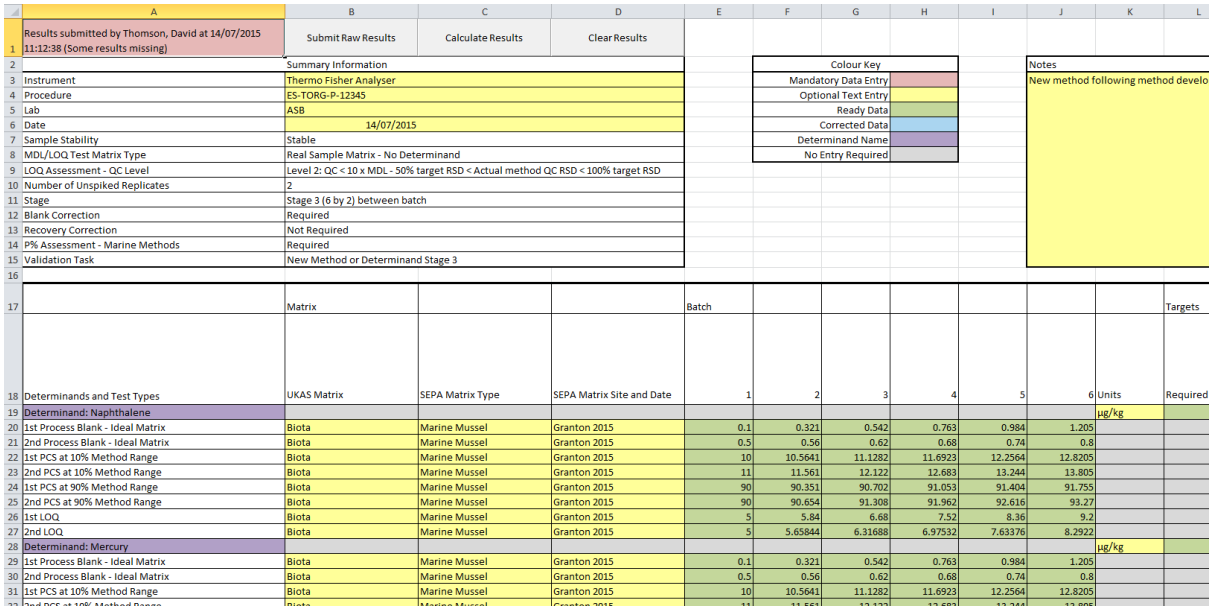
Colour Key
Mandatory Data Entry
Optional Text Entry
Ready Data
Corrected Data
Determinand Name
No Entry Required

Notes: New method following method dev

	Matrix			Batch							Targe
Determinands and Test Types	UKAS Matrix	SEPA Matrix Type	SEPA Matrix Site and Date	1	2	3	4	5	6	Units	Requ
Determinand: Naphthalene										µg/kg	
1st Process Blank - Ideal Matrix	Biota	Marine Mussel	Granton 2015	0.1	0.321	0.542	0.763	0.984	1.205		
2nd Process Blank - Ideal Matrix	Biota	Marine Mussel	Granton 2015	0.5	0.56	0.62	0.68	0.74	0.8		
1st PCS at 10% Method Range	Biota	Marine Mussel	Granton 2015	10	10.5641	11.1282	11.6923	12.2564	12.8205		
2nd PCS at 10% Method Range	Biota	Marine Mussel	Granton 2015	11	11.561	12.122	12.683	13.244	13.805		
1st PCS at 90% Method Range	Biota	Marine Mussel	Granton 2015	90	90.351	90.702	91.053	91.404	91.755		
2nd PCS at 90% Method Range	Biota	Marine Mussel	Granton 2015	90	90.654	91.308	91.962	92.616	93.27		
1st LOQ	Biota	Marine Mussel	Granton 2015	5	5.84	6.68	7.52	8.36	9.2		
2nd LOQ	Biota	Marine Mussel	Granton 2015	5	5.65844	6.31688	6.97532	7.63376	8.2922		
Determinand: Mercury										µg/kg	
1st Process Blank - Ideal Matrix	Biota	Marine Mussel	Granton 2015	0.1	0.321	0.542	0.763	0.984	1.205		
2nd Process Blank - Ideal Matrix	Biota	Marine Mussel	Granton 2015	0.5	0.56	0.62	0.68	0.74	0.8		
1st PCS at 10% Method Range	Biota	Marine Mussel	Granton 2015	10	10.5641	11.1282	11.6923	12.2564	12.8205		
2nd PCS at 10% Method Range	Biota	Marine Mussel	Granton 2015	11	11.561	12.122	12.683	13.244	13.805		
1st PCS at 90% Method Range	Biota	Marine Mussel	Granton 2015	90	90.351	90.702	91.053	91.404	91.755		

12.10.2 Submit Entered Raw Results

Once there are results ready to be assessed you can submit them onto the “Validation” sheet. To do this, press the “Submit Raw Results” button. Anything entered on the “Results” sheet will be copied across to the “Validation” sheet. Results submitted correctly will now be coloured as “Ready Data”. Any outstanding mandatory results will remain coloured as “Mandatory Data Entry”.



Results submitted by Thomson, David at 14/07/2015 11:12:38 (Some results missing)

Submit Raw Results | Calculate Results | Clear Results

	Summary Information
Instrument	Thermo Fisher Analyser
Procedure	ES-TORG-P-12345
Lab	ASB
Date	14/07/2015
Sample Stability	Stable
MDL/LOQ Test Matrix Type	Real Sample Matrix - No Determinand
LOQ Assessment - QC Level	Level 2: QC < 10 x MDL - 50% target RSD < Actual method QC RSD < 100% target RSD
Number of Unspiked Replicates	2
Stage	Stage 3 (6 by 2) between batch
Blank Correction	Required
Recovery Correction	Not Required
P% Assessment - Marine Methods	Required
Validation Task	New Method or Determinand Stage 3

Colour Key
Mandatory Data Entry
Optional Text Entry
Ready Data
Corrected Data
Determinand Name
No Entry Required

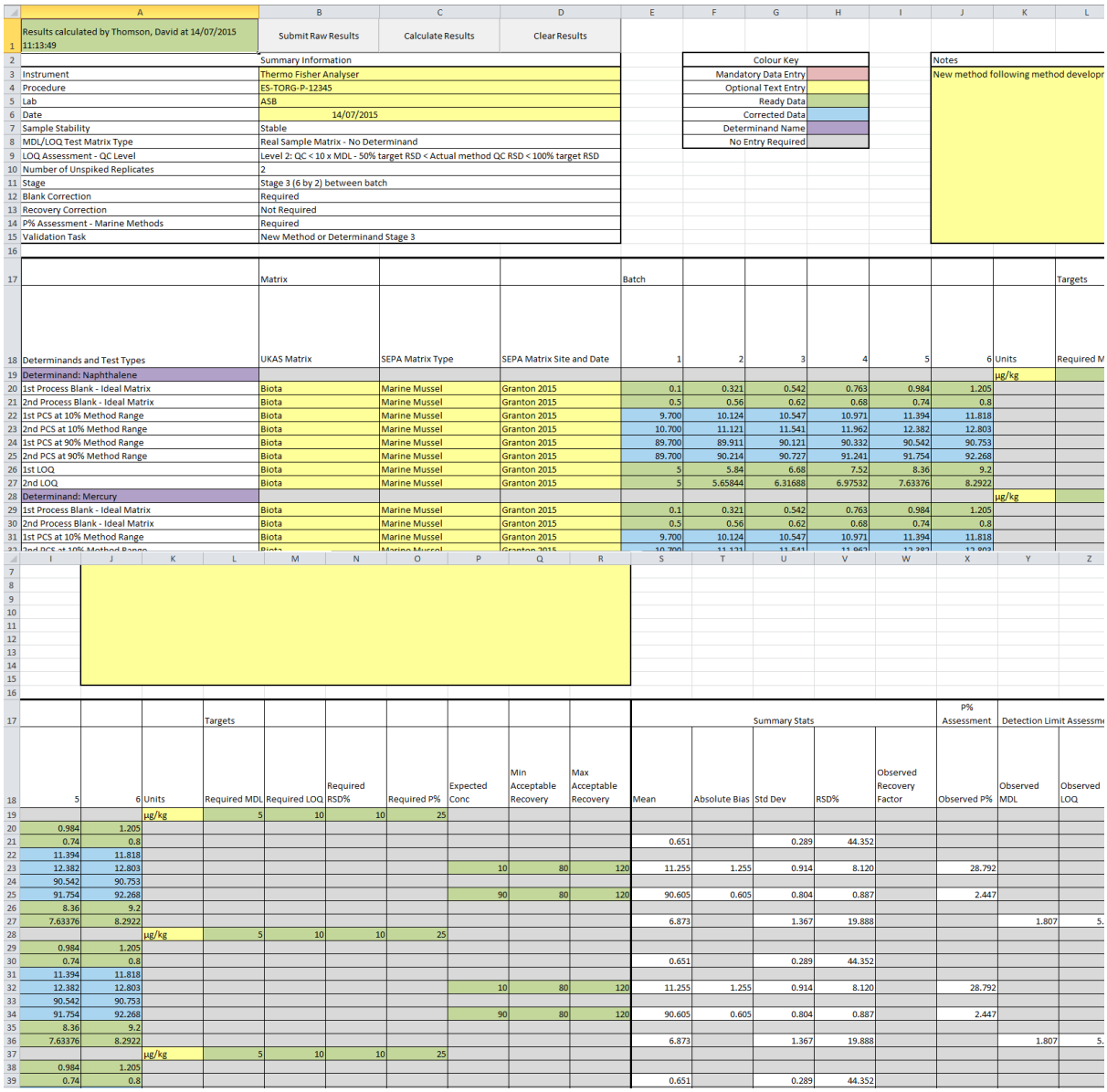
Notes: New method following method develo

	Matrix			Batch							Targets
Determinands and Test Types	UKAS Matrix	SEPA Matrix Type	SEPA Matrix Site and Date	1	2	3	4	5	6	Units	Required
Determinand: Naphthalene										µg/kg	
1st Process Blank - Ideal Matrix	Biota	Marine Mussel	Granton 2015	0.1	0.321	0.542	0.763	0.984	1.205		
2nd Process Blank - Ideal Matrix	Biota	Marine Mussel	Granton 2015	0.5	0.56	0.62	0.68	0.74	0.8		
1st PCS at 10% Method Range	Biota	Marine Mussel	Granton 2015	10	10.5641	11.1282	11.6923	12.2564	12.8205		
2nd PCS at 10% Method Range	Biota	Marine Mussel	Granton 2015	11	11.561	12.122	12.683	13.244	13.805		
1st PCS at 90% Method Range	Biota	Marine Mussel	Granton 2015	90	90.351	90.702	91.053	91.404	91.755		
2nd PCS at 90% Method Range	Biota	Marine Mussel	Granton 2015	90	90.654	91.308	91.962	92.616	93.27		
1st LOQ	Biota	Marine Mussel	Granton 2015	5	5.84	6.68	7.52	8.36	9.2		
2nd LOQ	Biota	Marine Mussel	Granton 2015	5	5.65844	6.31688	6.97532	7.63376	8.2922		
Determinand: Mercury										µg/kg	
1st Process Blank - Ideal Matrix	Biota	Marine Mussel	Granton 2015	0.1	0.321	0.542	0.763	0.984	1.205		
2nd Process Blank - Ideal Matrix	Biota	Marine Mussel	Granton 2015	0.5	0.56	0.62	0.68	0.74	0.8		
1st PCS at 10% Method Range	Biota	Marine Mussel	Granton 2015	10	10.5641	11.1282	11.6923	12.2564	12.8205		

12.10.3 Calculate Final Results and Summary

Once raw results are submitted the final results may be calculated by pressing the “Calculate Results” button. This will calculate all the required statistics for the validation exercise on the “Validation” sheet and it will also summarise an assessment of these on the “Summary” sheet. If there are any mandatory entries missing a message-box will inform you – this may have an impact on any calculations and could cause errors.

The “Validation” sheet contains a full set of all statistical calculations and results which are visible to the right of the analytical data. Any blank, recovery or spike corrected data will be coloured appropriately.



	A	B	C	D	E	F	G	H	I	J	K	L
1	Results calculated by Thomson, David at 14/07/2015 11:13:49	Submit Raw Results	Calculate Results	Clear Results								
2		Summary Information				Colour Key				Notes		
3	Instrument	Thermo Fisher Analyser				Mandatory Data Entry				New method following method developr		
4	Procedure	ES-TORG-P-12345				Optional Text Entry						
5	Lab	ASB				Ready Data						
6	Date	14/07/2015				Corrected Data						
7	Sample Stability	Stable				Determinand Name						
8	MDL/LOQ Test Matrix Type	Real Sample Matrix - No Determinand				No Entry Required						
9	LOQ Assessment - QC Level	Level 2: QC < 10 x MDL - 50% target RSD < Actual method QC RSD < 100% target RSD										
10	Number of Unspiked Replicates	2										
11	Stage	Stage 3 (6 by 2) between batch										
12	Blank Correction	Required										
13	Recovery Correction	Not Required										
14	P% Assessment - Marine Methods	Required										
15	Validation Task	New Method or Determinand Stage 3										
16												
17		Matrix			Batch							Targets
18	Determinands and Test Types	UKAS Matrix	SEPA Matrix Type	SEPA Matrix Site and Date	1	2	3	4	5	6	Units	Required M
19	Determinand: Naphthalene										µg/kg	
20	1st Process Blank - Ideal Matrix	Biota	Marine Mussel	Granton 2015	0.1	0.321	0.542	0.763	0.984	1.205		
21	2nd Process Blank - Ideal Matrix	Biota	Marine Mussel	Granton 2015	0.5	0.56	0.62	0.68	0.74	0.8		
22	1st PCS at 10% Method Range	Biota	Marine Mussel	Granton 2015	9.700	10.124	10.547	10.971	11.394	11.818		
23	2nd PCS at 10% Method Range	Biota	Marine Mussel	Granton 2015	10.700	11.121	11.541	11.962	12.382	12.803		
24	1st PCS at 90% Method Range	Biota	Marine Mussel	Granton 2015	89.700	89.911	90.121	90.332	90.542	90.753		
25	2nd PCS at 90% Method Range	Biota	Marine Mussel	Granton 2015	89.700	90.214	90.727	91.241	91.754	92.268		
26	1st LOQ	Biota	Marine Mussel	Granton 2015	5	5.84	6.68	7.52	8.36	9.2		
27	2nd LOQ	Biota	Marine Mussel	Granton 2015	5	5.65844	6.31688	6.97532	7.63376	8.2922		
28	Determinand: Mercury										µg/kg	
29	1st Process Blank - Ideal Matrix	Biota	Marine Mussel	Granton 2015	0.1	0.321	0.542	0.763	0.984	1.205		
30	2nd Process Blank - Ideal Matrix	Biota	Marine Mussel	Granton 2015	0.5	0.56	0.62	0.68	0.74	0.8		
31	1st PCS at 10% Method Range	Biota	Marine Mussel	Granton 2015	9.700	10.124	10.547	10.971	11.394	11.818		

	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z
17				Targets							Summary Stats					P% Assessment	Detection Limit Assessm	
18	5	6	Units	Required MDL	Required LOQ	Required RSD%	Required P%	Expected Conc	Min Acceptable Recovery	Max Acceptable Recovery	Mean	Absolute Bias	Std Dev	RSD%	Observed Recovery Factor	Observed P%	Observed MDL	Observed LOQ
19			µg/kg	5	10	10	25											
20	0.984	1.205																
21	0.74	0.8									0.651		0.289	44.352				
22	11.394	11.818																
23	12.382	12.803						10	80	120	11.255	1.255	0.914	8.120		28.792		
24	90.542	90.753																
25	91.754	92.268						90	80	120	90.605	0.605	0.804	0.887		2.447		
26	8.36	9.2																
27	7.63376	8.2922									6.873		1.367	19.888			1.807	5.
28			µg/kg	5	10	10	25											
29	0.984	1.205																
30	0.74	0.8									0.651		0.289	44.352				
31	11.394	11.818																
32	12.382	12.803						10	80	120	11.255	1.255	0.914	8.120		28.792		
33	90.542	90.753																
34	91.754	92.268						90	80	120	90.605	0.605	0.804	0.887		2.447		
35	8.36	9.2																
36	7.63376	8.2922									6.873		1.367	19.888			1.807	5.
37			µg/kg	5	10	10	25											
38	0.984	1.205																
39	0.74	0.8									0.651		0.289	44.352				

The summary sheet assesses the relevant statistical results from the “Validation” sheet against the entered targets and presents the results in a condensed format. An autofilter is enabled for the sheet which will allow subsets of the results to be viewed should this be convenient. Notes can also be made in the box at the top.

Summary Information	
Instrument	Thermo Fisher Analyser
Procedure	ES-TORG-P-12345
Lab	ASB
Date	14/07/2015
Sample Stability	Stable
MDL/LOQ Test Matrix Type	Real Sample Matrix - No Determinand
LOQ Assessment - QC Level	Level 2: QC < 10 x MDL - 50% target RSD < Actual method QC RSD < 100% target RSD
Number of Unspiked Replicates	2
Stage	Stage 3 (6 by 2) between batch
Blank Correction	Required
Recovery Correction	Not Required
P% Assessment - Marine Methods	Required
Validation Task	New Method or Determinand Stage 3

Notes

Assessment Type	Matrix					MDL Assessment			LOQ Assessment		
Determinands and Test Type	UKAS Matrix	SEPA Matrix Type	SEPA Matrix Site and Date	Units	Overall Assessment Satisfactory	Observed MDL	Required MDL	Performance Within Target?	Observed LOQ	Required LOQ	Performance Within Target?
Determinand: Naphthalene					No			Yes			Yes
PCS at 10% Method Range	Biota	Marine Mussel	Marine Mussel								
PCS at 90% Method Range	Biota	Marine Mussel	Marine Mussel								
LOQ	Biota	Marine Mussel	Marine Mussel			1.807	5.000	Yes	5.422	10.000	Yes
Determinand: Mercury					No			Yes			Yes
PCS at 10% Method Range	Biota	Marine Mussel	Marine Mussel								
PCS at 90% Method Range	Biota	Marine Mussel	Marine Mussel								
LOQ	Biota	Marine Mussel	Marine Mussel			1.807	5.000	Yes	5.422	10.000	Yes
Determinand: Gravy					No			Yes			Yes
PCS at 10% Method Range	Biota	Marine Mussel	Marine Mussel								
PCS at 90% Method Range	Biota	Marine Mussel	Marine Mussel								
LOQ	Biota	Marine Mussel	Marine Mussel			1.807	5.000	Yes	5.422	10.000	Yes

13 APPENDIX D
Statistical calculations used in determining method performance

A key aspect of the validation procedure is the requirement to measure performance across a number of batches of the various test types. In order to separate the variation in performance due to within-batch and between-batch factors we closely follow the methods described in NS30, any exceptions are explicitly noted. This involves the use of significance testing at various stages.

D1 Assessment of method performance

The approach to quantifying the within-batch and between-batch performance recognises that the total standard deviation or variance (s_t^2) of a method can be divided into two components, between-batch variance (s_b^2) and within-batch variance (s_w^2).

$$s_t^2 = s_b^2 + s_w^2$$

Understanding how the total standard deviation error is divided between these two components is important to understanding the sources of random error in any method.

D2 Calculation of within-batch, between-batch and total standard deviation

The phrase “within-batch” is potentially misleading as it suggests that we are solely concerned with performance within a *single* batch. In method validation “within-batch” is a pooled estimate of variance or standard deviation from *multiple* batches (i.e. 11 x 2 or 6 x 4). It is not permissible to use a single batch to calculate within-batch precision during method validation.

The assessment of between- and within-batch variances or standard deviations follows NS30 and uses an ANOVA approach. This calculates the mean square values, M_0 and M_1, which are the within-batch and between-batch mean squares respectively. M_0 is solely an estimate of the within-batch variance and M_1 is a function of both the within-batch and between-batch variances. They are calculated using the following:

$$M_0 = \sum_{i=1}^{m} \frac{s_i^2}{m}$$

where s_i is an estimate of standard deviation of the ith batch of m batches; and:

$$M_1 = n.s_{bm}^2$$

where s_{bm} is the standard deviation of n batch means. See p76 & p77 of NS30.

M_0 and M_1 are also used to calculate s_w, s_t and s_b using the equations shown on p77:

$$s_w = \sqrt{M_0}$$

$$s_b = \sqrt{(M_1 - M_0)/n}$$

$$s_t = \sqrt{(M_1 + (n-1)M_0)/n}$$

The within-batch standard deviation (s_w) is used when calculating detection limits – see below.

The total standard deviation (s_t) as calculated above is used to assess the method precision - see section below.

As an observation, s_t as calculated by NS30, is very similar to a simple standard deviation calculated from each of the individual data points across multiple batches. However, the calculation used by NS30 for s_t should always be used for calculating total standard deviation.

The between batch standard deviation is only used where samples show instability between batches – see below.

D3 Calculation of detection limits

When calculating detection limits (e.g. Method Detection Limit) it is important that the within-batch standard deviation is used, i.e. the pooled estimate standard deviation from multiple batches. Detection limit calculations in all validation work will always use multiple batches and use the within-batch standard deviation (s_w) as defined above.

NOTE: In the Preliminary Stages of method development (see Sections 4.3.2 and 4.3.3), it is permissible to run single batches of Blanks, IPS's, PCS's etc. to verify that the procedure is capable of meeting the performance requirements in the short term. This single batch approach must not be used for method validation; it should only be used during preliminary stages of method development.

D4 Method performance assessment of unstable samples

If the determinand concentration is not stable across multiple batches, the value for between-batch standard deviation will overestimate the true between-batch variability. The approach taken in NS30 and used here makes three assumptions:

1. That standard solutions prepared freshly for each analysis batch will not be affected by any between-batch instability that affects samples.
2. That the between-batch standard deviation of standards is directly proportional to determinand concentration of the standards.
3. The instability does not affect the within-batch variability of the samples.

The first two assumptions are well-founded. If the third assumption is not valid, the F-test used to compare M_0 and M_1 should identify this (see below).

In order to estimate the between-batch precision (s_b) of unstable samples, the s_b values of freshly prepared standards are plotted against their concentrations and a linear regression fitted through the origin. This linear relationship is then used to estimate s_b values for the samples based on the mean concentration of unstable samples across the validation batches. Only then is a final M_1 value calculated and significance testing carried out. NS30 gives a practical example of how unstable

samples are dealt with. Note that the within-batch standard deviation remains valid throughout this process to deal with unstable samples.

D5 Assessment of bias

Bias is measured by analysing a CRM or analysing spiked-unspiked sample matrix pairs and comparing the difference with the expected difference. In the case of a CRM the bias is expressed as a percentage difference from the accepted reference value. If this is lower than the target bias (positive or negative), then the result is acceptable. When analysing spiked-unspiked sample matrix pairs the bias is the difference (positive or negative) between the two results expressed as a percentage difference from the expected result. This is different to the approach taken by NS30, where bias is expressed as a "recovery" with for example 105% recovery being equivalent to a positive 5% bias. In practice there is no practical different in outcomes when the various statistical tests are applied.

Here we avoid the use of the term recovery when talking about spiked-unspiked sample pairs. The term recovery is reserved for pre-treatment methods. For example where an extraction as part of sample preparation may not recover all of the determinand of interest and a recovery correction may be required when calculating final results.

D6 Significance testing in method validation

Statistical significance testing should be used to assess method validation performance data, specifically:

1. When comparing the within-batch and between-batch mean squares – F-test.
2. When determining whether total precision is significantly greater than the target precision – F-test.
3. When assessing whether the measured bias lies significantly outside the target bias range – t-test.

Comparison between the within-batch and between-batch mean squares

NS30 uses a one-sided F-test to determine if M1 is significantly greater than M0 or that there is no significant difference. Here we use a two-sided F-test to compare the between-batch mean square (M_1) and the within-batch mean square (M_0), always ensuring that the calculated F value is greater than 1. There are three possible outcomes.

1) M_1 is significantly greater than M_0. This is a common situation found in many methods and is a pass if the target precision is met.
2) M_1 is not significantly different than M_0. This is also a pass if the target precision is met.
3) M_0 is significantly greater than M_1. This is a fail and is indicative of a potential problem with the method. For example a consistent drift within the analytical batches.

The number of degrees of freedom used to determine critical one-sided F-values are as follows: between-batch degrees of freedom = *m-1*; and within-batch degrees of freedom = *m(n-1)*. The confidence limit used is 95%.

Precision assessment against a target precision

A one-sided F-test is used to determine whether an estimated precision is significantly greater than the target precision. The F-test is only applied if the estimate of precision is greater than the target precision, if it is less it is a pass and no F-test is required. There are two possible outcomes to this one-sided F-test with 95% confidence limits.

1) The estimate of precision is not significantly different to the target precision. This is a pass; the target precision has been met.
2) The estimate of precision is significantly greater than the target precision. This is fail; the target precision has not been met.

The number of degrees of freedom for the total precision estimate is calculated using the following formula, with the final value rounded to the nearest whole number:

$$\text{Degrees of freedom} = \frac{m(m-1)(M_1 + (n-1)M_0)^2}{mM_1^2 + (m-1)(n-1)M_0^2}$$

The degrees of freedom for the target precision is infinite, although for calculation purposes a value of $\geq 10^{10}$ is sufficient. Again, the confidence limit used is 95%.

Significance testing in the assessment of bias

All bias determinations are based on the mean result from multiple batches (i.e. 11 x 2). Where the overall batch mean is within the limits of the target bias range the result is acceptable. If the batch mean lies outside the limits (either higher or lower), the individual batch means (11 in the case of an 11 x 2) are used to assess if the overall mean bias is significantly greater or less than the target range using a one-sided t-test. The t-value is calculated using the following equation:

$$t = (\bar{x} - bias\ target\ limit)\frac{\sqrt{m}}{s}$$

where $\bar{x}$ is the overall mean bias, m is the number of batches and s is the standard deviation of the batch means. The calculated t-value is then compared against a one-sided critical t-value at 95% confidence limits. If the calculated t-value is greater than the critical t-value then the bias must be significantly greater than the target bias.