Quality Control Document:

Assay Validation

# Purpose

Analytical assays performed to Good Clinical Practice (GCP) standards must be properly validated prior to undertaking the analysis of clinical trial samples to ensure data integrity. The performance characteristics of the assay must be demonstrated to be suitable and reliable for the intended application through the use of specific laboratory investigations. In addition, acceptance criteria for each assay must be transparent and clearly defined.

This document provides a guideline for assay validation and gives examples of assay parameters which may be considered as part of the validation process. This document is designed to be used for clinical trials of investigational medicinal products (CTIMPs) but could also be used for non-CTIMP trials and studies to support best practice.

# Instructions

1. Remove this first instruction page.
2. Update the header to include the trial/study ID.
3. Update the footer; include a version date and retain the document reference information relating to this quality control document (QCD).

## Assay Validation Plan

1. Remove red instruction text.
2. Complete an assay validation plan for each analytical assay of clinical trial samples to be completed by the laboratory.
3. Ensure the assay validation plan is approved by an appropriate member of the research team (e.g., the Laboratory Academic Lead (LAL), the Chief Investigator (CI).
4. File each approved validation plan in the ‘Assay Validation’ section of the Laboratory Master File (LMF); see quality control document (QCD) Setting Up a Laboratory Master File (UoB-CRL-QCD-001). Archive with the other trial records upon closure; see Archiving SOP (UoB-ARC-SOP-001).

## Assay Validation Report

1. Carry out the assay validation as described in Assay Validation Plan.
2. Record the outcomes in the Assay Validation Report. Note - all assay validation reports must be approved by an appropriate member of the research team (e.g., the LAL, the CI) before the assay can be used to analyse clinical trial samples.
3. File each approved validation report in the ‘Assay Validation’ section of the LMF; see QCD Setting Up a Laboratory Master File (UoB-CRL-QCD-00). Archive with the other trial records upon closure; see Archiving SOP (UoB-ARC-SOP-001).

# Related documents

* UoB-ARC-SOP-001 Archiving
* UoB-CRL-QCD-001 Setting Up a Laboratory Master File
* UoB-CRL-SOP-001 Laboratory Set Up and Management
* UoB-CRL-SOP-002 Laboratory Facilities
* UoB-CRL-SOP-003 Sample Management
* UoB-CRL-SOP-004 Laboratory Analysis
* UoB-CRL-SOP-005 Reportable Issues

UoB QMS documents can be found on the [CRCT website](https://www.birmingham.ac.uk/research/activity/mds/mds-rkto/governance/index.aspx). Internal work instructions can be obtained from the CRCT (<mailto:crct@contacts.bham.ac.uk>) and/or from the RGT ([researchgovernance@contacts.bham.ac.uk](mailto:researchgovernance@contacts.bham.ac.uk)).

Validation Plan

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| --- |
| Assay Name |
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| Assay details |
| *Method overview* |
| Assay Methodology |
| *List the trial-specific procedures involved, giving the code and title for each.* |
| Validation parameters being tested |
| *Describe how each of these parameters is being addressed during the validation. Justify any exclusion. It is accepted that not all performance parameters can be easily validated for some types of analytical assay e.g. some pharmacodynamic cell-based assays.* |
| Characterisation of controls |
| *Where possible samples will be the same biological matrix as the test samples*  *For many types of assay, data for each control sample can be derived from a calibration curve constructed using a zero control and 5 – 8 non-zero reference standards.*  *The data used from replicate analysis of control samples and reference standards (where appropriate and/or available) should be used to obtain intra- and inter-assay data on precision and accuracy.* |
| Precision *Precision is the closeness of replicate determinations. This parameter can be further subdivided into intra-assay precision and inter-assay precision.*  *Measure precision using a minimum of five determinations.*  *Where appropriate to the assay, carry this out at using at least three concentrations in the range expected.*  *Determine both the intra-assay precision and inter-assay precision.* |
| Accuracy *Accuracy is the closeness of the test results to the true value of the analyte.*  *Measure accuracy using a minimum of five determinations.*  *Where appropriate to the assay, carry this out for a minimum of three concentrations in the expected range.* |
| **Calibration (where appropriate)**  *Calibration is the relationship between the experimental value and the analytical concentration.*  *A calibration curve should be constructed which consists of a blank or negative sample and typically 5 - 8 non-zero samples.*  *A sufficient number of standards should be employed to adequately define the relationship between concentration and response, and also reflect the concentration range expected in a particular study.*  *Replicate samples may be used to improve accuracy, but the same number of replicates should be used when analysing unknown samples.* |

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| Specificity *Specificity is the ability of an analytical method to differentiate the analyte in the presence of other constituents in the sample.*  *This is particularly important for ligand binding assays (e.g. ELISAs, ELISPOT assays) due to potential problems associated with cross-reactivity and non-specific binding.*  *Where appropriate, test blank or normal samples of the biological matrix from a minimum of ten individuals. If more than 10% of blank samples give significant interference, test additional blank samples. If more than 10% of these samples give significant interference the method should be modified.* | |
| Stability *Stability is a function of the storage conditions of the analyte.*  *Stability experiments should address standards, control samples, test samples, and key test reagents.*  *Stability experiments on test samples should reflect situations likely to be encountered during actual sample handling i.e. from being taken from the patient, sample transit, short and long term storage at the intended temperature, and also freeze-thaw cycles if appropriate.* | |
| Acceptance criteria | |
| *Precision - Variability for low, medium and high concentrations should be <15% and at the limit of detection it should be <20%.*  *Accuracy - Mean experimental values should be within +15% of the nominal value at the low, medium and high concentrations and should not deviate more than +20% at the limit of detection.*  *Sensitivity - The lowest standard should be accepted as the limit of quantitation if the precision and accuracy are as defined above.*  *Specificity - should be >95%.*  *Acceptance criteria should be defined in the validation report.*  *The validation report should rationalise the acceptance of any reduced performance specifications.* | |
| Primary Data Storage | |
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| Storage and management of reagents | |
| *Key reagents, controls and standards should be managed and stored appropriately throughout. Try to ensure that the same lot or batch numbers of standards, control samples and key reagents are used for both the validation experiments and analyses throughout the trial. Substantial changes in any of these, or any important item of equipment, may mean that the method has to be re-validated during the study.* | |
| References | |
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| Appendices | |
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| Prepared by: | |
| Name: | Signature: |
| Function: | |
| Date (dd-mmm-yyyy): | |

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| Approved by: | |
| Name: | Signature: |
| Function: | |
| Date (dd-mmm-yyyy): | |

# Validation Report

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| Experimental results | |
| 1. Including characterisation of controls. | |
| Conclusions | |
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| Recommendations and acceptance criteria | |
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| References | |
|  | |
| Prepared by: | |
| Name: | Signature: |
| Function: | |
| Date (dd-mmm-yyyy): | |
| Approved by: | |
| Name: | Signature: |
| Function: | |
| Date (dd-mmm-yyyy): | |