Pediatric Blood and Marrow Transplant
Adult Blood and Marrow Transplant
Stem Cell Laboratory

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Analytical Validation Protocol

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Validation Protocol Title:

Prepared By:	Date:
Approved By: Department Supervisor/Manager	Date:
Approved By: Medical Director	Date:
Approved By: APBMT Clinical Quality Program (CQP)	Date:

This form is to be used as a guide in the development of an analytical validation. Applicable contents can be imported into an MS Word document to create the validation protocol and final report.

1. Table of Contents

2. Objective and Scope

Clearly state the objective of the validation to validate a particular analytical method. List names/numbers of relevant SOPs to be followed during this validation. Insert information about scope, such as how many samples will be used or how many runs will be included, etc.

2.1. Validation Execution Prerequisites

Detail any pre-execution requirements for the validation protocol. This may include verification of qualification status of any equipment/systems required in the execution of protocol, any change controls necessary.

3. Introduction and Background

Provide details of the analytical method being validated, what it is used to assess, and principle for use. Validation parameters should meet the requirements of ICH Guideline Q2 (R1) and Guidance for Industry: Analytical Procedures and Methods Validation for Drugs and Biologics (July 2015).

Type of Analytical Procedure *Characteristics*	*IDENTIFICATION*	*TESTING FOR IMPURITIES* *Quantitation limit*		*ASSAY* *- dissolution (measurement only)* *- content/potency*
Accuracy	-	+	-	+
Precision	-	+	-	+
Repeatability	-	+ (1)	-	+ (1)
Interim. Precision	+	+	+	+
Specificity (2)	-	- (3)	+	-
Detection Limit				
Quantitation Limit	-	+	-	-
Linearity				
Range	-	+	-	+
	-	+	-	+

Key:

- *- signifies that this characteristic is not normally evaluated*
- *+ signifies that this characteristic is normally evaluated*
- *(1) in cases where reproducibility has been performed, intermediate precision is not needed*
- *(2) lack of specificity of one analytical procedure could be compensated by other supporting analytical procedure(s)*
- *(3) may be needed in some cases*

Example:
This validation protocol was generated in accordance with ICH Guideline Q2 (R1), Validation of Analytical Procedures and SOP COMM-QA-044, Approaches to Validation. The following analytical performance parameters will be assessed:

1. *Accuracy*
2. *Precision*
   a. *Repeatability*
   b. *Intermediate Precision*
3. *Specificity*
4. *Limit of Quantitation*
5. *Linearity*
6. *Range*

Details regarding each of these parameters and how they will be assayed, the equipment used, reagents, and how samples are prepared are provided in the sections below.

When switching to a new analytical method, a comparability study should be considered to verify that the new method performs comparably or better than the previously validated method. Every effort should be made to ensure that any outside sources of variation between methods are minimized. When making changes to a validated method, an assessment should be made to determine if re-validation is needed. This may include comparability of one or more parameters, as applicable.

Procedures will be performed per the following SOPs.

Table 3.1

SOP Number	SOP Title

Following execution of this protocol, a final report will be generated, including data and final conclusions. Data must be available to establish that the analytical procedures used in testing meet proper standards of accuracy, sensitivity, specificity, and reproducibility (as applicable) and are suitable for their intended purpose.

4. List of Equipment and Reagents

List and describe equipment and reagents to be used in the analytical validation. Equipment should be appropriately qualified prior to use in the validation.

Example:

The following equipment and reagents will be used during execution of this validation protocol. This equipment is used when performing this XXX procedure during standard practice in the XXX Laboratory. The validation will be performed with appropriately sized, calibrated pipettes.

Table 4.1

Equipment/Reagent	Serial Number/Label	Location

5. Accuracy

Accuracy is defined as the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found.

5.1. Sample Preparation

Describe sample preparation.

Example:
Spiked samples will be prepared at three concentrations over the range of 50 to 150% of the target concentration. Three individually prepared replicates at each concentration will be analyzed. When it is impossible or difficult to prepare known placebos, a low concentration of a known standard will be used.

5.2. Acceptance Criteria

Describe acceptance criteria. This is typically reported as CV, % change, or % difference.

Example:
The mean recovery must be within 90 to 110% of the theoretical value.

5.3. Analysis Methodology

List and describe statistical analyses here.

Example:
For each sample, the theoretical value and the assay value will be reported. The mean, standard deviation, and percent recovery will be calculated for all samples.

6. Precision

6.1. Repeatability

Repeatability is defined as the precision under the same operating conditions over a short interval of time. Repeatability will be demonstrated by a single operator.

6.1.1. Sample Preparation

Describe sample preparation.

6.1.2. Acceptance Criteria

Provide details of the acceptance criteria. Refer to ICH guidance to define acceptance criteria.

Example:
The acceptance criteria for repeatability are XXX.

6.1.3. Analysis Methodology

List and describe statistical analyses here.

Example:
Variability will be assessed by comparing triplicate results at each sample concentration. The standard deviation, relative standard deviation (coefficient of variation), and confidence interval will be reported.

Table 6.1

Test Sample	***Results % viability***	***Standard deviation, relative standard deviation or coefficient of variation, and confidence interval***	***Specification***
High-1			*≤5% CV*
High-2			
High-3			
Intermediate-1			*≤5% CV*
Intermediate-2			
Intermediate-3			
Low-1			*≤5% CV*
Low-2			
Low-3			

6.2. Intermediate Precision

Intermediate precision is defined as the within-laboratory variations: different days, different analysts, different equipment, etc.

Provide details of intermediate precision.

It was decided to have 3 operators test intermediate precision on the same day in order to allow for the use of a single, fresh, processed cord blood test sample.

6.2.1. Sample Preparation

Describe sample preparation.

Example:
The first test sample, high viability, will consist of fresh processed cord blood alone. The low viability test sample will consist of fresh processed cord blood diluted 1:10 with the 1% DMSO frozen/thawed, processed cord blood. Specifically, 10 µL of fresh, processed cord blood will be mixed with 90 µL of the thawed 1% DMSO sample, yielding a sample with low viability.

Operator 1 will first create each of the high and low viability samples based on the instructions above. Samples will be processed according to the SOP and then tested in triplicate. Operator 2 will prepare and test the same two samples, using a different hemocytometer from operator 1 and a different lot of trypan blue. Operator 3 will prepare and test the same two samples, randomly choosing the hemocytometer and the lot of trypan blue.

6.2.2. Acceptance Criteria

Describe acceptance criteria.

Example:
The target value for intermediate precision is ≤10% variability for the same sample between different operators/days.

6.2.3. Analysis Methodology

List and describe statistical analyses here.

Variability will be assessed by comparing all results at each sample concentration. The standard deviation, relative standard deviation (coefficient of variation), and confidence interval will be reported.

Table 6.2

Test Sample	***Operator #1 % Viability***	***Operator #2 % Viability***	***Operator #3 % Viability***	***Standard deviation, relative standard deviation or coefficient of variation, and confidence interval***	***Specification***

High-1					*≤10% CV*
High-2					*≤10% CV*
High-3					*≤10% CV*
Low-1					*≤10% CV*
Low-2					*≤10% CV*
Low-3					*≤10% CV*

7. Specificity

Specificity is defined as the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Specificity will be demonstrated by a single operator.

7.1. Sample Preparation

Provide details of the sample preparation.

Example:
Two possible components in cord blood that could impact the assessment of viability are high levels of dead cells or the presence of high levels of red blood cells. In the assessment of linearity (Section 9), viability will be measured over a range of approximately 10-100% viable cells, and thus specificity with respect to high levels of dead cells will be determined.

Processed cord blood typically has a hematocrit of 20-30%. For this assessment of specificity, processed cord blood (low red blood cells) will be diluted with red blood cells derived from the same cord blood unit at a ratio of 1:1 to create a sample with high red blood cells. Specifically, the hematocrit of the processed cord blood will be measured along with that of the isolated red blood cells. Fifty microliters of red blood cells will be added back to 50 µL of the processed cord blood, and the expected hematocrit of the new solution will be documented. We expect this value to be around 60%.

Each sample will be prepared as defined in the SOP and tested in triplicate. Specificity will be determined by comparing the variability of viability between test conditions.

7.2. Acceptance Criteria

Describe acceptance criteria.

Example:
The target value for specificity is ≤5% variability between test results.

7.3. Analysis Methodology

List and describe statistical analyses here.

Example:
Variability will be assessed by comparing triplicate results at each sample concentration. Specificity will be assessed by calculating variability between each test condition (high, intermediate, and low RBCs). The standard deviation, relative standard deviation (coefficient of variation), and confidence interval will be reported.

Table 7.1

Test Sample	***Results % viability***	***Standard deviation, relative standard deviation or coefficient of variation, and confidence interval***	***Specification***
High-1			*≤5% CV*
High-2			
High-3			*≤5% CV*
Low-1			
Low-2			*≤5% CV*
Low-3			

8. Limit of Quantitation

Quantitation limit is defined as the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. Limit of quantitation will be demonstrated by a single operator.

Provide details of the quantitation limit.

Example:
Based on the current SOP, a visual evaluation is performed to quantitate the results of this assay. A technician must count at least 100 cells. If the number of cells is not sufficient in four large squares of one chamber, both chambers of the hemocytometer must be inoculated. Then, all 8 sides must be counted before performing viability calculations.

8.1. Sample Preparation

Describe sample preparation.

Example:
Three different fresh processed cord blood samples will be independently prepared according to the SOP and diluted to give approximately 100 cells in the 8 squares to be counted per the SOP. Each sample will be tested in triplicate. This will be done on a single instrument.

8.2. Acceptance Criteria

Describe acceptance criteria.

Example:
The precision between the replicates of each sample must achieve a target value of ≤5%.

8.3. Analysis Methodology

List and describe statistical analyses here.

Example:
Precision will be calculated as indicated above. Variability will be assessed by comparing duplicate results for each sample. The standard deviation, relative standard deviation (coefficient of variation), and confidence interval will be reported.

Table 8.1

Test Sample	***Results (% viability)***	***Standard deviation, relative standard deviation or coefficient of variation***	***Specification***
A-1			*≤5% CV*
A-2			
A-3			
B-1			*≤5% CV*
B-2			
B-3			
C-1			*≤5% CV*
C-2			
C-3			

9. Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample. Linearity will be tested by a single operator.

9.1. Sample Preparation

Provide details of the sample preparation.

Example:
The trypan blue assay is used to measure dead cells as a component of live and dead cells to calculate viability. To test linearity, a sample of post-processed cord blood will be frozen in 1% DMSO to -150°C and thawed to room temperature to create a sample of increased dead cells. This sample with low cell viability will be mixed with a fresh sample in the ratios listed in the table below to create a range of 5 different sample concentrations. These samples will then be prepared according to the SOP and tested in triplicate. This will be done on a single instrument.

9.2. Acceptance Criteria

Describe acceptance criteria.

Example:
Acceptable linearity is the measurement of non-viable cells in increasing numbers of viable cells (from A to E in the table below). Furthermore, the viability variability of each sample should be within a target value of 5% of the estimated viability based on the initial readings of the fresh and frozen samples.

9.3. Analysis Methodology

List and describe statistical analyses here.

Example:
The viability percentages from the linearity assessment will be plotted. A regression line will be calculated, and the correlation coefficient, y-intercept, slope of the regression line, and residual sum of the squares will be submitted.

Table 9.1

Test Sample	***Fresh sample mixed with frozen/thawed sample (with 1% DMSO) Fresh/frozen thawed***	***Specification***	***Results % Viability***	***% Recovery***
A-1	*100% fresh/0% frozen-thawed*	*>90%*		
A-2				
A-3				
B-1	*75% fresh/25% frozen-thawed*	*75%*		
B-2				
B-3				
C-1	*50% fresh/50% frozen-thawed*	*45%*		
C-2				
C-3				
D-1	*25% fresh/75% frozen-thawed*	*25%*		
D-2				
D-3				
E-1	*0% fresh/100% frozen-thawed*	*5%*		
E-2				
E-3				

10. Range

Range is defined as the interval between the upper and lower concentration (amounts) of analyte in the sample (including those concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy, and linearity.

Provide details of the analyte range.

Example:
Based on the current SOP, a visual evaluation is performed to quantitate the results of this assay. A technician must count at least 100 cells. If the number of cells is not sufficient in four large squares of one chamber, both chambers of the hemocytometer must be inoculated. Then, all 8 sides must be counted before performing viability calculations. Thus, because a technician must count at least 100 cells in order for the assay result to be valid, a formal range for this assay has not been established.

Furthermore, if cells are overlapping or too dense to distinguish between viable and non-viable cells, a greater dilution is prepared, and counting then proceeds using the standard protocol. This upper limit has not been defined but is left to the analyst's discretion.

10.1. Sample Preparation

Describe sample preparation.

Example:
The linearity assessment in Section 9 should help demonstrate that the trypan blue assay can make viability determinations over a wide range of values.

10.2. Acceptance Criteria

Describe acceptance criteria.

Example:
Not applicable since range will not be determined.

10.3. Analysis Methodology

List and describe statistical analyses here.

Example:
Not applicable since range will not be determined.

11. Amendment Revision History (as applicable)

Provide a brief description of the change(s) from the previous review and approval. Include justification for the change(s).

12. Final Report

A final report will be generated after execution of this protocol and provided to the Medical Director and CQP for review. The final report will include completed tables with data and other information as described in this protocol. In addition, summaries, conclusions, and recommendations will be included.

12.1. Non-Conformances

Describe any protocol generation errors, non-conformances, or deviations that occurred during execution of the protocol. Minimally, this will also include an assessment/justification of any perceived impact to the validation. If no instances of non-conformance are noted, thus deviations are not required, this rationale should be stated within the summary report.

If, during the execution of a validation protocol, there is a deviation from an existing, effective version of an SOP in MasterControl, a formal deviation and investigation report, per COMM-QA-042 Deviations and Investigations, will be launched and referenced. All events associated with the execution of a validation protocol must be closed before signoff of the associated validation summary report.

NOTE: If a deviation is launched within MasterControl, this number must be referenced in the validation summary report. See COMM-QA-044 Approaches to Validation for clarification on requirements for situations when a MasterControl event is required.

12.2. Post-Execution Requirements

Following execution of the validation protocol, describe in the report if any change control requests are necessary as a result of this validation in order to ensure the validated parameters/processes are incorporated into applicable SOPs/batch records. If change controls are not determined to be necessary, this rationale should be stated within the summary report.

NOTE: If a change control request is launched within MasterControl, this number must be referenced in the validation summary report.

Signature Manifest

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