Method Validation for *Canadian Methods and Procedures For Testing Seed* and In-house Developed Method for Seed Grading

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1. General

Seed quality testing methods should be reliable and reproducible so that any decision based on it can be taken with confidence. The standard testing methods specified in the *Canadian Methods and Procedures for Testing Seed* (hereafter referred to as M&P) are used to obtain uniform testing results across different accredited seed testing laboratories in Canada. With new technology applications, new needs, or new development, sometimes, in-house developed methods, such as a modification of the standard methods, can be used for grading a seed lot by the *Seed Regulations*. Whether a new method proposal to the M&P, or an in-house developed method for their own use, they will require a scientific evaluation for method reliability, equivalence, or improvement. Method validation is an evidence-based process and its goal is to conduct a formal evaluation, which allows amending of existing methods, adding new methods, developing in-house methods, comparing test methods for their equivalency, and removing sub-optimum methods for seed quality testing in seed grading.

Scope:

- Method amendment for the *Canadian Methods and Procedures for Testing Seeds* (M&P).
- In-house method development in Canadian accredited laboratories for their own needs related to testing under the Seed Regulations.

Objectives:

- To specify method validation requirements
- To illustrate a method validation process
- To provide guidance to conduct method validation

Definitions

Method validation:
Validation is defined by ISO as *Confirmation by examination and provision of objective evidence that the particular requirements for a specified intended use are fulfilled* (ISTA, 2007). The Standard Council of Canada (SCC) defined method validation as *the process of ensuring that a method has the capabilities and performance characteristics consistent with specified requirements and that it's fit for the intended use and designed application* (SCC, 2021).

Method validation can be interpreted as a process to 1) define its intended use or method specifications and requirements, and 2) to evaluate the method's performance, e.g.,. accuracy, reducibility, repeatability, and detection limits.

Method verification
The process of ensuring that the laboratory has the ability to perform a previously validated method consistent with the requirements of the method and the needs of the laboratory (SCC, 2021). When the intended use is the same as the standard method, modified methods may consist of non-significant deviation(s) from a standard reference method or a prescriptive procedure for use on an ongoing basis.

In-house developed method
An In-house method contains a combination of variables not prescribed in the M&P but developed in one or more accredited laboratories based on their own situation or own use. The In-house method cannot be used for seed grading purposes unless they are verified or validated. The in-house method is deemed capable of meeting the analytical requirement if the results of validated in-house methods are equivalent to the results of the standard Canadian M&P method.

2. Method validation category and requirement

The outcomes of method validation for Canadian M&P are expected to be new or improved test methods accepted for inclusion in the M&P Rules.

- Compare different standard methods to remove test method(s) from M&P
- Replace the current standard M&P method(s) with a new method
- Add a new method as one of the standard methods in the M&P

The outcomes of method validation for in-house developed methods are to validate the non-standard method developed by and to be used by individual accredited laboratories for grading purposes with their special needs.

Regardless of the category, the requirement of following the process must be completed before the usage of the method as an accredited test.

a. Method selection and development;
b. Method validation as specified in section 3;
c. Submission of the method validation report (see section 3);
d. Review of validation study by SSTS and its assigned reviewers
e. Approval for:
    - € Final acceptance of the in-house validated method to be used for the accredited test by the laboratories that validated the method.
    - € A rule proposal to be submitted using the M&P proposal process for acceptance in the M&P.
f. Laboratory keeps the approved testing plan and the validation study report as records as long as the method is in use, and is subject to an audit.

3. Method validation guidance

3.1 There are two major stages in the validation of seed quality testing methods:

a. Establishing the method requirements or specifications and the determination of acceptable performance parameters within a single laboratory;

b. Demonstration of acceptable performance in an inter-laboratory collaborative study (multi-laboratory) or peer validation.

Stage b is not required for in-house developed methods if the method is only for their own use and not intended for an amendment of the M&P.

3.2 Types of an inter-laboratory collaborative study

a. Multi-laboratory collaborative studies

A comparative test is conducted by six or more laboratories during the method validation process to characterize test method performance. The participating labs shall be experienced and accredited for testing the crops or the methods, e.g., accredited testing laboratories with the testing scope. Preferably six samples representing three levels of the quality component should be assessed. Each sample shall be mixed and divided to the required sample quantity according to the testing plan. Sample homogeneity must be tested and passed before the distribution. This is typically used for the validation of percentage testing results such as germination or purity where the known value is an estimation.

b. Peer-validation studies

A comparative test is conducted by a minimum of two collaborating laboratories during the method validation process to characterize test method performance. The sample number is determined by performance claims but should include samples of known characteristics chosen by the test organizer. The selection of the peer validation is based on specifications of method performance and method assessment needs. When the variation between laboratories is not a significant factor for the method performance or specifications, peer validation is normally used.

3.3 Method validation procedures (see the flow chart in Appendix I)

a. Notification

The applying laboratory, i.e., the applicant, notifies SSTS (via email: ssts@inspection.gc.ca) that a test method is planned to be submitted for validation, especially when the applicants want to have assistance or suggestions from SSTS on test plan development.

b. Test plan submission

Applicant submits a test plan using ***Appendix II: Method validation application*** to ssts@inspection.gc.ca. All sections of the appendix shall be filed and specified, and detailed enough for the evaluation.

c. Test plan review

SSTS assigns reviewers to review the test plan, using ***Appendix IV Instruction for Reviewers – Test plan***. SSTS will conclude the submitted test plan as:

- € Test plan approved
- € Test plan needs to be modified. The applicant incorporates reviewers' comments into the test plan for further evaluation. This process will be repeated until the test plan is approved.
- € Test plan rejected and the validation process stops

d. Conduct validation study and data collection

When the test plan is approved, the applicant shall appoint a test organizer as a contact person who will be responsible for:

- Call for participating laboratories. The call letter shall provide sufficient information for a laboratory to determine their participation, such as the objectives, testing schedules, workload, testing equipment, and requirements.
- Prepare and distribute seed samples
- Provide support documents to participants, such as testing instructions with a data submission timeline and data record sheets.

Participants should be committed to providing the raw data to the test organizer at the defined timeline.

The applicant, as well as the participating laboratories, performs a validation study according to the test method illustrated in the test plan. Any amendment of the testing plan shall be approved by a re-submission.

e. Validation report development

After checking and analyzing the data, the test organizer will produce a validation report using ***Appendix III Validation Technical Report***. The validation report shall be available for all participating laboratories for comments. Participants need to send any comments back to the test organizer within one month. With received comments, the test organizer will finalize the report and submit it to SSTS for review.

f. Validation report review

SSTS assigns reviewers to review the validation report, using ***Appendix V: Instruction for Reviewers – Validation technical report***. The validation report will be determined as:

- € Validation report approved
- € Validation report needs further revision. For example, more data analysis is needed or the conclusion is not well supported by the data obtained. The applicant incorporates reviewers' comments into the validation report for further evaluation. This process will be repeated until the validation report is approved.
- € Validation report rejected and the validation process stops

SSTS M&P Committee will review the comments and recommendations from technical reviews and make a final decision. The test organizer will be informed of the committee's decision by SSTS Section Head.

4. Method amendment with the Validation study

Method amendment for the M&P:

Following the completion of the validation study, the applicant will then prepare an M&P proposal and submit the proposal with the supporting evidence of the validation study according to M&P amendment procedures.

In-house method development
The approved method validation report will grant the in-house developed method to be used in the applicant laboratories for seed grading purposes. Method validation application (Appendix II) and Validation Technical Report (Appendix III) shall be kept as records for audits as long as the method is in use.

5. Reference:

International Seed Testing Association (ISTA), 2007, ISTA Method Validation for Seed Testing, V.1

Standard Council of Canada (SCC), 2021, SCC Requirements and Guidance for Method Verification and Validation in Testing Laboratories

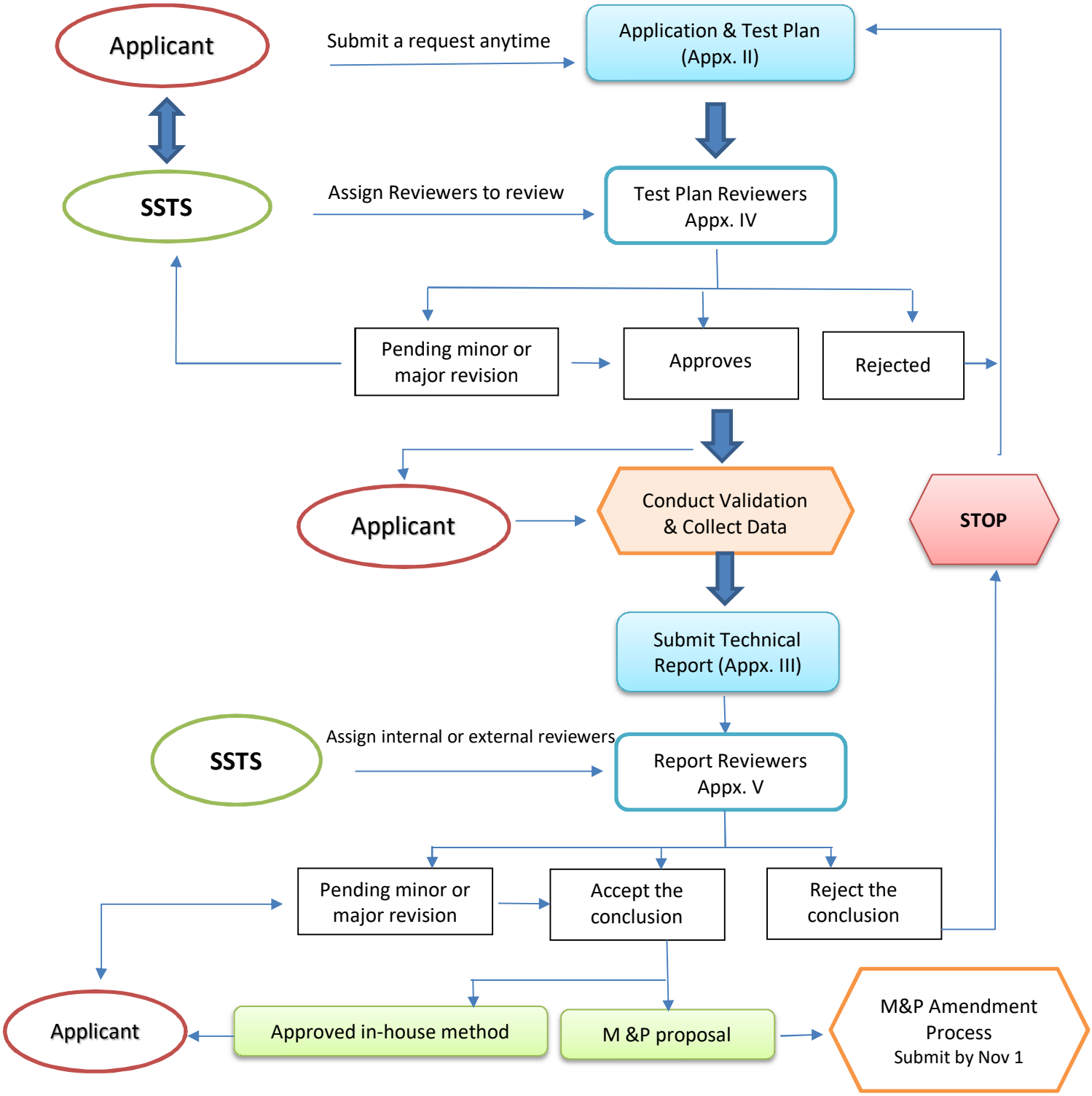
Revision Table

Version	Version date	Revision and Purpose	Author
V 1.0	2022/10	Provide a standard process and guide for validation of methods for testing seed according to Seed Regulations	R. Wang L. Ren J. Maruschak

List of Appendices:

Appendix I: Flow chart of the general method validation process
Appendix II: Method validation application
Appendix III: Validation Technical Report
Appendix IV: Instruction for Reviewers – Test plan
Appendix V: Instruction for Reviewers – Validation technical report

Appendix I: Flow chart of the method validation submission and evaluation processes



Appendix II: Method Validation Application & Testing Plan

Title:

Applicant (Applying laboratory's name):
Name of Test Organizer (contact person):
Email address:

Submission date:		
Reviewer name:		
Review returned date:		

The method described in the test plan should be considered as a (Check if it is applied):

☐For M&P Amendment

- ☐Add a new method (e.g., new crops added to M&P)
- ☐Replace a method (e.g., prechilling replace KNO_3 for dormancy breaking)
- ☐Add options to a method (e.g., add organic media to a germination media)
- ☐Delete options to a method (e.g., delete a temperature to a germination method)
- ☐Others, please specify

☐In-house developed method for lab's own use

- ☐New method (e.g., new to the M&P, such as auto planting instead of analyst planting)
- ☐Others, please specify

1. **Background:**
   Outline:
   1) the method needs, changes, or specifications,
   2) intended use or application scopes, including if it is an in-house method or for the M&P amendment, and
   3) describe the deviation from the standard method, if applicable.

2. **Objectives**:
   Outline the purpose of the study and the expected outcomes

3. **Validation study design:**

   Outline of the test method as follows:
   a. Intended species or crops
   b. Scope of study
   c. Study organizer and participant requirement
   d. Performance indicators
   e. Multi-lab study needed or not (the in-house method for lab's own use is generally not needed). Specify multiple lab study requirements, such as the participating lab's qualification, how many participating labs are needed, etc..

4. **Material and methods:**
   a. Materials used for the study e.g.,
      - sample quality indicated by germination range,
      - sample quantity indicated by how many lots will be used
      - sample preparation and homogeneity test
   b. Full details of the test methods to be followed
   c. Data analysis for method performance indicators

5. **Data collection sheet and instruction (if needed)**:
   According to the objectives, study design, and anticipated application of the validated method, please specify data to be collected in a form. The following is an example of germination validation

Lab names:

Corp Kinds:

Generic Germination condition:

Temperature (°C)	Media	Light	Prechill T°C & days

Germination data:

Seed lot	Treatment	Rep	First count			First count		
			Normal	Normal	Dead	Normal	Normal	Dead

6. List of reference documentation

a. preliminary studies, if available
b. published papers or methods
c. Other supporting documents

Appendix III: Validation Technical Report

Title

Author names
Laboratory name and address:
Email address:

Submission date:		
Reviewer name:		
Review returned date:		

The method described in the test plan should be considered as (Please check if it is applicable):

☐For M&P Amendment

☐Add a new method (e.g., new crops added to M&P)

☐Replace a method (e.g., prechilling replace KNO_3 for dormancy breaking)

☐Add options to a method (e.g., add organic media to a germination method)

☐Delete options to a method (e.g., delete a temperature to a germination method)

☐Others, please specify

☐In-house developed method for lab's own use

☐New method (e.g., new to the M&P, such as auto planting instead of analyst planting)

☐Others, please specify

1. **Background:**
   Outline: 1) the method needs, changes, or specifications, 2) intended use or application scopes, 3) describe the deviation from the standard method, if applicable; 4)participants (if multiple parties involved);

2. **Objectives**:
   Outline the purpose of the study and the expected outcomes

3. **Materials and Methods**:

   a. Summary of study design
   b. Materials used for the study e.g.,
      - sample quality indicated by germination range,
      - sample quantity indicated by how many lots were used
      - sample preparation
   c. Detailed test methods
   d. Data analysis approaches

4. **Data Analysis and Results:**

   Please specify what data is collected for what purposes or evaluations? What is the result of the data analysis?
   Identification of the following parameters of the proposed study, where it applicable:
   a) Accuracy.
   b) Precision (repeatability and reproducibility).
   c) Characterization/specifications of the proposed method.
   d) Appendix of data collection sheets

5. **Discussion & conclusion:**
   Discussion of the method performance including comments from participants and how they were addressed. A clear statement of the conclusions of the validation and recommendations for actions.

6. **Accredited method description, if applicable:**

   Specify the in-house validated method or method amendment proposal for M&P for purposes of official tests.

7. **Acknowledgements:**

8. **References**

Appendix IV: Instruction for Reviewers – Test Plan

Please review the enclosed draft test plan with reference to the evaluation criteria below, making comments on additional sheets as appropriate.

Test plan title:

Appling Laboratory name:
Name Test Organizer:
Submission date:
Reviewer name:

The method described in this draft test plan should be considered as a:

☐For M&P Amendment

- ☐Add a new method (e.g., new crops added to M&P)
- ☐Replace a method (e.g., prechilling replace KNO_3 for dormancy breaking)
- ☐Add options to a method (e.g., add organic media to a germination method)
- ☐Delete options to a method (e.g., delete a temperature to a germination method)
- ☐Others, please specify

☐In-house developed method for lab's own use

- ☐New method (e.g., new to the M&P, such as auto planting instead of analyst planting)
- ☐Others, please specify

Evaluation Criteria (not all aspects will necessarily apply):

	Yes	No	Comments
Is the purpose of the method and the need for validation adequately explained?			
Is the method description clear and unambiguous?			
Are parameters for accuracy, repeatability, reproducibility, and uncertainty of the test method identified?			
Is the method described suitable for meeting the objective(s) of the test?			
Are there any omitting of critical steps/parameters identified?			
Are potential participating laboratories identified?			
Are data record sheets and instructions for their completion included?			
Are the comments to the former test plan be addressed? (if applicable)			

Please make any additional comments on a separate sheet.

Recommendation (delete as applicable).

- € Approve the Test Plan without revision.
- € Approve the Test Plan following minor revisions.
- € Defer a decision pending major revisions.
- € Reject the Test Plan.

Appendix V: Instruction for Reviewers – Validation Report

Please review the enclosed validation report with reference to the evaluation criteria below, making comments on additional sheets as appropriate. Please indicate any aspects on which you do not feel qualified to comment.

Validation report title:

Applicant name:
Submission date:
Reviewer name:

The method described in this draft test plan should be considered as a:

☐For M&P Amendment

- ☐Add a new method (e.g., new crops added to M&P)
- ☐Replace a method (e.g., prechilling replace KNO_3 for dormancy breaking)
- ☐Add options to a method (e.g., add organic media to a germination method)
- ☐Delete options to a method (e.g., delete a temperature to a germination method)
- ☐Others, please specify

☐In-house developed method for lab's own use

- ☐New method (e.g., new to the M&P, such as auto planting instead of analyst planting)
- ☐Others, please specify

Evaluation Criteria (not all aspects will necessarily apply):

	Yes	No	Comments
Is the title appropriate?			
Is the summary clear/adequate?			
Is the reason for the study clearly stated? (i.e. objective(s), aim, questions, the hypothesis that the test organizer wishes to address)			
Has/have previous literature/data been reviewed adequately?			
Have the comments of participants been reported/addressed?			
Was the design of the validation appropriate?			
Were the controls adequate to ensure repeatability and reproducibility of the data reported?			
Were reference materials included and are their results reported?			
Were steps taken to ensure the integrity of the data, i.e. blind testing/coding of samples?			
Were checks included to ensure that each participant followed the protocol?			
Has a statistical analysis been performed?			
Is the statistical analysis appropriate to the data, and has the approach been justified?			
Is the conclusion clear and well align with the results?			
Are the comments to the former validation report be addressed? (if applicable)			

Please make any additional comments on a separate sheet.

Recommendation (delete as applicable).

- Approve the validation report without revision.
- Defer a decision, more information is needed.
- Reject the validation report.