



Canadian Food
Inspection Agency

Agence canadienne
d'inspection des aliments

Canadian Food Inspection Agency

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To excel as a science-based regulator, trusted and respected by Canadians and the international community.

Our Mission:

Dedicated to safeguarding food, animals and plants, which enhances the health and well-being of Canada's people, environment and economy.



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Canadian Food Inspection Agency Agence canadienne d'inspection des aliments

Lentil Germination Method Referee

Seed Science and Technology Section

Saskatoon Laboratory

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Canada

Objectives

- To promote precision, standardization and uniformity among seed testing laboratories.
- To evaluate the method variation of seed testing rules in M&P, AOSA and ISTA.
- To provide data to be used as supporting evidence for testing procedure or rule changes.
- To identify specific areas that research is needed to promote uniformity among laboratories.

Background

1. Testing Rule Comparison

Rules	Media	T (°C)	1 st Count	Final Count	General Requirements
M&P	BP, S, RT	20	-	7	
AOSA	B, T	20	5	10	Hard seeds - See 6.2d and 6.9m (6)
ISTA	BP, S	20	5	10	Prechill

2. Lack of uniformity in test results reported

Materials and Methods

Methods Used for the Referee

Method	Instructions
1	<ul style="list-style-type: none">•Prechill for 4 days in rolled towel•Germinate at 20°C•Count at 5, 7 and 10 days.•Remove any dead seeds; continue the test; count at 15 days.
2	<ul style="list-style-type: none">•Germinate at 20°C in rolled towel (without a prechill)•Count at 5, 7 and 10 days.•Remove any dead seeds; continue the test; count at 15 days.

Materials and Methods

Seed Lots Used

Lot 1: Harvested in fall 2010

Variety: CDC Peridot

Lot 2: Suspected chemical damage (glyphosate or a desiccating agent)

Blended sample

The two lots were sub-sampled at the SSTS and passed a homogeneity test using 10 random samples of 100 seeds.

Referee Participants

Total Participating Labs: 27		Experience indicated by the number of Samples Tested by Participants		
		No. of Samples Tested	No. of Participants	Participant %
Participants: Canadian Labs	17 (63%)	0	4	15%
		1-50	7	26%
		51-100	1	4%
		101-200	2	7%
		>200	3	11%
Participants: U.S. labs	10 (37%)	0	7	26%
		1-50	2	7%
		51-100	0	0%
		101-200	0	0%
		>200	1	4%



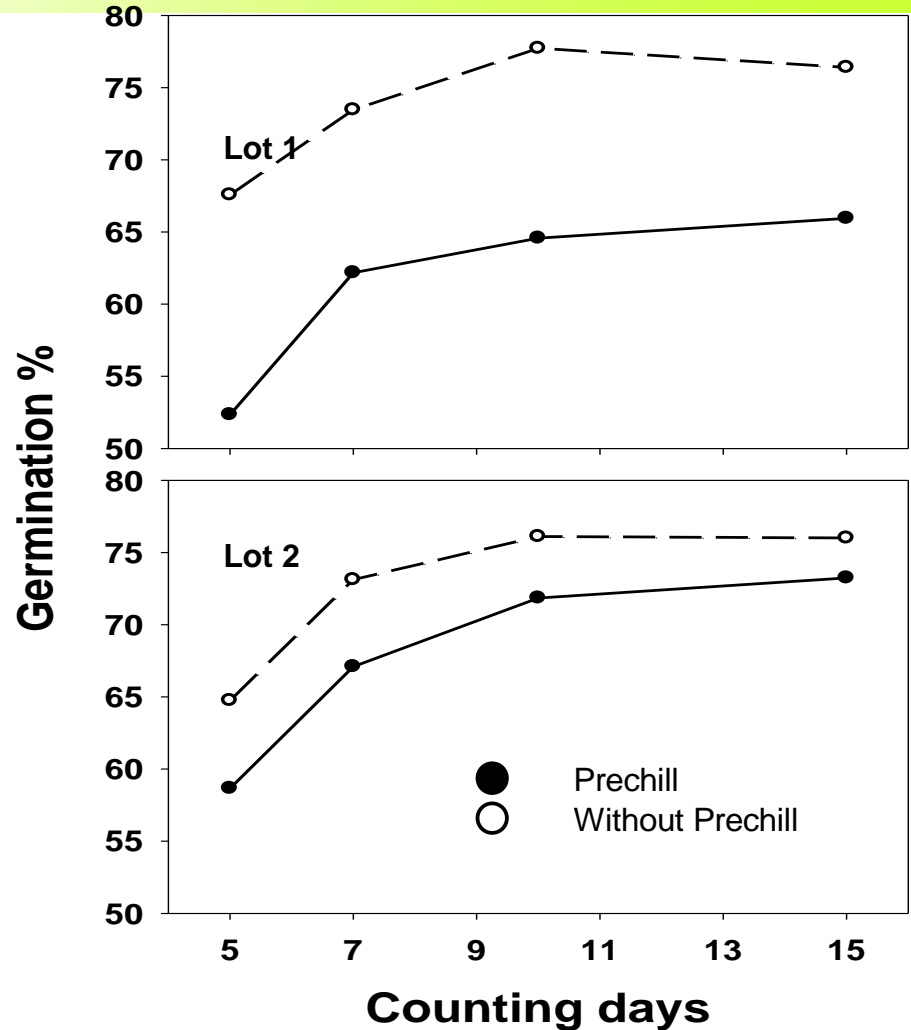
Preliminary Data Analysis

Germination at variable counting days

Germination potential reached at 10 days

Prechill treatment

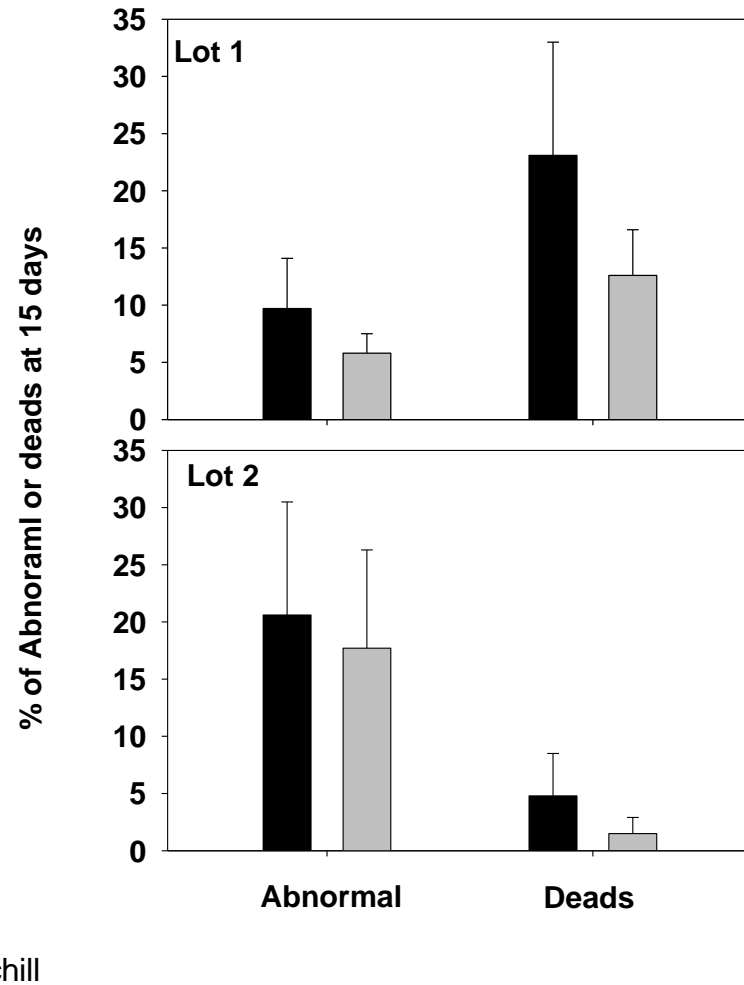
Prechill reduced the germination



Abnormals and Deads

Both Seed Lots

- **Prechill treatment**
 - Increased abnormals and deads
- **Hard seed**
 - Less than 1% at the end of the germination test



Lab variations

Z-score for lab variation at 7 days

21 (78%) labs were within 1 std. deviation for lot 1 without prechill

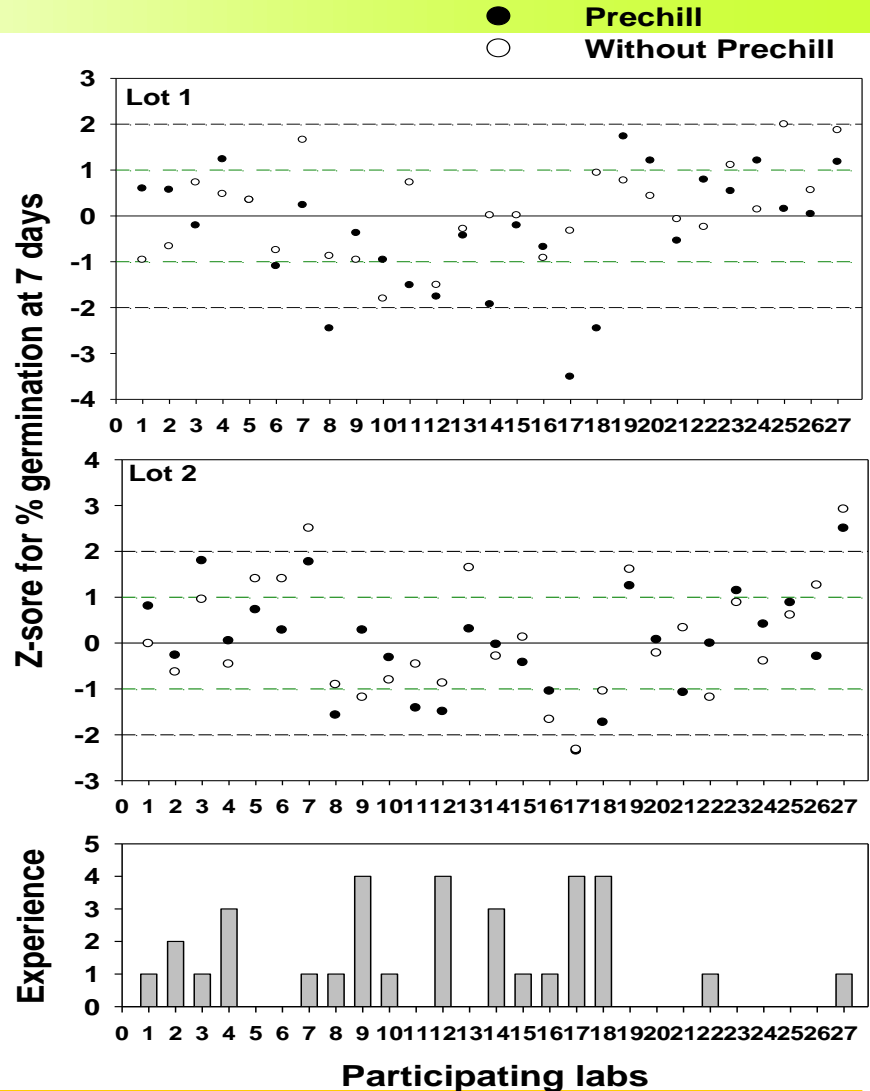
15 (55%) labs were within 1 std. deviation for lot 2 with and without prechill

2-3 testing results being outliers (z score > 2)

Experience did not have a strong impact on the performance

Experience assigned value:

- Do not test = 0
- 1-50 samples = 1
- 51-100 samples = 2
- 101-200 = 3
- >200 samples = 4



Lab variations

Z-score for lab variation at 10 days

15 (55%) labs within 1 std. deviation for both lots without prechill.

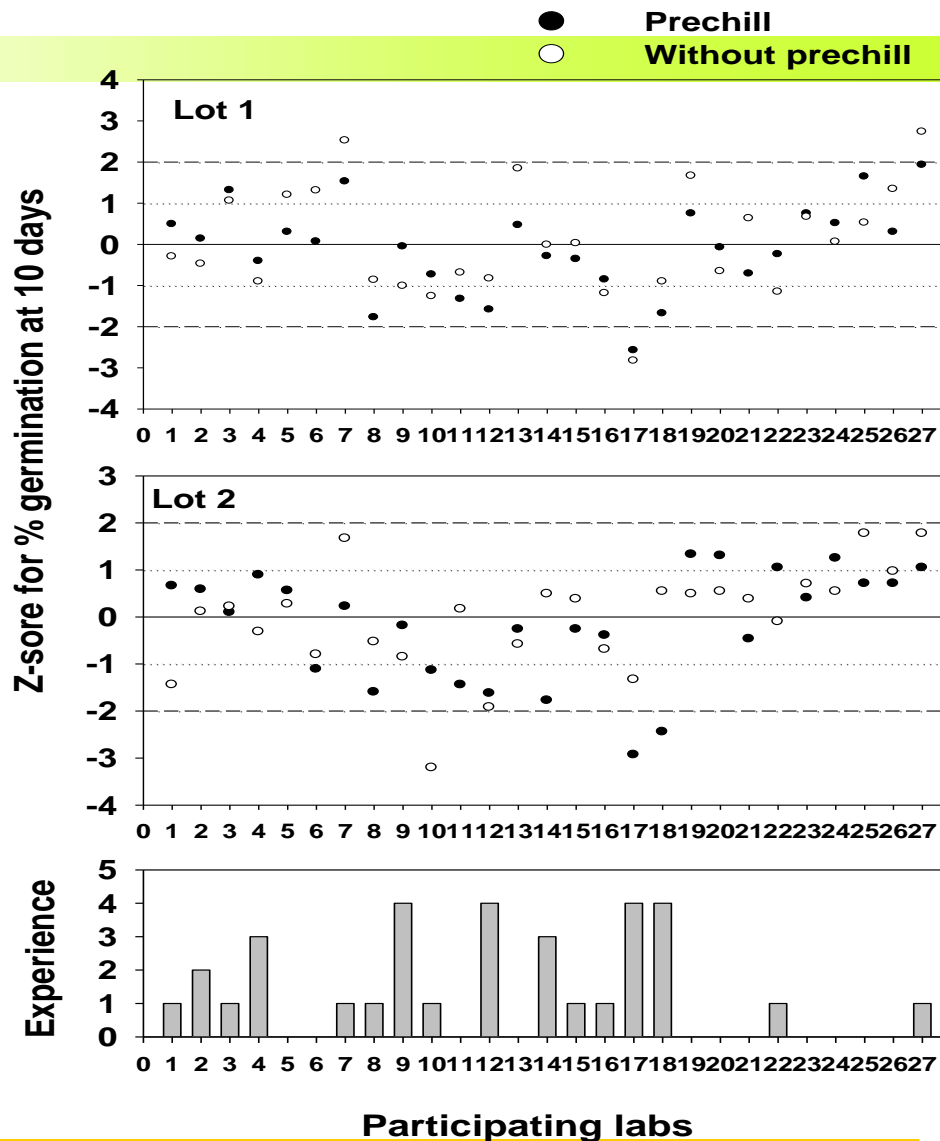
18 (67%) labs within 1 std. deviation for lot one with prechill.

1-3 testing results been outliers (z score > 2).

Experience did not have a strong impact on the performance

Experience assigned value:

- Do not test = 0
- 1-50 samples = 1
- 51-100 samples = 2
- 101-200 = 3
- >200 samples = 4



Lab variations

Germination variation at 7 days

range, difference, mean

Prechill

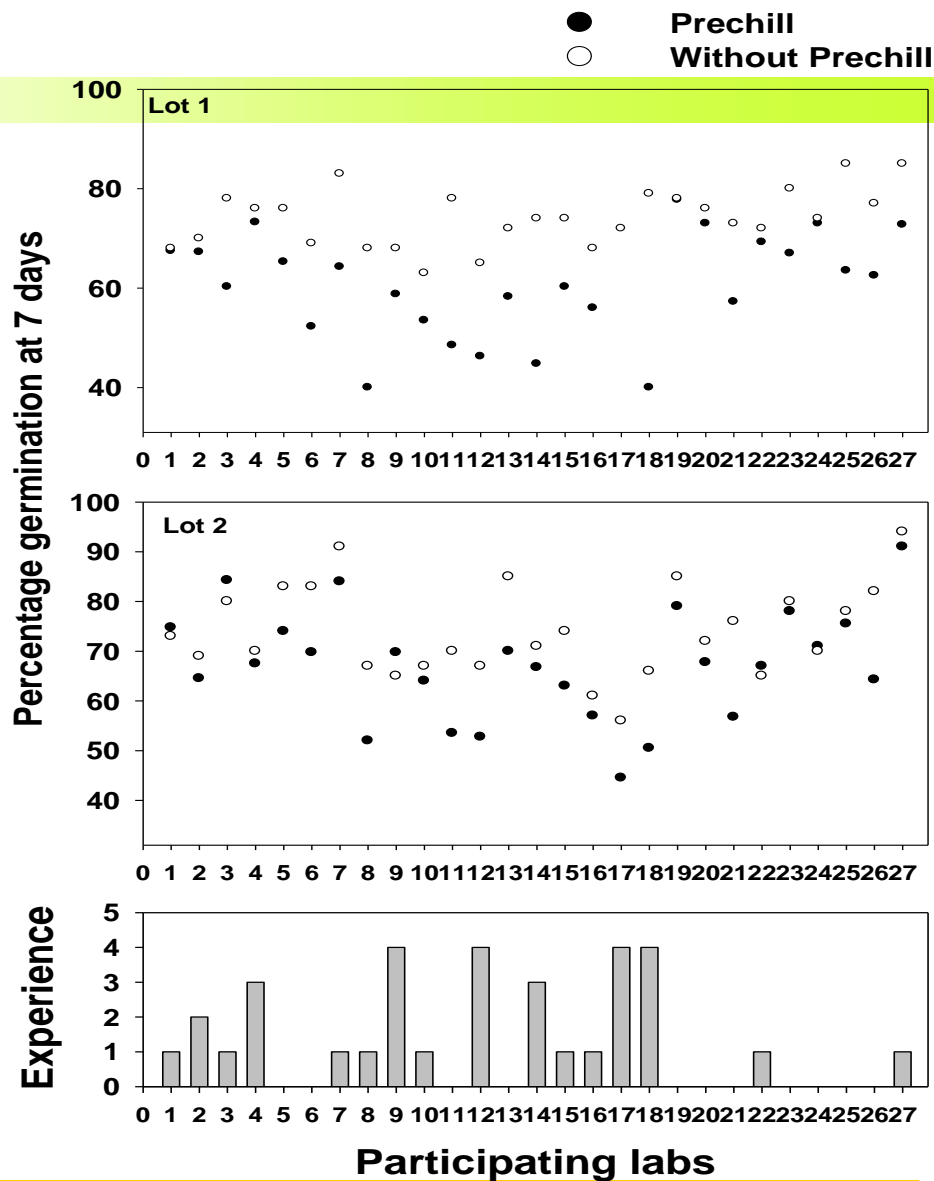
Lot 1: 30-78%, 47%, 62%

Lot 2: 44-91%, 47%, 67%

Without prechill

Lot 1: 63-85%, 22%, 73%

Lot 2: 56-94%, 38%, 73%



Lab variations

Germination variation at 10 days

range, difference, mean

Prechill

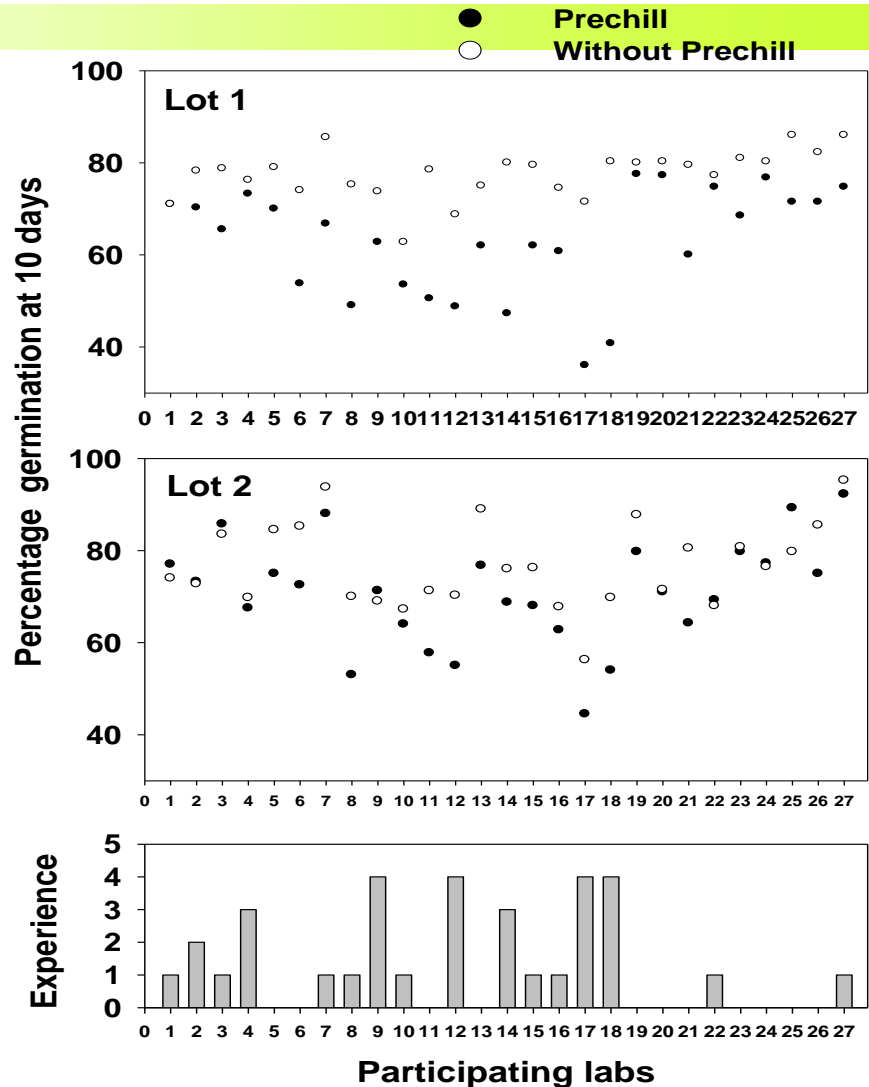
Lot 1: 36-77%, 41%, 64%

Lot 2: 44-92%, 48%, 71%

Without prechill

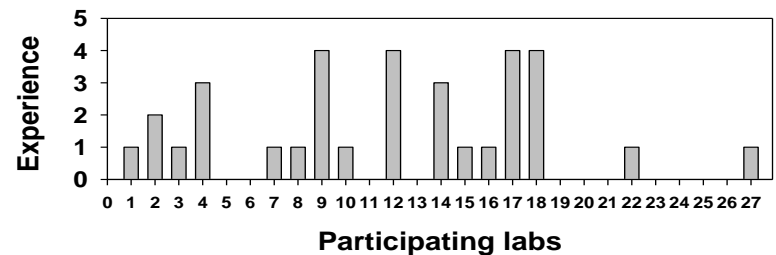
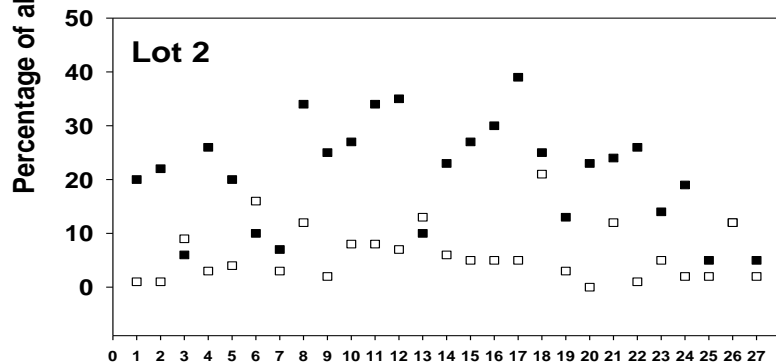
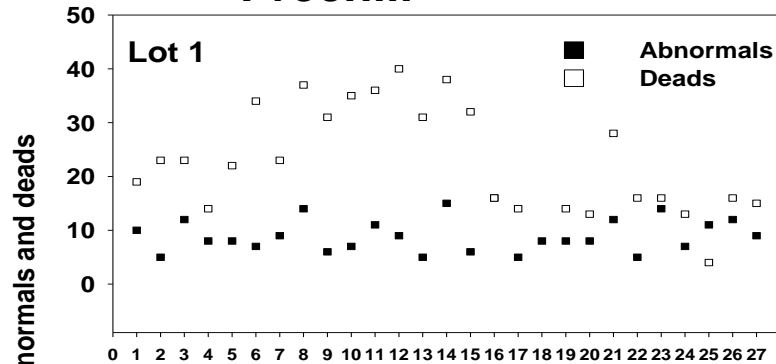
Lot 1: 62-86%, 23%, 77%

Lot 2: 56-95%, 39%, 76%

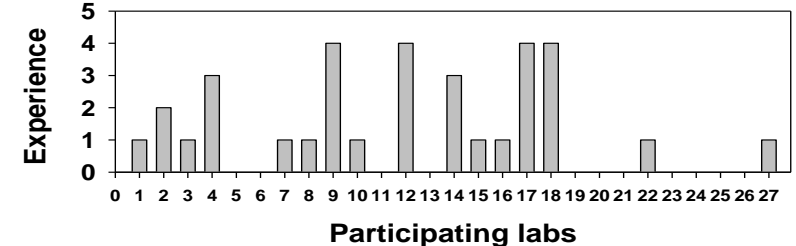
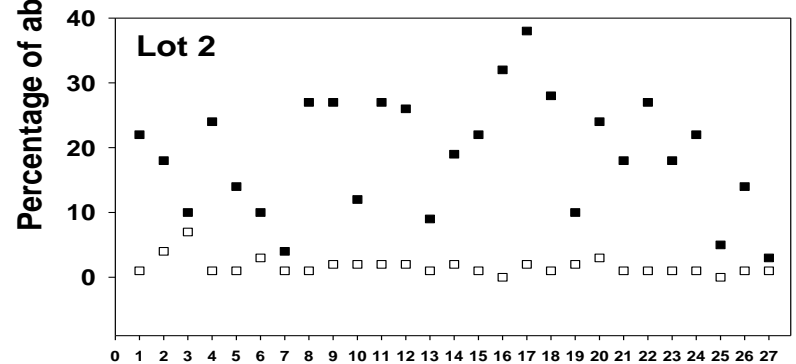
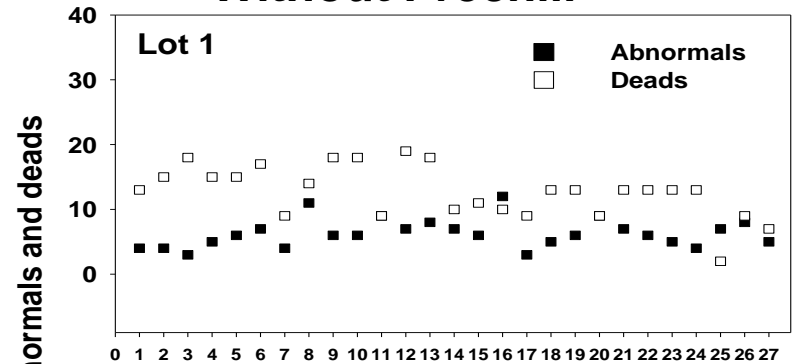


Lab variations

Prechill



Without Prechill



Normal Seedlings



Abnormal Seedlings

Epicotyl lesion



**Weak secondary roots
Suspect Chemical Damage**



**Stubby primary root with weak
secondary roots/Shortened
and thickened epicotyl.
Suspect Chemical damage**



Abnormal Seedlings Descriptions

Reported in this referee but not in the rules

- Watery or glassy epicotyl
- Glyphosate or chemical damage
- Wiry primary roots
- Mechanical damage
- Damping off
- Spindly
- No hypocotyl

Summary

- Referee had good participation from labs using AOSA and M&P rules.
- Prechill treatment will reduce germination where there is no dormancy in lentil seeds
- **Further investigation shall support rules harmonization on:**
 - Final counting days to 10 days
 - Dormancy breaking method options: e.g., scarification, prechill or just reporting hard seed %
- **Further investigation and training to reduce lab variation**
 - Method induced abnormal seedlings and death
 - Abnormal seedlings with chemical damage
 - Rule clarity and training for germination method and seedling evaluation

Acknowledgments

- ▶ **For preparation of the referee:**
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