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Seed Quality Assurance

A.I. chatbots in seed testing – possibilities and pitfalls

Examples and **Discussion**

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Assumptions

- All information on testing (e.g. germination, moisture etc) seeds is available in some way at the internet
- Information on Official Seed Testing (ISTA) is available on line
- Methods for seed testing data analysis are available on line

Conclusion

- **Theoretically** A.I. chatbots could tell you how to perform and official seed test for a specific species
- **Theoretically** A.I. chatbots could be used do do the data analysis and present a conclusion on the outcome of the test.



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Conclusion

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- **Theoretically** A.I. chatbots could be used do do the data analysis and present a conclusion on the outcome of the test.

Does this work in practice?

Do people have experience with this?

Is this useful?

How to deal with these developments?



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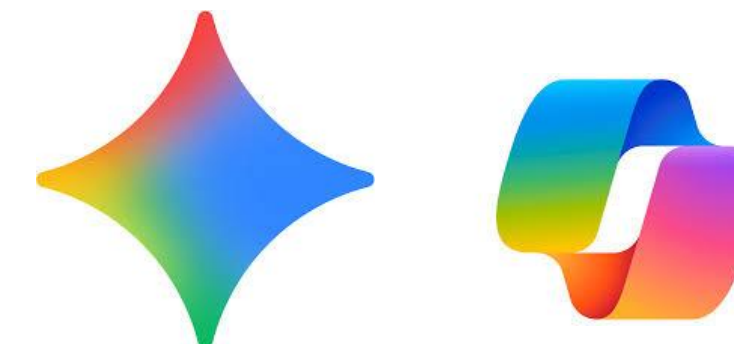
Examples to be discussed

- **SH Test**
- **Germination test**
- **Data statistical analysis**

Development of a not existing SH Rule (a tutorial)

Aim: Produce an A.I. driven SH protocol for the detection of *Xanthomonas axonopodis* pv. *allii* on onion seed.

Use : AI chat bots Gemini (google) and Copilot (microsoft)



Questions asked:

1. Draft me an ISTA Chapter 7-style rule for the detection of *xanthomonas axonopodis* pv. *allii* on onion seed.
2. Add the exact recipes for the growth media used, specific primer sequences and thermocycling profiles, and specific Critical Control Points (CCPs) for laboratories to this protocol.
3. Also add possible Taqman PCR assays to the protocol.
4. Now combine all the above information into an ISTA Chapter 7 Rule Stee protocol.
5. Integrate a list of references in the correct place in the protocol in accordance with ISA Chapter 7 rules.

7-0XX: Detection of *Xanthomonas axonopodis* pv. *allii* on *Allium cepa* (Onion) seed

Object

Detection of the bacterial blight pathogen *Xanthomonas axonopodis* pv. *allii* (Xaa) in onion seed lots.

Principle

The method is based on the extraction of bacteria from the seed sample followed by a workflow consisting of a **molecular screening assay** (either Duplex Nested-PCR or Triplex TaqMan Real-Time PCR), followed by **isolation on semi-selective media** for validation, and finalized by **confirmation of suspect colonies** via molecular or pathogenicity testing.

Sample Size and Subsample Size

- **Minimum working sample:** 30,000 seeds.
- **Maximum subsample size:** 2,500 seeds (divided into 12 independent subsamples). [1]

1. Materials, Equipment and Media

1.1 Reference Material

- A known positive reference strain of *Xanthomonas axonopodis* pv. *allii* (e.g., CIRAD or CFBP reference strain).
- A known negative control strain (e.g., *Pseudomonas fluorescens*). [1]

1.2 Equipment

- Stomacher® homogenizer and sterile filter-bags.
- Incubator operating at 28 ±2 °C.
- Thermal cycler or Real-Time PCR instrument.
- Calibrated data loggers for temperature monitoring.
- Post-PCR workstation with UV-C decontamination lamps. [1, 2]

1.3 Buffers and Media Recipes

1.3.1 Extraction Buffer

- Sterile saline (0.85% NaCl) containing Tween™ 20 (0.02% v/v) or 10 mM sterile phosphate buffer (pH 7.2).

1.3.2 NCTM1 Medium (Semi-selective Isolation Medium)

Basal Medium Ingredients (per liter distilled water):

- Yeast extract: 7.0 g
- Peptone: 7.0 g
- Glucose: 7.0 g
- Agar: 15.0 g



Chapter 7: Detection of *Xanthomonas axonopodis* pv. *allii* on *Allium cepa* (onion) seed

7.X.1 Scope

This method describes procedures for the detection of *Xanthomonas axonopodis* pv. *allii* (Xaa), the causal agent of bacterial blight of onion, in seeds of *Allium cepa*.

The protocol includes:

- Seed extraction
- Isolation on semi-selective media
- Conventional PCR
- TaqMan real-time PCR (qPCR)
- Confirmation procedures

7.X.2 Principle

Bacteria associated with seeds are extracted by agitation in buffer. The extract is analysed using:

- **Isolation** on semi-selective media [Ref. 3, 6]
- **Molecular detection** (PCR/qPCR) [Ref. 4, 7]

Positive detections are confirmed by molecular or phenotypic methods.

7.X.3 Definitions

- **Working sample:** subsample used for testing
- **Subsample:** portion tested independently
- **Detection limit:** lowest detectable contamination level

7.X.4 Reagents and Materials

Differences: Asked to Copilot

Aspect	Gemini document	Copilot document
Purpose	Near-final ISTA method / SOP	Draft ISTA-style guideline
Detail level	High (lab-ready)	Medium (framework)
Validation readiness	High	Moderate
Complexity	High	Moderate

Conclusion:

Gemini document =

👉 *Technically complete, validation-oriented, close to an ISTA submission-ready method*

Copilot document =

👉 *Structured, readable, but simplified version for concept development or early drafting*



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Examples to be discussed

Germination test

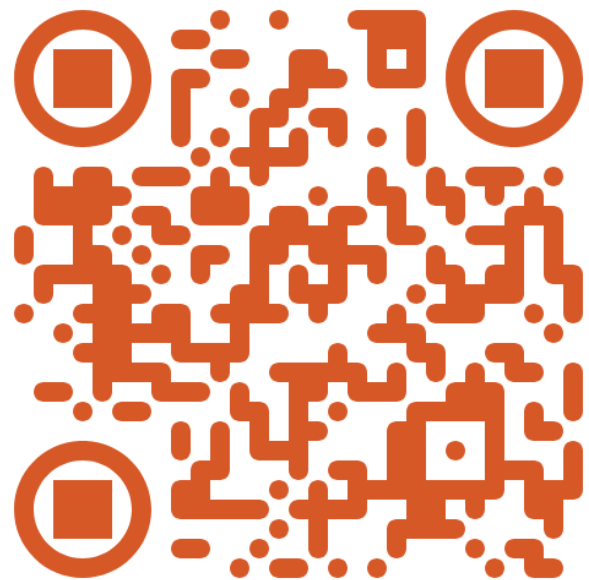
Exercise:

ask ChatGPT or Gemini: give me a protocol for a germination test for cabbage seeds

ask: give me a protocol for an **official** germination test for cabbage seeds

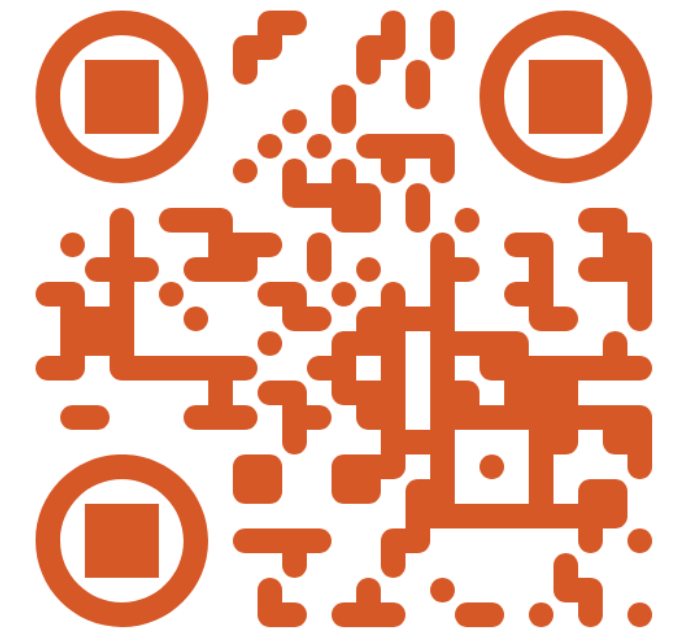
Are the answers the same for both chatbots?

Are the answers different for an “official” germination test?



Gemini

Chat GPT





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Examples to be discussed

Germination test

Example

Asked ChatGPT or Gemini: give me a protocol for an **official** germination test for sugar beet seeds

ChatGPT: Here's a **standard (official-style) germination test protocol for sugar beet seeds** based on methods used by International Seed Testing Association (ISTA) and Association of Official Seed Analysts.

Sample size: 400 seed units

Replicates:

4 × 100 seeds

If pelleted:

Test as received (do not remove coating)

4. Materials

Germination paper (top of paper or between paper) OR sand

Distilled water

Evenly moist (not saturated)

Alternative: Sand

Fine, sterilized sand

Moisture: ~60% water holding capacity

6. Sowing Procedure

Place seeds evenly spaced

Ensure good contact with medium

Avoid overcrowding (important for multigerm seeds)

Label replicates clearly

7. Environmental Conditions

Temperature: Alternating: 20°C (16 hours, dark) 30°C (8 hours, light)

8. Duration of Test

First count: Day 4

Final count: Day 14

Gemini: For sugar beet (*Beta vulgaris* L. subsp. vulgaris), official germination protocols (ISTA/AOSA) are highly specialized because the "seed" is actually a multigerm fruit (a corky cluster containing several true seeds).

In commercial quality control, the primary goal is to manage the naturally occurring inhibitors in the corky fruit and ensure high-precision results, as sugar beet is often precision-planted.

Test Parameters

Parameter	Requirement
Substrate	Pleated Paper (PP) or Between Paper (BP)
Temperature	Constant 20°C or Alternating 20/30°C
First Count	4 days
Final Count	14 days

Pre-treatment Pre-washing is mandatory to remove inhibitors

Step-by-Step Protocol

1. Pre-Washing (The Most Critical Step)

Sugar beet fruits contain water-soluble phenolic compounds (like ferulic acid) that inhibit germination.

- **Procedure:** Place the seeds in a mesh bag and wash them under running water (approx. 25°C) for 2 to 4 hours.
- **Drying:** After washing, the seeds must be blotted or lightly dried to prevent "sopping" during the actual test.



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Examples to be discussed

Data analysis

The Heterogeneity Testing Calculator for seed lots in multiple containers

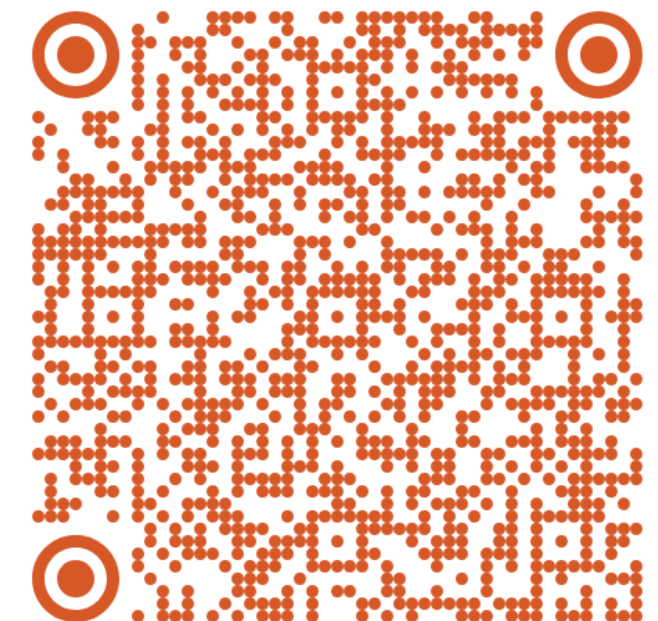
The sampling rules and procedures described in the current ISTA Rules and ISTA Handbook on Seed Sampling are based on the assumption that the seed lot is as uniform as practically possible with good seed production practice. If the seed lot is too heterogeneous, there is a risk that the sample drawn does not represent the whole seed lot.

Consequently, if there is evidence of heterogeneity, sampling must be stopped and the sample drawn must be refused. In the case of doubt, if the seed lot is in multiple containers heterogeneity can be tested as described in the ISTA Rules under Chapter 2.9.

Presentation of the Heterogeneity Testing Calculator

The Heterogeneity Testing Calculator is a Microsoft® Excel workbook which can be used to detect heterogeneity which would make a seed lot technically unacceptable for sampling for the issuance of an Orange International Seed Lot Certificate (OIC).

Statistical Tools for Seed Testing



ISTA Tool (Approved Analysis):



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Examples to be discussed

Data analysis

The H and R values **do not indicate significant heterogeneity**. The seed lot fulfils the uniformity requirement. Note: the number of seeds tested for purity analysis per container-sample is 1000.

Purity - Total pure seeds (%): Heterogeneity testing for seed lots in multiple containers			
ISTA Rules - Chapter 2 - 2.9			
		X	Container sample #
Number of containers in the lot	64	98.8	1
		98.5	2
Minimum number of independant container samples to be drawn	20	97.9	3
		99	4
Number of independant container-samples	20	99.1	5
		97.8	6
Number of seeds tested from each container-sample	1000	99	7
		98.4	8
Chaffy seeds (Y/N)	Y	98.8	9
		98	10
Reported mean value	98.6	99	11
		97.7	12
		97.8	13
		97.9	14
		98	15
		98.6	16
		99.3	17
		99.1	18
		99.2	19
		99.1	20
			21
			22
			23
			24
			25
			26
			27
			28
			29

H-value test	
W	0.17
V	0.31
Calculated H value	0.63
Critical H-value	1.09
This H value does not indicate significant heterogeneity.	

R value test	
R	1.60
Critical R value	2.4
This R value does not indicate significant heterogeneity.	

Gemini:



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Examples to be discussed

Data analysis

Asked Gemini:

I have a seed lot in 64 containers. From 20 containers I took samples of 1000 seeds and tested purity for these samples. The purities were in %: 98.8, 98.5, 97.9, 99, 99.1, 97.8, 99, 98.4, 98.8, 98, 99, 97.7, 97.8, 97.9, 98, 98.6, 99.3, 99.1, 99.2, 99.1 . **Is there heterogeneity in these seed lots?**

Answer: To determine if there is significant heterogeneity (statistically significant variation) among the containers in your seed lot, we can perform an **H-test** (often used in seed testing, such as ISTA rules) or a standard **Chi-Square (χ^2)** test for homogeneity.

Because your sample sizes are identical ($n = 1000$ seeds per container), we can analyze the variation of the percentage data directly.

Here is the step-by-step statistical evaluation of your data.

Gemini:

Should be:

classical Pearson chi-square statistic: $\chi^2 = 41.64$

Why 48.08 ??? AI Hallucination... 😊

Result: Because your calculated chi² value (48.08) is significantly greater than the critical value (30.14), we **reject the hypothesis of homogeneity.**

R: qchisq(0.95,19)
[1] 30.14353

Gemini:

Answer: ISTA Evaluation Criteria

ISTA does not use a raw H-value in isolation; instead, it converts this into an adjusted value to compare against a strict critical limit (H_{lim}) based on the number of containers sampled ($N = 20$) and total containers (64).

Alternatively, the standard ISTA direct χ^2 check for 20 samples relies on the same mathematical relationship:

$$\chi^2 = 48.07 \text{ ???}$$

Table 2E. Factors for additional variation in seed lots to be used for calculating W and finally the H value

Attributes	Non-chaffy seeds	Chaffy seeds
Purity	1.1	1.2
Other seed count	1.4	2.2
Germination	1.1	1.2

Gemini completely missed the point. According to the ISTA Rules, heterogeneity is assessed using the H test and the R test, and extra variation is accounted for through the f factor.

Gemini:



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99.2, 99.1 . **Is there heterogeneity in these seed lots**

according to ISTA?

Answer: To determine heterogeneity specifically according to the International Seed Testing Association (ISTA) Rules, a specialized H-test (Heterogeneity test) is used.

The ISTA H-test uses a modified Chi-Square approach that compares the calculated H-value against an officially formatted critical value table. If the calculated H-value exceeds the critical value for your number of containers, the lot is declared significantly heterogeneous, and an international certificate cannot be issued for the lot as a single, uniform unit.

Here is how your data evaluates under the ISTA framework.

.....

Gemini:



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Examples to be discussed

Data analysis

Answer: 3. Final ISTA Verdict

The seed lot fails the ISTA homogeneity requirement. Because $48.07 > 36.19$, the variation between your containers is too high to be permitted under ISTA rules.

Consequence: Under ISTA rules, this lot cannot be issued an Orange International Seed Lot Certificate in its current state. To obtain certification, the seed lot must be thoroughly re-blended/re-mixed to achieve uniformity, or it must be split into smaller, more homogenous separate lots and re-tested.

ChatGPT:



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Examples to be discussed

Data analysis

Asked Chat GPT:

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Answer: Yes — the data provide evidence of ****heterogeneity among the containers****.

And according to ISTA?

Answer: According to the [ISTA International Rules for Seed

Testing](https://www.seedtest.org/en/international-rules-for-seed-testing-_content---1--1083.html?utm_source=chatgpt.com), heterogeneity of a seed lot in multiple containers is evaluated with the H-value (or R-value) heterogeneity test. ([International Seed Testing Association][1]

No, should be **AND**

ChatGPT:



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99.2, 99.1 . **Is there heterogeneity in these seed lots?**

And according to ISTA?

ISTA conclusion

Under the ISTA heterogeneity procedure, this seed lot would be regarded as:

No significant heterogeneity detected.

In practical ISTA terms, the lot would generally be considered sufficiently uniform for sampling and certification.

ISTA Tool	Gemini	Gemini ACCORDING ISTA	Chat GPT	Chat GPT ACCORDING ISTA
No heterogeneity	Heterogeneity	Heterogeneity	Heterogeneity	No heterogeneity

Our Recommendation on Statistical Data Analysis with AI

Generative AI is an extremely powerful tool for science and engineering. However, it should not be treated as an autonomous reasoning system. Rather, it is a tool for scientists who have a solid understanding of the subject matter, are able to critically evaluate and challenge its output, and remain ultimately responsible for the conclusions. **If you are not an expert in statistics for seed testing, use it with great caution.**



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Does this work in practice? Are there any risks?

Do people have experience with this?

Is this useful?

How to deal with these developments?



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ANNUAL MEETING

22-25
June 2026



Calgary, Canada