

Appendix13: 2016 Proposal 13 Supporting Evidence

Tomato Referee

A national referee was conducted to determine the equivalency of agar as a germination media when compared to traditional AOSA approved media. This referee was conducted in the fall of 2014 and the summer of 2015.

Hypothesis: Using Agar as a planting media will give germination results that are equivalent in germination tests performed on current AOSA approved media.

2014 Protocol:

We are conducting a national referee on standard germination of *Solanum lycopersicum var. lycopersicum* (tomato) on agar media. In your packet there should be 6 samples of tomato, agar powder (if requested) and data forms.

For this referee you will perform a standard germination of 400 seeds using the AOSA rules on the current media used in your lab. You will also conduct a side by side 400 seed test within the same time duration and temperature constraints using agar as the germination media. This will be done on samples 1-3. Samples 4-6 will be planted following the same procedure 5-7 days later. Please note dates planted on the data sheet. Attached are instructions for preparing the agar media. The concentration of agar is to your preference within a range of 0.6% to 1% concentration.

*Note - Tomato samples are not fresh seed lots, if KNO_3 is used please note on the data sheet

Samples were sent out September of 2014 to 19 laboratories with a deadline of December 19, 2014. Results were submitted as late as April 2015.

11 labs returned data for tomato. One set of data was thrown out as replicates did not add up to 100 and was unreliable.

Data was analyzed for repeatability within each laboratory to repeat the initial scores on both agar and current media. The data was also analyzed for reproducibility of similar scores across the participating labs. It was expected that agar would be as repeatable and as reproducible as the approved media currently being used in each lab.

2014 Results:

Figure 1. 2014 Repeatability Results (Within the same lab)

| Crop | Method | Percent Germination | f_r^2 | SR |
|--------|---------|---------------------|---------|------|
| Tomato | Agar | 92.66 | 0.19 | 1.14 |
| Tomato | Current | 92.72 | 0.22 | 1.21 |

The repeatability is the closeness of the agreement between the results of successive measurement of the same measure and carried out in the same conditions of measurement.

f_r^2 –Reproducibility within a lab (> 1 is a sign of more variability than expected)

Sr-The average of the variance of results within laboratories (similar number to the other method is desired.)

Figure 2. 2014 Reproducibility Results (across many labs)

| Crop | Method | Percent Germination | fR^2 | SR |
|--------|---------|---------------------|--------|------|
| Tomato | Agar | 92.66 | 0.84 | 0.99 |
| Tomato | Current | 92.72 | 0.66 | 0.78 |

The reproducibility is the precision under reproducibility conditions, i.e. conditions where test results are obtained with the same method on identical test items in different laboratories with different operators using different equipment.

fR^2 –Reproducibility (> 1 is a sign of more variability than expected)

SR- The average of the variance of results across all labs (similar number to the other method is desired.)

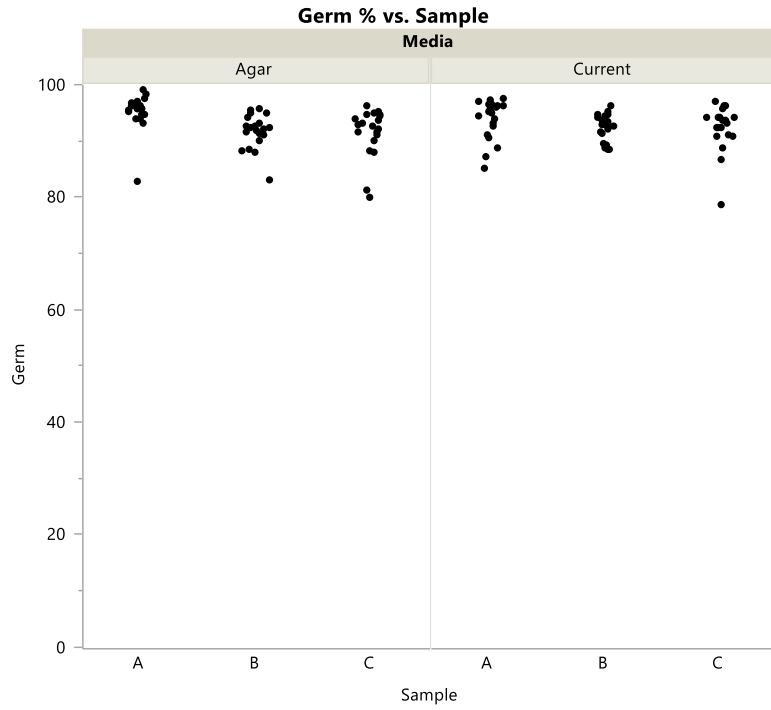


Figure 3 2014 Sample average by media

The conclusion looking at the data mean germination, repeatability, and reproducibility is all comparable for the agar and current methods.

2015 Protocol:

We are conducting a national referee on standard germination of *Solanum lycopersicum var. lycopersicum* (tomato) on agar media. In your packet there should be 6 samples of corn and/or tomato (depending on the crops you selected), agar powder (if requested) and data forms.

For this referee you will perform a standard germination of 400 seeds using the AOSA rules on the current media used in your lab. You will also conduct a side by side 400 seed test within the same time duration and temperature constraints using agar as the germination media. This will be done on samples 1-3. Samples 4-6 will be planted following the same procedure 5-7 days later. Please note dates planted on the data sheet. Attached are instructions for preparing the agar media. The concentration of agar is to your preference within a range of 0.8% to 1% concentration for tomato**.

**Note- If you germinate your tomato on a slant a higher concentration (0.9% or higher) is recommended.

Samples were sent out June, 2015 to 13 laboratories with a deadline of August 31st, 2015. Results were submitted as late as September. 8 labs returned data for the referee.

Data was analyzed for repeatability for within each laboratory to repeat the initial scores on both agar and current media. The data was also analyzed for reproducibility of similar scores across the participating labs. It was expected that agar would be as repeatable and as reproducible as the approved media currently being used in each lab.

Figure 4. 2015 Repeatability Results (Within the same lab)

| Crop | Method | Percent Germination | fr ² | Sr |
|--------|---------|---------------------|-----------------|-------|
| Tomato | Agar | 89.60 | 1.79 | 40.88 |
| Tomato | Current | 89.86 | 1.29 | 33.79 |

The repeatability is the closeness of the agreement between the results of successive measurement of the same measure and carried out in the same conditions of measurement.

fr² –Reproducibility within a lab (> 1 is a sign of more variability than expected)

Sr-The average of the variance of results within laboratories (similar number to the other method is desired.)

Figure 5. Reproducibility Results (across many labs)

| Crop | Method | Percent Germination | fR ² | SR |
|--------|---------|---------------------|-----------------|------|
| Tomato | Agar | 89.60 | 0.24 | 1.50 |
| Tomato | Current | 89.86 | 0.48 | 2.09 |

The reproducibility is the precision under reproducibility conditions, i.e. conditions where test results are obtained with the same method on identical test items in different laboratories with different operators using different equipment.

fR² –Reproducibility (> 1 is a sign of more variability than expected)

SR- The average of the variance of results across all labs (similar number to the other method is desired.)

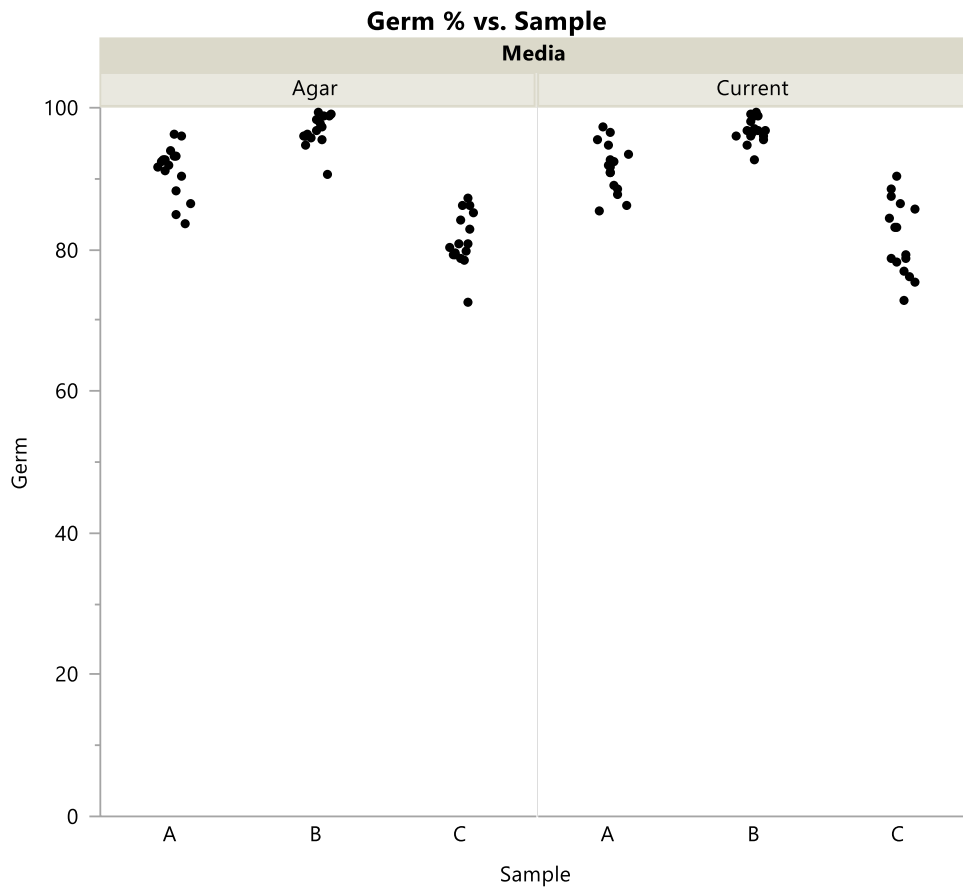


Figure 6 2015 Sample average by media

The conclusion of the 2015 referee is that tomato does give repeatable results within a laboratory and the results are reproducible across many laboratories. This conclusion was proven in two different referees using different lots of seed for each round. The data from 2015 actually shows that the agar reduced the variability across labs. This would suggest the potential for increased uniformity of results.