

## 2012 Western Wheatgrass Referee

Thank you to everyone who participated in the western wheatgrass referee. There were 10 responses submitted whose data were used to create this summary.

I asked if any of the participant labs had ever used the alternate 10/30C method allowed in the M&P. I was curious as no other rules allow this option and I was wondering how it had come to be in our rules and if anyone was using it. There was one lab that had done so in the past but not within the last year.

The referee consisted of 3 lots of western wheatgrass tested by 3 different methods each.

Method 1 was a straight TZ of 200 seeds in two replicates.

Method 2 was a germination of 200 seeds in dark with KNO<sub>3</sub> at 15/25C with a final count at 28 days and TZ of ungerminated seeds.

Method 3 was a germination of 200 seeds in dark with KNO<sub>3</sub> at 15/25C with a final count of 14 days and TZ of ungerminated seeds.

### **Method 1:**

Lot 1 TZ average of 80%.

2 results were more than 25% out from the average so when those are removed the average of the other 8 results is 87%.

Lot 2 TZ average of 69%.

2 results were more than 20% out from the average so when those are removed the average of the other 8 results is 75%.

Lot 3 TZ average of 57%.

3 results were more than 20% out from the average so when those are removed the average of the other 7 results is 63%.

Conclusion- TZ is a skill that requires practice and more workshops and resources may be helpful for analysts who do not do TZ on a regular basis and also for trainees and for those who wish to maintain proficiency in this area.

### **Method 2:**

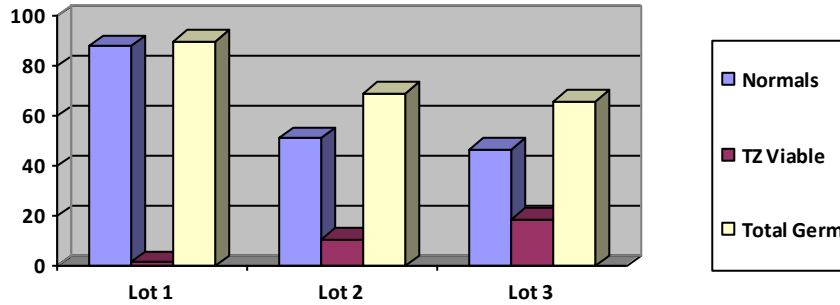
The average for Lot 1: 88% Normal seedlings, 2% TZ Viable and a Total Germ of 90%.

The average for Lot 2: 51% Normal seedlings, 11% TZ Viable and a Total Germ of 69%.

The average for Lot 3: 47% Normal seedlings, 19% TZ Viable and a Total Germ of 66%.

The following chart gives a visual display of this data.

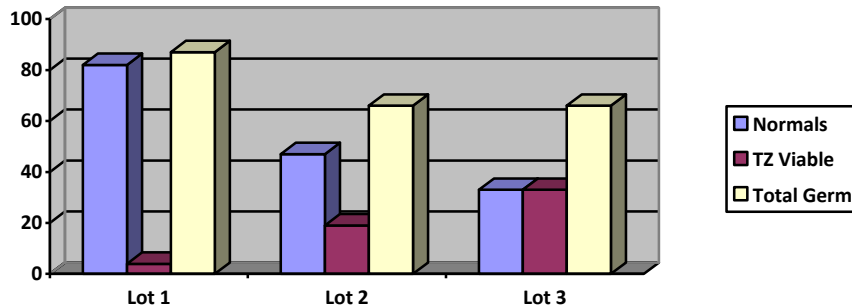
**Method 2:**



**Method 3:**

The average for Lot 1: 82% Normal seedlings, 4% TZ Viable, and a Total Germ of 87%.  
 The average for Lot 2: 47% Normal seedlings, 19% TZ Viable, and a Total Germ of 66%.  
 The average for Lot 3: 33% Normal seedlings, 33% TZ Viable, and a Total Germ of 66%.

The following chart gives a visual display of this data.

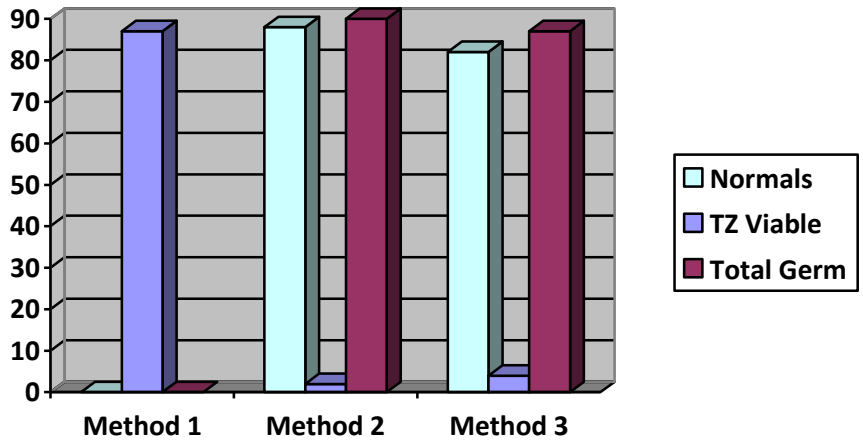


**Comparison of the three Methods:**

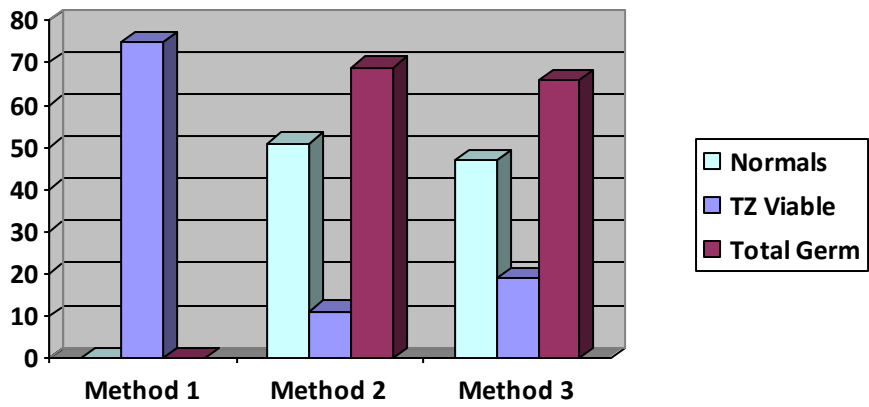
LOT	Method 1 Total Viable	Method 2 Total Viable	Method 3 Total Viable
<b>1</b>	87%	90%	87%
<b>2</b>	75%	69%	66%
<b>3</b>	63%	66%	66%

Method 1 averages are not including outliers.

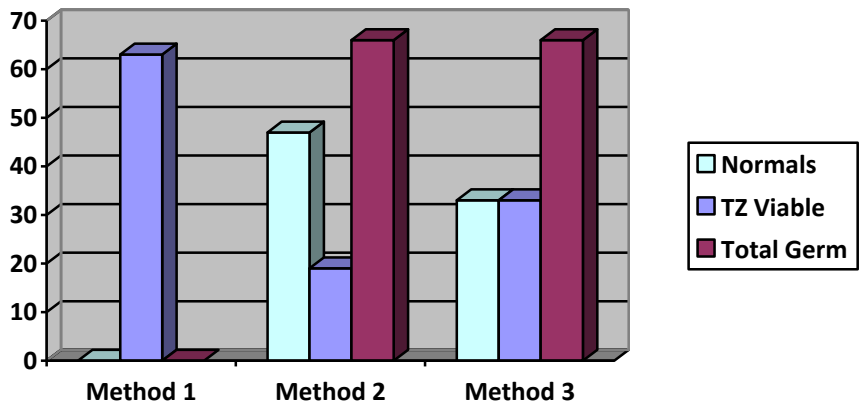
**Lot 1: Method 1(TZ) & Method 2(28 days) & Method 3(14 days)**



**Lot 2: Method 1(TZ) & Method 2(28 days) & Method 3(14 days)**



**Lot 3: Method 1(TZ) & Method 2(28 days) & Method 3(14days)**



### **In Summary:**

Overall the germination results were very consistent. In conclusion TZ of ungerminated seed after 14 or 28 days does not make a significant difference in the germination on average. The straight TZ was only higher on average for Lot number 2 and not significantly so (would be in tolerance if it was two germination tests). This may indicate seeds do not typically die during the test period and the TZ of remaining seeds is as accurate as a TZ beforehand.

The straight TZ method presented the most varied results and this may be due to participants who are not accustomed to TZ or the crop kind under analysis. The concentration of TZ solution used varied from 0.1% to 1% and did not seem to affect the end result. I have tried different concentrations and feel most comfortable with 0.5%. It seems like you get used to whatever concentration you are accustomed to just like your microscope or any other tool it just requires practice.

This referee proves that both methods in the M&P (doing a TZ test prior to the germination or on remaining seeds after 28 days) is accurate and repeatable. I propose the method for 10/30C with light and a final count at 14 days should be removed from the rules as uniformity in seed testing is our primary goal and it has not been proven that this method would achieve that as no labs are using it. I also propose that the use of KNO<sub>3</sub>, and TZ should be moved from the fresh and dormant seed column to the general requirements on Table 5 of the M&P.

Thank you to everyone who participated in this labour intensive referee. I hope it was an interesting/educational experience.

Lisa Greenan  
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