

Using JMP® Data Visualization to Teach a Teachable Moment: A Case Study

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Abstract

The cancer research program at Bob Jones University has as its primary function the pedagogic goal of giving undergraduate biology majors a true laboratory research experience to prepare for graduate school or for entering the job market in the biological sciences. The students design, execute, and analyze their own experiments using cancer biology as the area of study.

Two cancer cell lines were treated with almond extracts from almonds stored at various temperatures to see if storage temperature would affect the anticancer activity in the almonds. All the necessary control treatments were incorporated into the students' design. Upon evaluating the results, one cell line presented control data that was completely consistent with expectations and led to conclusions that could be consistently interpreted from the expected biology. In contrast, the other cell line yielded data that was in apparent total disarray.

Using the analysis and data visualization capabilities of JMP, the students were led through a troubleshooting exercise and were able to draw conclusions explaining what went wrong in the experimental execution with the second cell line that were consistent with the data obtained.

Methods

Cell Lines

AGS is a human gastric cancer line and LoVo is a human colon adenocarcinoma. Both cell lines were maintained under identical conditions using the standard cell culture practices appropriate for these cell lines. Both cells are adherent and are removed from their culture flask wall by brief trypsinization.

Almond Storage

Almonds were stored for four months at room temperature (~21°C), 2-8°C, noncycling -20°C, and cycling -20°C. (Cycling/noncycling refers to the type of freezer; cycling goes through wider temperature fluctuations over time to reduce frost buildup.) Fresh almonds were bought just before the experiment was done.

Almond Digestion

Ten whole almonds were added to 200 mL dH₂O and blended in a Waring blender for 5 minutes at room temperature. The pH was adjusted to 2.8 with HCl and incubated for 2 hours at 37°C with pepsin. Following this incubation, the pH was adjusted to 5.7 with NaOH and a mixture of pancreatin and bile salts were added and incubated an additional 2 hours at 37°C. The digests were clarified by centrifugation, sterile filtered through a 0.2 micron filter, and stored at 2-8°C until use (within one week's time).

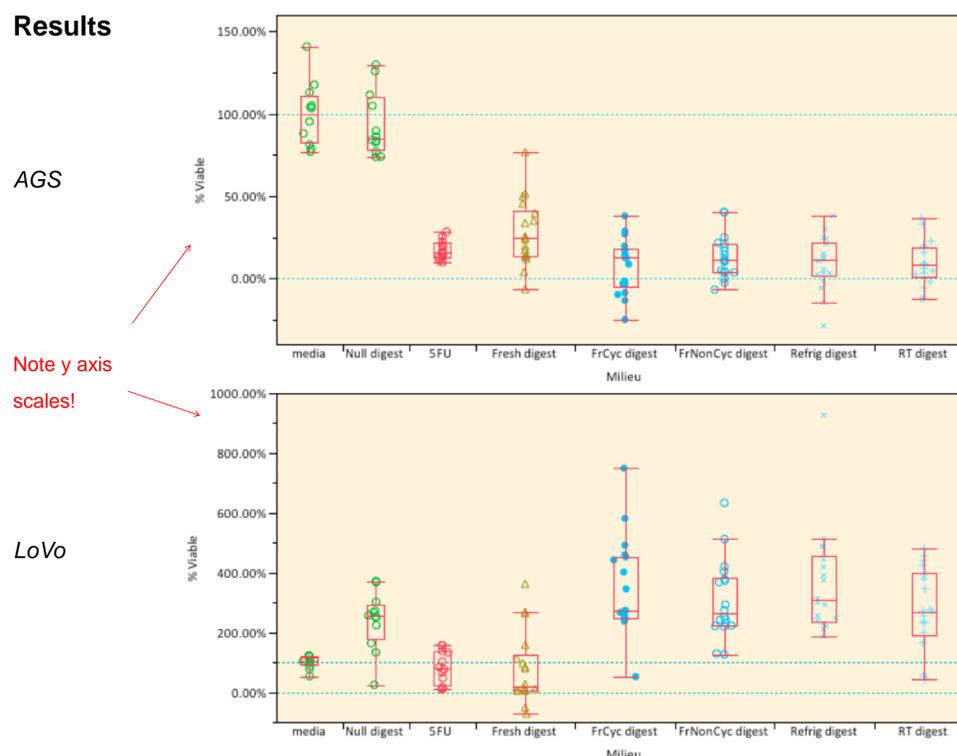
Cell Assay

Cells were harvested by trypsinization, counted to determine concentration, and plated into a sterile microtiter plate at 100 µL per well at the optimum density for each cell line. Plates were incubated at 37°C for 24 hours to establish growth, the media removed and 100 µL of treated media (95% growth media + 5% test solution) as shown in Table 1 was added to the appropriate wells. After 72 hours incubation at 37°C, 20 µL of MTS dye (this dye is metabolized by living cells to a product that has an absorbance at 490 nm; the higher the absorbance, the greater the number of viable cells present) was added to each well and incubated for 2 hours at 37°C. The absorbance at 490 nm of the solutions in each well was then measured in a GENios Plus Tecan plate reader and the % viability calculated. Treatment groups were compared by oneway ANOVA using the Fit Y by X platform in JMP 10.0.0.

Table 1

Milieu	Description	Role	Expected Results
media	growth media only	negative control	100% viable
Null digest	enzymatic digest w/o almonds	digest control	100% viable (hopefully!)
5FU	5-fluorouracil, anticancer drug	positive control	< 100% viable
Fresh digest	freshly purchased almond digest	experimental	TBD
FrCyc digest	cycling -20°C almond digest	experimental	TBD
FrNonCyc digest	noncycling -20°C almond digest	experimental	TBD
Refrig digest	2-8°C almond digest	experimental	TBD
RT digest	room temp almond digest	experimental	TBD

Results



Discussion/Conclusions

- Results with the AGS cell line were consistent** with the hypothesis of an anticancer agent being present in the almonds. All three controls behaved as expected. No significant difference was found between the various storage conditions.
- Results with the LoVo cell line were highly irregular** with the controls behaving inconsistently with expectations and drastically departing from their behavior seen with the AGS cell line.
- Data visualization with JMP** allowed us to see a dramatically different spread in the data ranges for the different cell lines. The greater variation in the LoVo data was tracked back to inadequate trypsinization of the cells, resulting in a nonhomogenous stock of clumped cells being distributed unevenly in the wells of the microtiter plate used for the assay.

References

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