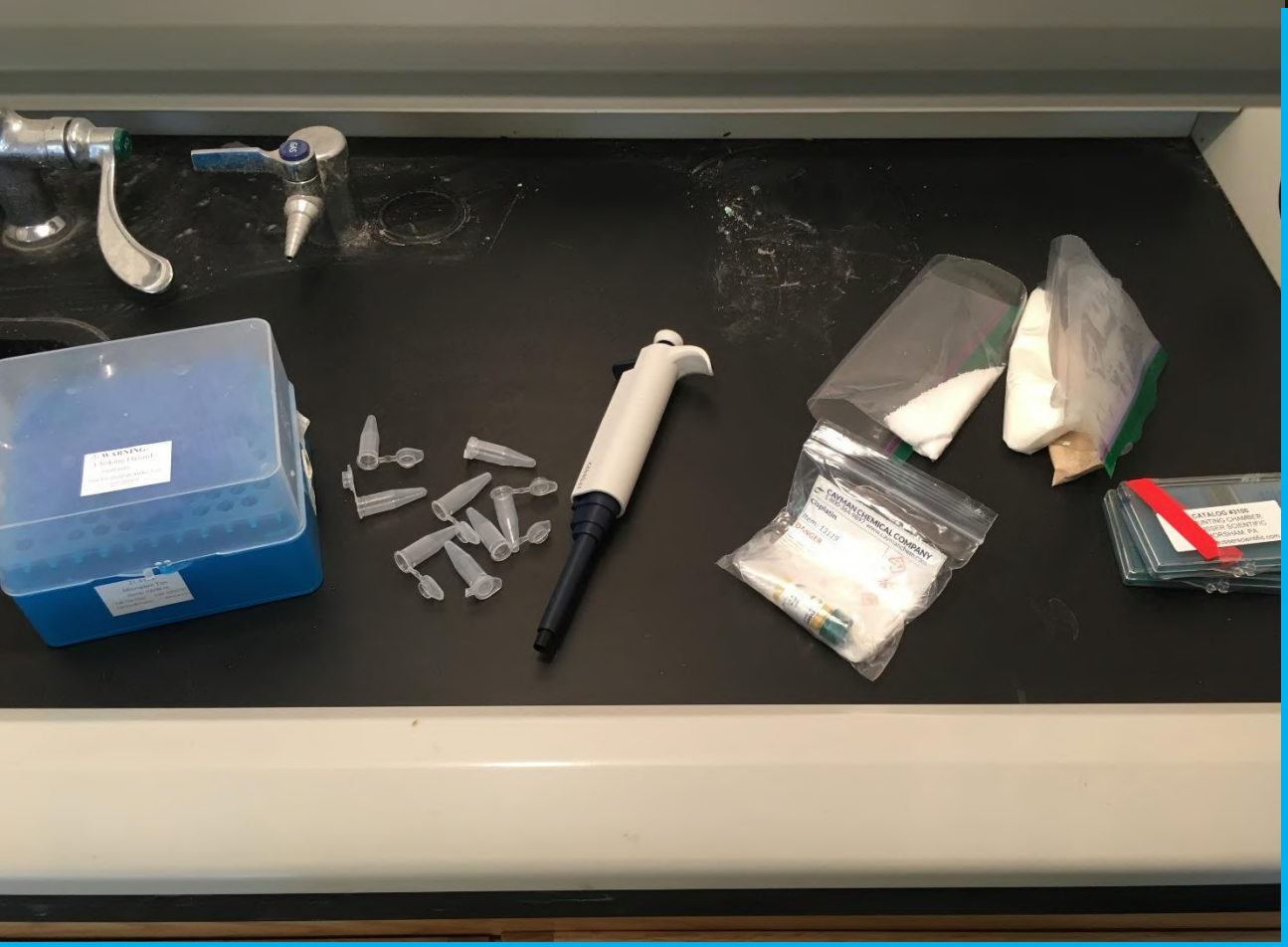
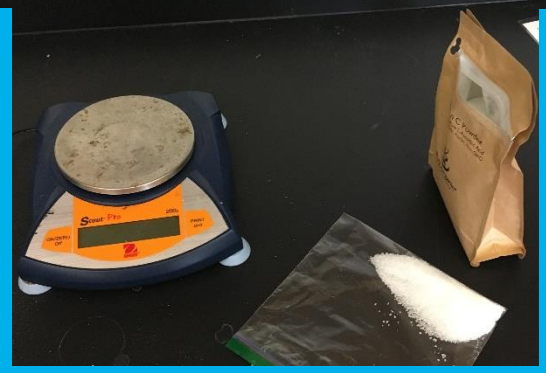
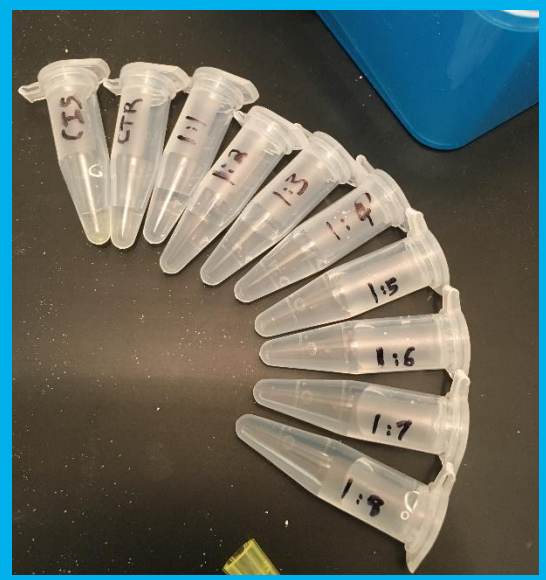
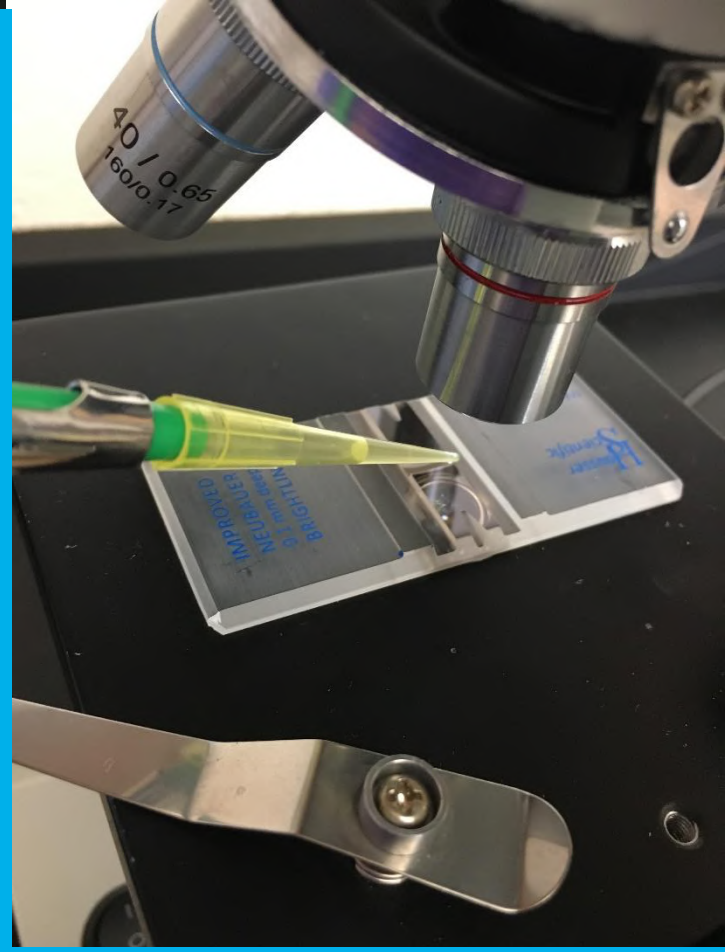






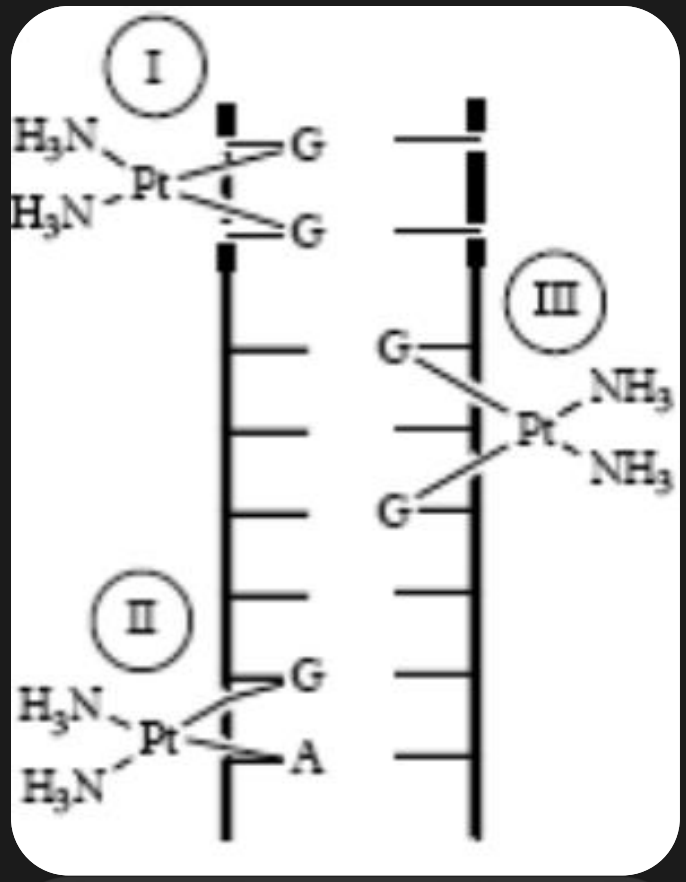


Antioxidative Effects of Ascorbic Acid on Cisplatin-Induced Cytotoxicity

Project experimentation and analysis done by Andrew Yang

Research and Background

- Cisplatin (cis-diamminedichloriidoplatinum (II))
  - Chemotherapeutic drug, acts through purine intrastrand crosslinks, typically GpG
  - GpXpG undergoes NER, GpG and ApG are irreparable and trigger apoptosis
- Recent studies show collateral side effects of cisplatin-generated oxidative stress
  - Creates excessive ROS, cytotoxic
    - DNA backbone breaks, peroxidation of lipid membranes, nephrotoxicity
- Most common and effective way to counter ROS: antioxidants
  - Ascorbic Acid (AA), commonly known as Vitamin C
    - Antioxidant: converts various high ROS into poor ROS
    - Prooxidant: reduces metal ions, produces hydrogen peroxide



How does the addition of AA affect cisplatin-induced cytotoxicity on model yeast cells?

Question

H_0: It is hypothesized that if AA is added, there will be no statistical difference between presence and absence of AA in terms of cisplatin-induced apoptosis in yeast.

H_1: It is hypothesized that if AA is added, then cisplatin-induced apoptosis in yeast will decrease.

Hypotheses

Materials

Cisplatin	Ascorbic Acid
Sodium Chloride	Baker’s Yeast
Water	Digital Balance
Hot Plate	Stirring Rods
Camera Light Microscope	Hemocytometer Grids
Graduated cylinders	Beakers
Micropipettes	Microtubes

Procedure

Step 1

- 0.9% saline solution
- 500mg NaCl to 35ml warm water, add water to make 50ml

Step 2

- 1mg/1ml cisplatin solution
- 10mg of cisplatin to 5mL saline, make 10ml

Step 3

- Activate yeast
- 100mg each of sugar and yeast in 10ml hot water, make 20ml

Step 4

- AA solution
- 100mg AA to 70ml warm water, make 100ml

Step 5

- 9 base micro tubes
- Each has 100µl cisplatin, and 50µl yeast

Step 6

- CTR tube, has no AA
- Subsequent tubes will have 100µl more AA, 9th has 800µl AA

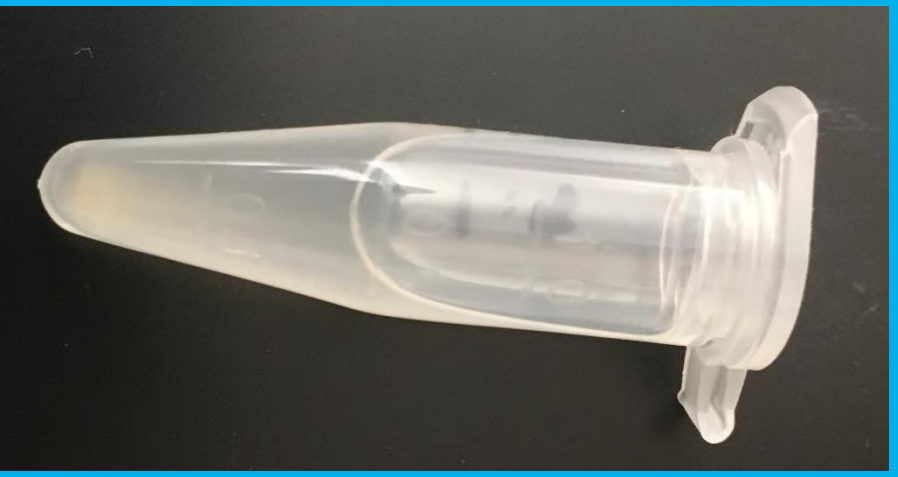
Step 7

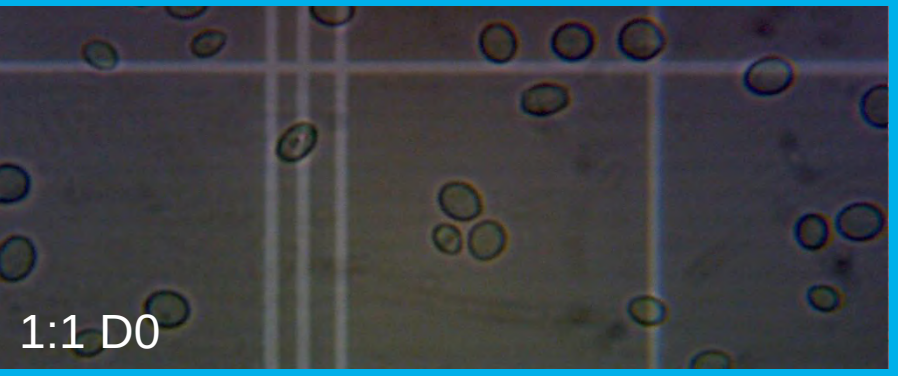
- 2 more CTRs
- 2nd CTR tube, has no cisplatin
- 3rd CTR tube, has only yeast

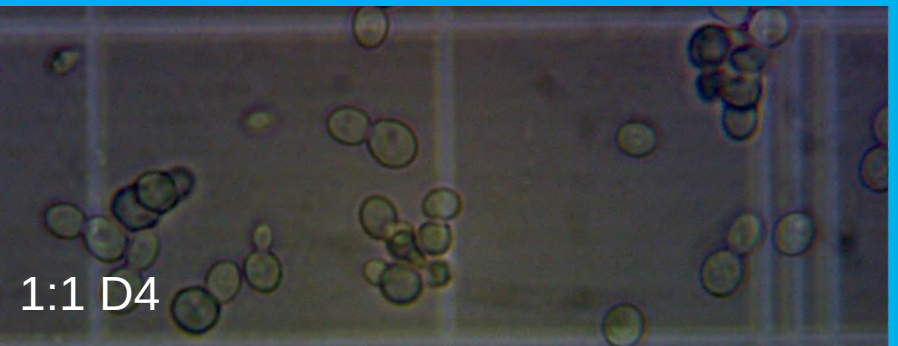
Step 8

- Each day, take 50µl CTR and examine on hemocyto-meter grid
- Repeat rest

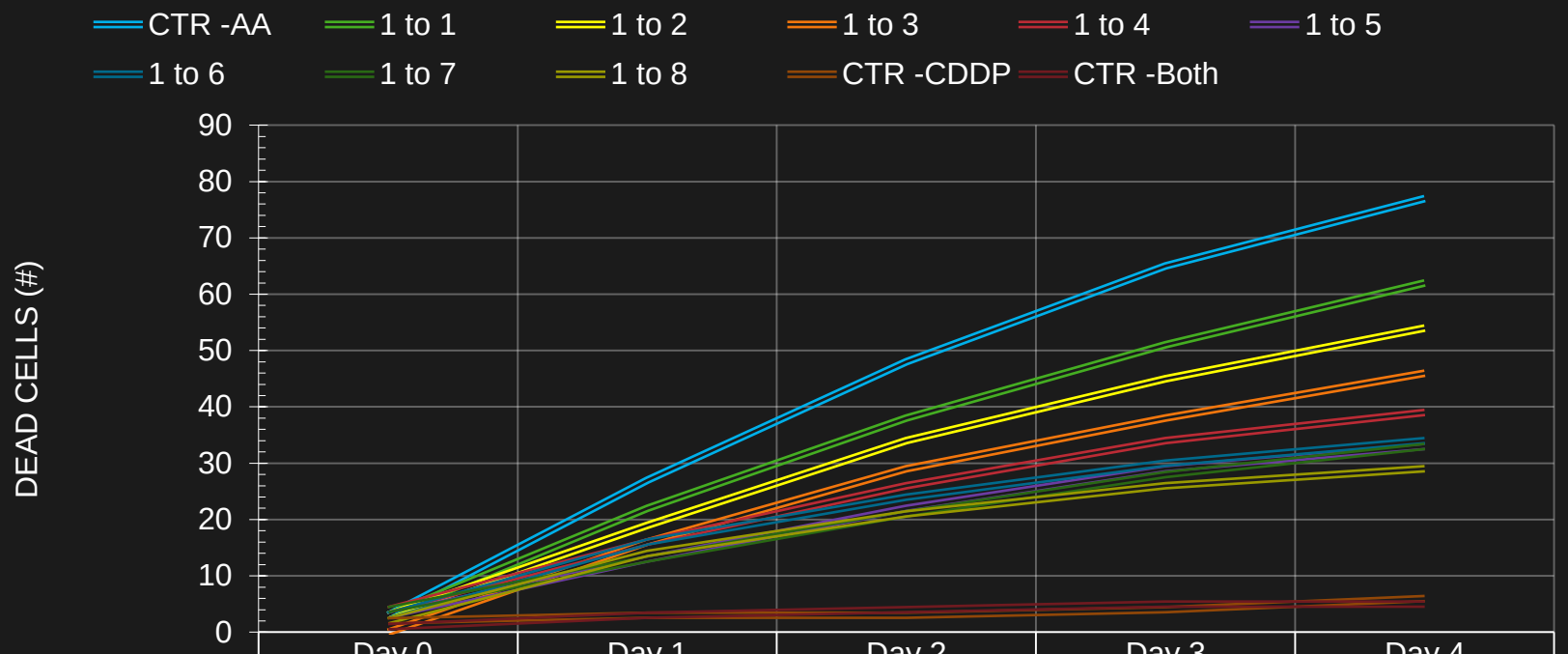
Data/Observations







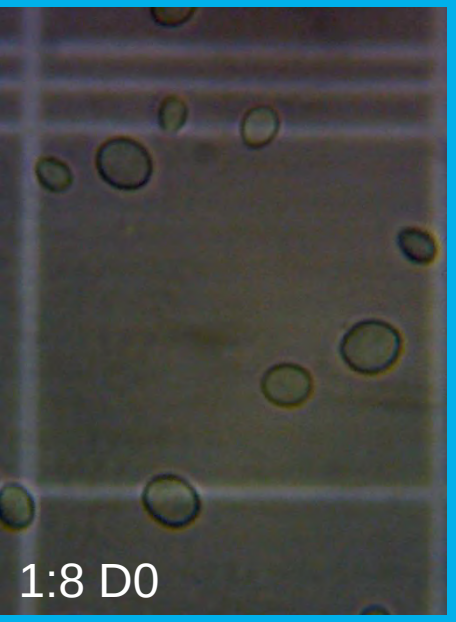
NUMBER OF DEAD CELLS BASED ON TIME AND AA CONCENTRATION

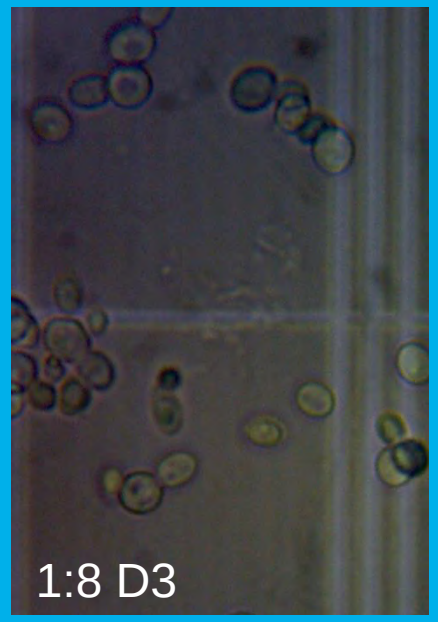


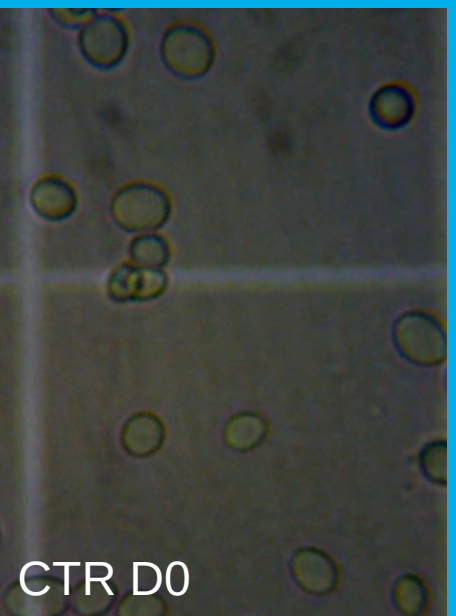
	Day 0	Day 1	Day 2	Day 3	Day 4
CTR -AA	3	27	48	65	77
1 to 1	3	22	38	51	62
1 to 2	3	19	34	45	54
1 to 3	0	16	29	38	46
1 to 4	4	16	26	34	39
1 to 5	3	13	22	29	33
1 to 6	3	16	24	30	34
1 to 7	4	13	21	28	33
1 to 8	2	14	21	26	29
CTR -CDDP	2	3	3	4	6
CTR -Both	1	3	4	5	5

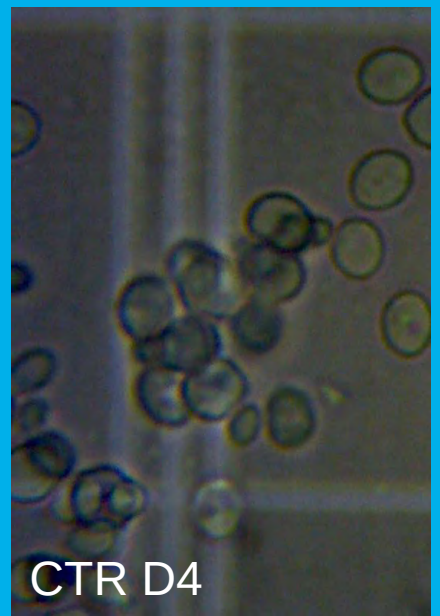
TIME (DAYS)

Data/Observations

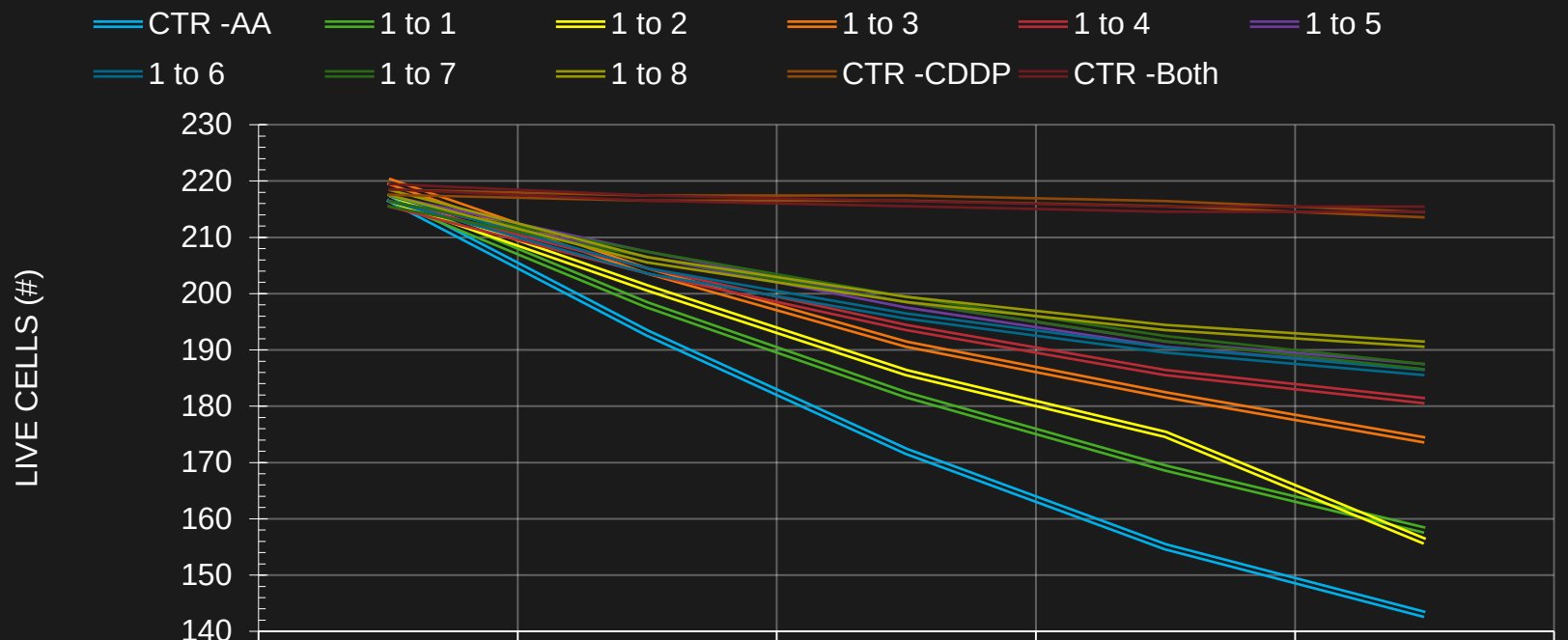








NUMBER OF LIVE CELLS BASED ON TIME AND AA CONCENTRATION

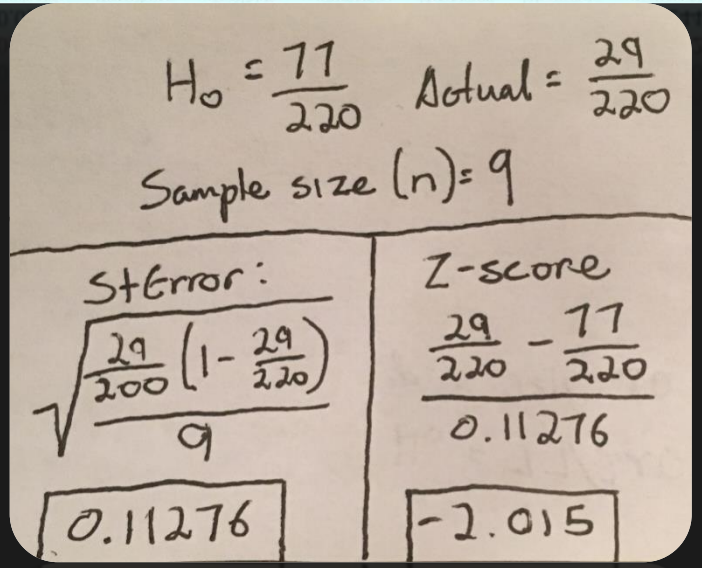


	Day 0	Day 1	Day 2	Day 3	Day 4
CTR -AA	217	193	172	155	143
1 to 1	217	198	182	169	158
1 to 2	217	201	186	175	156
1 to 3	220	204	191	182	174
1 to 4	216	204	194	186	181
1 to 5	217	207	198	191	187
1 to 6	217	204	196	190	186
1 to 7	216	207	199	192	187
1 to 8	218	206	199	194	191
CTR -CDDP	218	217	217	216	214
CTR -Both	219	217	216	215	215

Statistical Analysis

- Standard error: 11.276%
- Z-score: -2.015
- P value: 0.0202
- Smaller than 0.05
  - Rejects the null hypothesis
  - It is hypothesized that if AA is added, there **IS** a statistical difference presence and absence of AA in terms of cisplatin-induced apoptosis in yeast.

z	0.00	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09
-3.4	0.0003	0.0003	0.0003	0.0003	0.0003	0.0003	0.0003	0.0003	0.0003	0.0002
-3.3	0.0005	0.0005	0.0005	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004	0.0003
-3.2	0.0007	0.0007	0.0006	0.0006	0.0006	0.0006	0.0006	0.0005	0.0005	0.0005
-3.1	0.0010	0.0009	0.0009	0.0009	0.0008	0.0008	0.0008	0.0008	0.0007	0.0007
-3.0	0.0013	0.0013	0.0013	0.0012	0.0012	0.0011	0.0011	0.0011	0.0010	0.0010
-2.9	0.0019	0.0018	0.0018	0.0017	0.0016	0.0016	0.0015	0.0015	0.0014	0.0014
-2.8	0.0026	0.0025	0.0024	0.0023	0.0023	0.0022	0.0021	0.0021	0.0020	0.0019
-2.7	0.0035	0.0034	0.0033	0.0032	0.0031	0.0030	0.0029	0.0028	0.0027	0.0026
-2.6	0.0047	0.0045	0.0044	0.0043	0.0041	0.0040	0.0039	0.0038	0.0037	0.0036
-2.5	0.0062	0.0060	0.0059	0.0057	0.0055	0.0054	0.0052	0.0051	0.0049	0.0048
-2.4	0.0082	0.0080	0.0078	0.0075	0.0073	0.0071	0.0069	0.0068	0.0066	0.0064
-2.3	0.0107	0.0104	0.0102	0.0099	0.0096	0.0094	0.0091	0.0089	0.0087	0.0084
-2.2	0.0139	0.0136	0.0132	0.0129	0.0125	0.0122	0.0119	0.0116	0.0113	0.0110
-2.1	0.0179	0.0174	0.0170	0.0166	0.0162	0.0158	0.0154	0.0150	0.0146	0.0143
-2.0	0.0228	0.0222	0.0217	0.0212	0.0207	0.0202	0.0197	0.0192	0.0188	0.0183
-1.9	0.0287	0.0281	0.0274	0.0268	0.0262	0.0256	0.0250	0.0244	0.0239	0.0233



Conclusion

- Supports alternative hypothesis
  - It is hypothesized that if AA is added, then cisplatin-induced apoptosis in yeast will decrease.
- Decreased cell death from addition of AA
  - Yeast models healthy human cells, equipped with various normal cell functions that counter AA-generated ROS, especially normal peroxisomes
  - AA acts as suppressor for cisplatin-induced oxidative stress
  - Based on extra controls, AA alone has no effect on yeast
- Cell death still occurs when AA and cisplatin are present, indicating unrepaired DNA crosslinks

Applications

- Possible change of cisplatin chemotherapy regimens
  - Reduces collateral cell damage to healthy tissue, renal failure from nephrotoxicity
  - Maintains overall cytotoxicity towards cancerous cells
    - Cells with unrestricted proliferation lack measures against AA prooxidative effects
    - Studies on leukemia cell lines, 10 mM AA solution → excess H_2O_2 → p53 apoptosis
  - Possibility of increased dosage
    - Lowered risk for:
      - Cisplatin resistance, combination therapy
      - Secondary cancers, leukemia or solid state tumors
      - Cancer relapse

Future Improvements and Experimentation

- Improved equipment that can measure smaller amounts
  - Less chemical waste
- More trials to incorporate other statistical tests
- Yeast → human tissue samples
- Additional tests on cancer cultures (testicular, ovarian, bladder, squamous)
- Different methods of testing
  - Direct measurement of ROS
  - More accurate measurement of biological damage
    - Assays using colormetric and fluorometric detection

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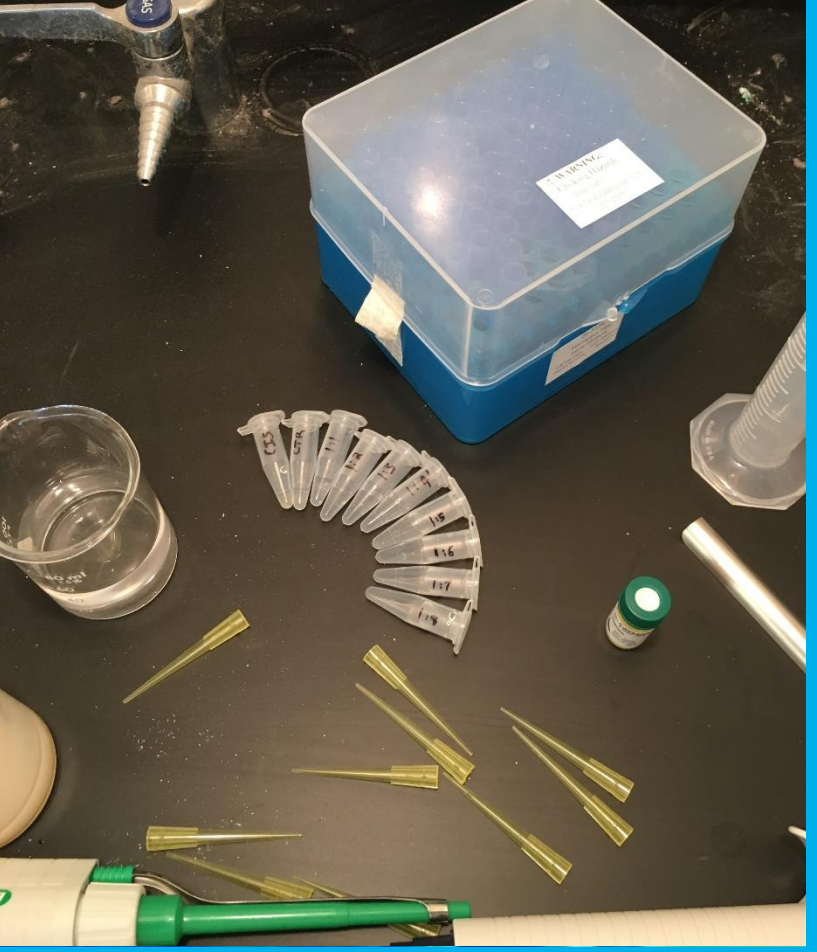

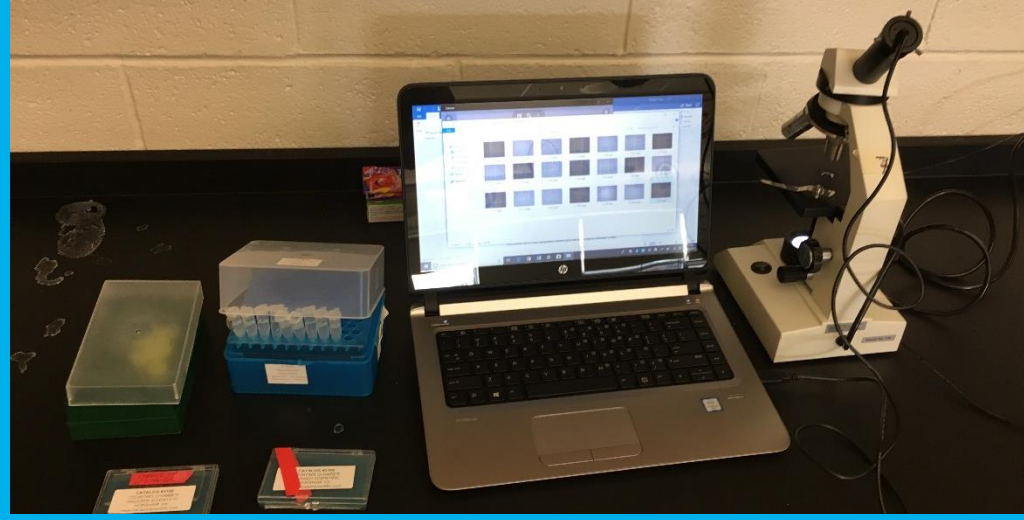

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