



Interlab Study



Bacteria Aimed at Removing and Terminating Intestinal Invaders

The Interlab Study was an initiative started by iGEM in 2014. This initiative examines the replicability of fluorescence measurements and identifies likely sources of error. This year, the Interlab Study focused on how close can these measurements be when measuring fluorescence around the world. In order to compare measurements around the world a standardized protocol was put in place to limit variances between measurements in different laboratories.

The FSU iGEM team participated in this measurement study using the Plate Reader Protocol. Here you can find the devices that were tested, a summary of the protocol, and graphs showing raw plate reader measurements of the OD600 and fluorescence of our samples over time.

Tested Devices

Competent *E. coli* DH5 α cells were transformed with the following plasmids:

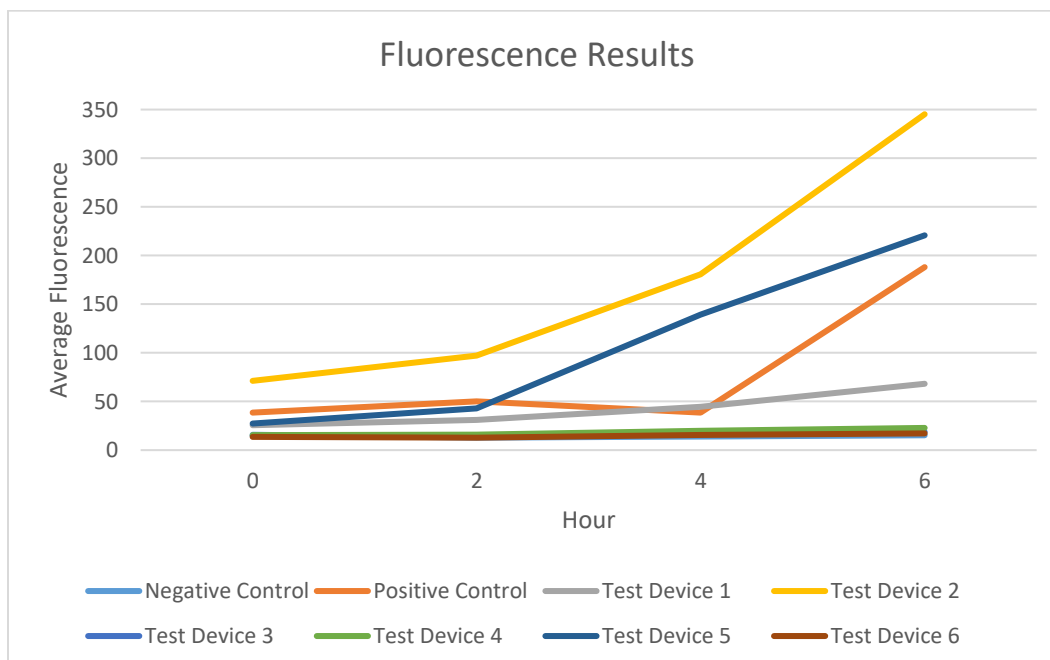
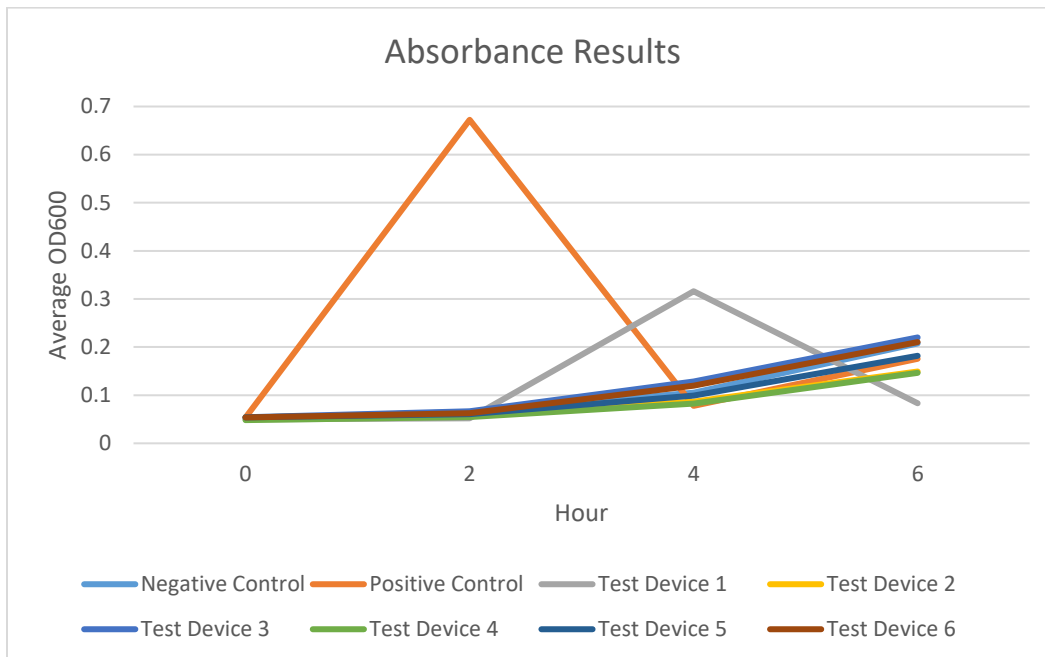
- Positive Control (**BBa_I20270**) – J2311.B0032.E0040.B0010.B0012 in pSB1C3
- Negative Control (**BBa_R0040**) – R0040 in pSB1C3
- Test Device 1 (**BBa_J364000**) – J23101.B0034.E0040.B0010.B0012 in pSB1C3
- Test Device 2 (**BBa_J364001**) – J23106.B0034.E0040.B0010.B0012 in pSB1C3
- Test Device 3 (**BBa_J364002**) – J23117.B0034.E0040.B0010.B0012 in pSB1C3
- Test Device 4 (**BBa_J364003**) – J23101.J364100.E0040.B0010.B0012 in pSB1C3
- Test Device 5 (**BBa_J364004**) – J23106.J364100.E0040.B0010.B0012 in pSB1C3
- Test Device 6 (**BBa_J364005**) – J23117.J364100.E0040.B0010.B0012 in pSB1C3

Protocol

- 2 colonies from each plate were inoculated in 5 mL LB + Chloramphenicol. Cells were grown overnight at 37°C and 220 rpm.
- Measured OD600 of overnight cultures
- Diluted the cultured to an OD600 of 0.01 in 12 mL LB + Chloramphenicol and placed in thermo shaker at 37°C and 220 rpm with a tray shielding thermo shaker from light.
- Samples of 500 μ L taken at 0, 2, 4, and 6 hours of incubation.
- OD and fluorescence measured of each sample.

Results

- Most samples grew exponentially other than the positive control and Test Device 1
- Test Devices 2 and 5 had the greatest increase in fluorescence over time
- The negative control did not express fluorescence, Test Devices 3, 4, and 6 also had little to no expression of fluorescence



Fluorescence Raw Readings:

Hour 0:	Neg. Control	Pos. Control	Device 1	Device 2	Device 3	Device 4	Device 5	Device 6	LB + Chlor (blank)
Colony 1, Replicate 1	18.174	39.761	23.02	51.452	14.502	16.617	28.324	14.721	11.768
Colony 1, Replicate 2	19.052	39.36	28.366	59.175	15.46	17.217	26.873	13.862	11.522
Colony 1, Replicate 3	18.188	36.729	29	64.12	14.504	16.284	25.818	12.391	11.453
Colony 1, Replicate 4	18.399	41.505	28.438	62.963	14.96	16.408	26.05	13.065	11.379
Colony 2, Replicate 1	13.301	38.146	23.902	86.13	13.992	14.241	26.784	13.684	11.054
Colony 2, Replicate 2	13.65	37.812	24.501	82.14	14.05	14.073	25.799	13.833	12.074
Colony 2, Replicate 3	12.044	37.961	23.455	77.648	14.213	14.063	28.696	13.983	11.723
Colony 2, Replicate 4	13.672	37.269	24.07	86	14.529	14.407	30.144	14.25	12.575

Hour 2:	Neg. Control	Pos. Control	Device 1	Device 2	Device 3	Device 4	Device 5	Device 6	LB + Chlor (blank)
Colony 1, Replicate 1	13.447	51.756	27.612	97.409	14.793	14.346	48.095	13.543	11.266
Colony 1, Replicate 2	14.342	54.116	33.732	113.75	15.273	15.144	47.634	14.315	11.86
Colony 1, Replicate 3	13.767	52.123	33.554	118.31	13.929	14.75	45.282	12.29	11.855
Colony 1, Replicate 4	11.037	42.307	25.319	88.713	10.984	10.552	34.657	10.047	8.544
Colony 2, Replicate 1	12.725	51.445	32.823	94.5	13.517	19.355	41.901	13.089	12.269
Colony 2, Replicate 2	13.125	53.08	33.641	92.755	13.249	18.92	41.887	14.158	11.941
Colony 2, Replicate 3	12.86	52.196	31.842	90.925	13.433	18.611	42.588	13.611	11.532
Colony 2, Replicate 4	11.248	43.084	28.672	81.134	12.171	16.656	41.907	11.335	10.373

Hour 4:	Neg. Control	Pos. Control	Device 1	Device 2	Device 3	Device 4	Device 5	Device 6	LB + Chlor (blank)
Colony 1, Replicate 1	13.646	94.945	38.891	155.51	14.383	21.23	123.48	14.39	9.043
Colony 1, Replicate 2	13.8	117.94	46.573	189.74	16.74	23.691	126.71	15.298	11.391
Colony 1, Replicate 3	13.302	117.79	42.729	191.85	16.15	24.626	122.13	13.738	11.227
Colony 1, Replicate 4	14.036	120.72	24.139	186.12	15.552	23.214	123.98	14.696	6.895
Colony 2, Replicate 1	13.669	119	50.36	22.59	15.905	16.255	145.94	16.373	11.842
Colony 2, Replicate 2	14.398	123.37	51.53	232.42	16.61	16.848	152.22	16.888	11.985
Colony 2, Replicate 3	14.132	122.71	51.388	228.96	17.875	15.851	156.28	16.954	11.829
Colony 2, Replicate 4	14.568	122.03	51.457	238.68	17.552	17.092	163.44	16.801	11.961

Hour 6:	Neg. Control	Pos. Control	Device 1	Device 2	Device 3	Device 4	Device 5	Device 6	LB + Chlor (blank)
Colony 1, Replicate 1	15.073	188.471	58.43	269.8	17.385	27.12	188.44	15.612	10.963
Colony 1, Replicate 2	15.961	195.19	67.832	309.57	18.895	28.29	198.68	16.909	11.268
Colony 1, Replicate 3	15.468	191.28	67.65	310.99	17.725	27.957	188.92	15.596	11.433
Colony 1, Replicate 4	15.737	191.66	69.445	302.77	17.292	28.335	193.61	15.913	11.376
Colony 2, Replicate 1	15.149	184.88	70.814	396.34	18.6	17.806	243.75	18.248	11.754
Colony 2, Replicate 2	14.34	181.11	68.729	379.33	18.703	17.515	240.45	17.912	11.985
Colony 2, Replicate 3	14.66	186.92	72.287	398.14	20.947	17.54	250.99	19.004	12.08
Colony 2, Replicate 4	14.965	185.05	70.247	394.66	20.462	17.968	261.05	18.277	11.877

Abs600 Raw Readings

Hour 0:	Neg. Control	Pos. Control	Device 1	Device 2	Device 3	Device 4	Device 5	Device 6	LB + Chlor (blank)
Colony 1, Replicate 1	0.055	0.056	0.053	0.056	0.057	0.054	0.056	0.055	0.045
Colony 1, Replicate 2	0.053	0.053	0.051	0.054	0.055	0.052	0.054	0.053	0.04
Colony 1, Replicate 3	0.054	0.055	0.052	0.054	0.054	0.052	0.053	0.053	0.041
Colony 1, Replicate 4	0.052	0.052	0.052	0.053	0.054	0.051	0.053	0.053	0.039
Colony 2, Replicate 1	0.053	0.049	0.049	0.056	0.054	0.045	0.055	0.052	0.038
Colony 2, Replicate 2	0.05	0.047	0.047	0.055	0.053	0.044	0.054	0.055	0.038
Colony 2, Replicate 3	0.05	0.046	0.046	0.054	0.053	0.044	0.054	0.055	0.039
Colony 2, Replicate 4	0.05	0.071	0.048	0.053	0.053	0.044	0.053	0.054	0.041

Hour 2:	Neg. Control	Pos. Control	Device 1	Device 2	Device 3	Device 4	Device 5	Device 6	LB + Chlor (blank)
Colony 1, Replicate 1	0.062	0.06	0.055	0.062	0.068	0.051	0.064	0.066	0.04
Colony 1, Replicate 2	0.059	0.059	0.053	0.061	0.072	0.063	0.065	0.068	0.039
Colony 1, Replicate 3	0.089	0.148	0.047	0.056	0.06	0.048	0.059	0.065	0.04
Colony 1, Replicate 4	0.055	2.359	0.05	0.061	0.065	0.05	0.064	0.067	0.041
Colony 2, Replicate 1	0.06	0.053	0.052	0.057	0.069	0.058	0.059	0.061	0.038
Colony 2, Replicate 2	0.07	0.068	0.056	0.058	0.067	0.056	0.056	0.056	0.04
Colony 2, Replicate 3	0.076	1.989	0.054	0.063	0.069	0.057	0.059	0.058	0.038
Colony 2, Replicate 4	0.052	0.643	0.05	0.054	0.064	0.056	0.057	0.059	0.038

Hour 4:L26:U34	Neg. Control	Pos. Control	Device 1	Device 2	Device 3	Device 4	Device 5	Device 6	LB + Chlor (blank)
Colony 1, Replicate 1	0.103	0.083	0.062	0.09	0.137	0.103	0.106	0.121	0.042
Colony 1, Replicate 2	0.113	0.091	0.063	0.091	0.144	0.106	0.107	0.123	0.041
Colony 1, Replicate 3	0.108	0.085	0.059	0.089	0.127	0.091	0.103	0.115	0.04

Colony 1, Replicate 4	0.101	0.08	2.108	0.084	0.127	0.102	0.097	0.11	0.052
Colony 2, Replicate 1	0.108	0.079	0.059	0.084	0.119	0.063	0.09	0.11	0.04
Colony 2, Replicate 2	0.103	0.072	0.059	0.081	0.117	0.064	0.096	0.117	0.04
Colony 2, Replicate 3	0.09	0.059	0.058	0.08	0.12	0.063	0.1	0.124	0.06
Colony 2, Replicate 4	0.113	0.077	0.06	0.091	0.137	0.068	0.094	0.137	0.037

Hour 6:	Neg. Control	Pos. Control	Device 1	Device 2	Device 3	Device 4	Device 5	Device 6	LB + Chlor (blank)
Colony 1, Replicate 1	0.182	0.149	0.087	0.137	0.203	0.179	0.171	0.186	0.051
Colony 1, Replicate 2	0.191	0.297	0.081	0.138	0.203	0.179	0.171	0.197	0.048
Colony 1, Replicate 3	0.209	0.16	0.084	0.156	0.227	0.191	0.186	0.214	0.042
Colony 1, Replicate 4	0.204	0.154	0.082	0.149	0.216	0.189	0.182	0.214	0.46
Colony 2, Replicate 1	0.219	0.141	0.083	0.159	0.238	0.108	0.194	0.223	0.052
Colony 2, Replicate 2	0.226	0.142	0.085	0.157	0.233	0.121	0.191	0.224	0.044
Colony 2, Replicate 3	0.226	0.233	0.085	0.156	0.235	0.108	0.188	0.223	0.042
Colony 2, Replicate 4	0.204	0.13	0.079	0.142	0.205	0.097	0.172	0.202	0.042