InterLab Study

InterLab Study was very helpful before the formal experiment started. By following InterLab protocols, we can easily establish standardized, repeatable and reliable measurement tools. The aim of this year's InterLab study was to establish standard curves of particles and fluorescein using a plate reader and measure GFP fluorescence.

Materials

- Plate reader: PerkinElmer EnSpire
- 96 well plates (transparent plates with clear flat bottom)
- Devices: Positive control: BBa_I20270

　　　　　　　Negative control: BBa_R0040

　　　　　　　Device 1: BBa_J364000

　　　　　　　Device 2: BBa_J364001

　　　　　　　Device 3: BBa_J364002

　　　　　　　Device 4: BBa_J364007

　　　　　　　Device 5: BBa_J364008

　　　　　　　Device 6: BBa_J364009

- Fluorescein (provided in kit)
- 10ml 1xPBS pH 7.4-7.6 (phosphate buffered saline)
- 300 μL Silica beads - Microsphere suspension (provided in kit, 4.7x10^8 microspheres)
- 1ml LUDOX CL-X (provided in kit)
- ddH2O

Methods

At first, Competent DH5a cells were prepared and then transformed with 8 test devices

according to protocol, the transformation was successful, a number of colonies were grown on all plates. Therefore, 2 colonies from each plate were picked and inoculated in LB medium with 0.25mg/ml chloramphenicol for approximately 16-18 hours at 37°C and 220 rpm.

Before measuring cells, calibration needed to be done. Thus, we made 3 sets of measurements: OD600 reference point, a particle standard curve and a fluorescein standard curve according to protocol.

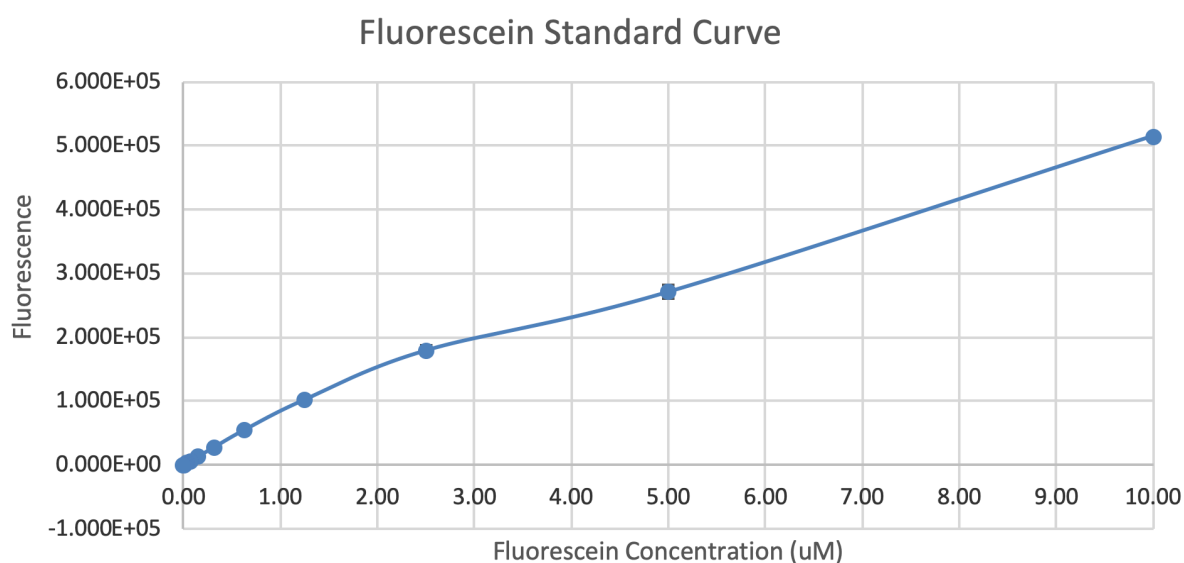After calibration, OD600 and fluorescence of transformed cells were measured.

Result

Figure 1. A Fluorescein standard curve generated by measuring the fluorescence of serial dilution of PBS stock.
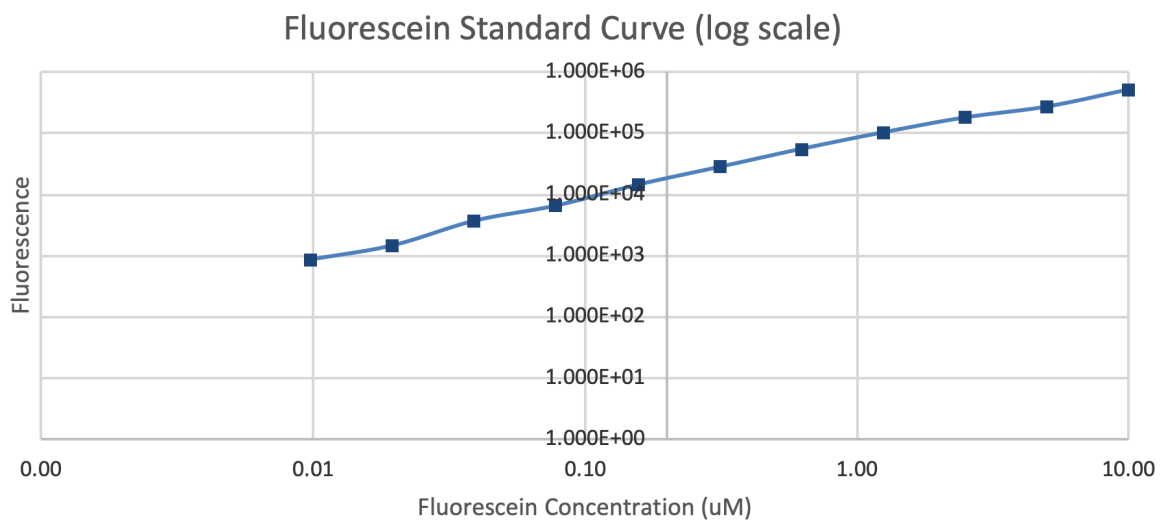
Fluorescein Standard Curve (log scale)

Figure 2. A Fluorescein standard curve displayed in log scale.

Particle Standard Curve
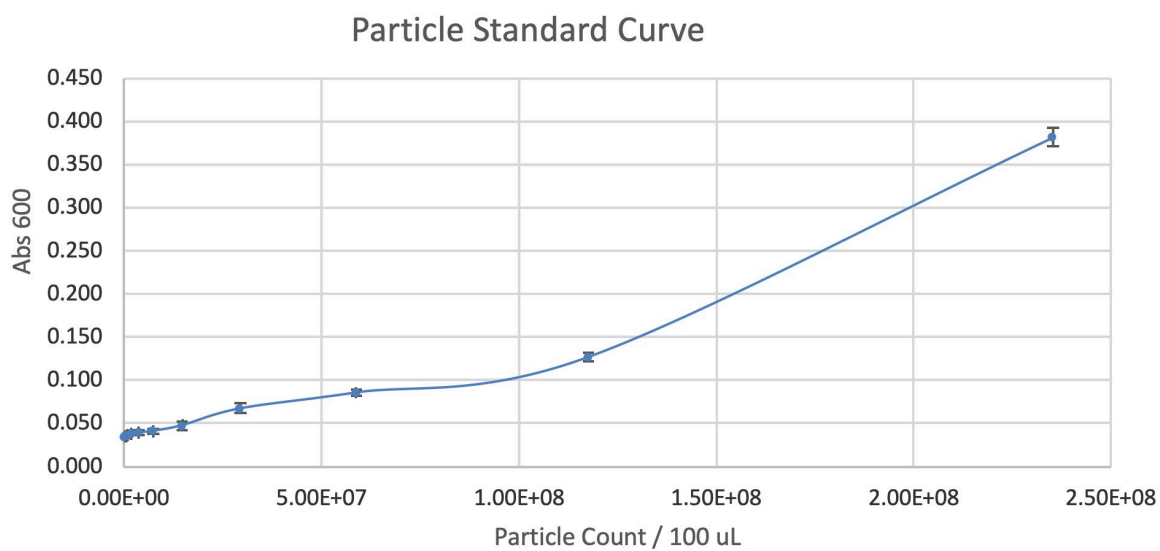

Figure 3. A Particle standard curve generated by measuring the Silica beads of serial dilution of ddH2O.
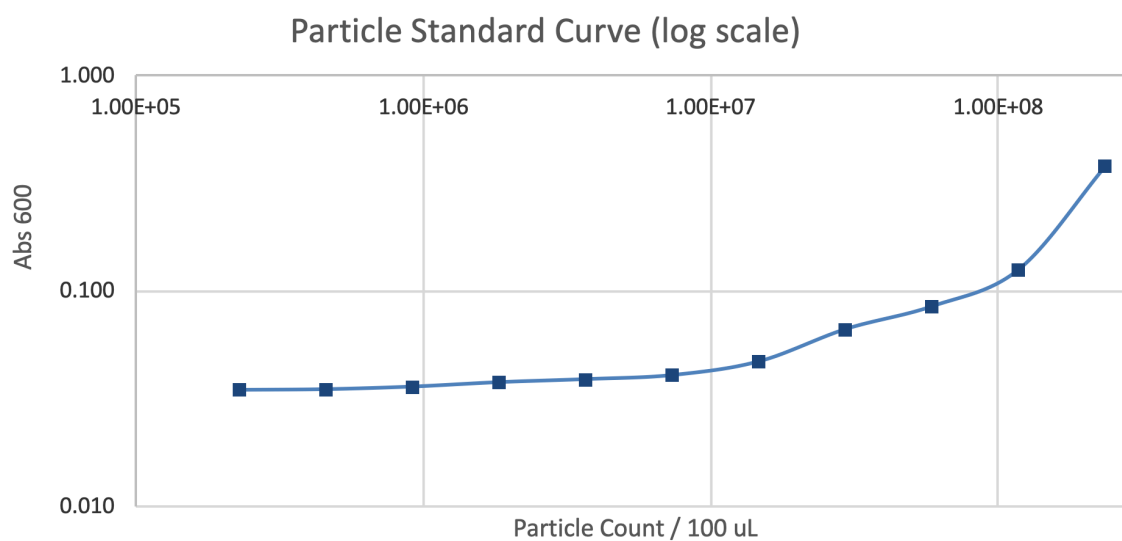
Particle Standard Curve (log scale)

Figure 4. A Particle standard curve displayed in log scale.

Net Fluorescein a.u.

Hour 0:	Neg.	Pos.	Device 1	Device 2	Device 3	Device 4	Device 5	Device 6
Colony 1, Replicate 1	190.00	3263.00	2265.00	2835.00	318.00	9765.00	2531.00	815.00
Colony 1, Replicate 2	149.00	3559.00	2342.00	2685.00	230.00	9396.00	2399.00	689.00
Colony 1, Replicate 3	-5.00	1969.00	2406.00	2720.00	244.00	10214.00	2323.00	725.00
Colony 1, Replicate 4	64.00	3356.00	2208.00	2675.00	119.00	10396.00	2833.00	711.00
Colony 2, Replicate 1	-136.00	2681.00	1442.00	3172.00	159.00	10786.00	5563.00	1022.00

	Neg.	Pos. Cl	Device 1	Device 2	Device 3	Device 4	Device 5	Device 6
Colony 2, Replicate 2	109.00	2368.00	2617.00	3377.00	234.00	10549.00	5797.00	982.00
Colony 2, Replicate 3	-20.00	3608.00	2646.00	3056.00	241.00	10090.00	5347.00	1150.00
Colony 2, Replicate 4	43.00	3091.00	2808.00	3223.00	233.00	10990.00	5845.00	1020.00
Hour 6:	Neg.	Pos. Cl	Device 1	Device 2	Device 3	Device 4	Device 5	Device 6
Colony 1, Replicate 1	1082.00	38545.00	15923.00	59298.00	9902.00	103019.00	5722.00	11402.00
Colony 1, Replicate 2	801.00	48468.00	16758.00	66061.00	1619.00	125605.00	6112.00	11911.00
Colony 1, Replicate 3	724.00	48474.00	16604.00	60395.00	1439.00	94196.00	5851.00	10790.00
Colony 1, Replicate 4	906.00	51659.00	17012.00	67682.00	1213.00	123862.00	6376.00	12983.00
Colony 2, Replicate 1	795.00	41178.00	10357.00	69304.00	1923.00	64063.00	11200.00	14434.00
Colony 2, Replicate 2	779.00	44495.00	9248.00	66054.00	1544.00	52732.00	10642.00	12858.00
Colony 2, Replicate 3	1184.00	43575.00	10287.00	68020.00	1981.00	58935.00	10682.00	13332.00
Colony 2, Replicate 4	839.00	41498.00	9887.00	67237.00	1750.00	58845.00	10275.00	13080.00

Table 1. Net Fluorescein a.u. of 8 devices in 0 hour and 6 hours

Net Abs 600

Hour 0:	Neg. Control	Pos. Control	Device 1	Device 2	Device 3	Device 4	Device 5	Device 6
Colony 1, Replicate 1	0.048	0.039	0.016	0.036	0.028	0.029	0.036	0.040
Colony 1, Replicate 2	0.061	0.042	0.015	0.030	0.040	0.027	0.032	0.037
Colony 1, Replicate 3	0.067	0.030	0.015	0.030	0.036	0.029	0.032	0.039

Colony 1, Replicate 4	0.052	0.035	0.010	0.025	0.032	0.021	0.031	0.032
Colony 2, Replicate 1	0.041	0.033	0.010	0.031	0.045	0.025	0.016	0.034
Colony 2, Replicate 2	0.045	0.027	0.017	0.032	0.037	0.028	0.017	0.034
Colony 2, Replicate 3	0.041	0.039	0.017	0.029	0.039	0.026	0.017	0.036
Colony 2, Replicate 4	0.044	0.033	0.016	0.033	0.039	0.027	0.020	0.035

Hour 6:	Neg. Control	Pos. Control	Device 1	Device 2	Device 3	Device 4	Device 5	Device 6
Colony 1, Replicate 1	0.406	0.351	0.212	0.343	0.381	0.278	0.290	0.327
Colony 1, Replicate 2	0.384	0.365	0.188	0.393	0.357	0.419	0.289	0.316
Colony 1, Replicate 3	0.380	0.389	0.222	0.381	0.353	0.356	0.285	0.333
Colony 1, Replicate 4	0.337	0.367	0.205	0.391	0.279	0.389	0.261	0.324
Colony 2, Replicate 1	0.330	0.334	0.189	0.420	0.378	0.317	0.358	0.350
Colony 2, Replicate 2	0.363	0.364	0.177	0.399	0.380	0.264	0.329	0.280
Colony 2, Replicate 3	0.336	0.328	0.193	0.446	0.434	0.318	0.375	0.342
Colony 2, Replicate 4	0.329	0.315	0.164	0.414	0.372	0.295	0.316	0.317

Table 2. Net Abs 600 of 8 devices in 0 hour and 6 hours

Unit Scaling Factors:

OD600 / Abs600	3.60
uM Fluorescein / a.u.	1.34E-05

Table 3. Unit Scaling factors

Colony 1 CFU						
Device	Device 1	Device2	Device 3	Device4	Device5	Device6
Part Number	Bba_J364000	Bba_J364001	Bba_J364002	Bba_J364007	Bba_J364008	Bba_J364009
8.00E-03	0	1408	1880	908	1260	1168
8.00E-04	0	644	1680	170	416	256
8.00E-05	0	334	327	54	70	167
Colony 2 CFU						
Device	Device 1	Device2	Device 3	Device4	Device5	Device6
Part Number	Bba_J364000	Bba_J364001	Bba_J364002	Bba_J364007	Bba_J364008	Bba_J364009
8.00E-03	0	2376	1104	434	3	744
8.00E-04	0	1648	288	132	2	228
8.00E-05	0	86	180	4	0	104

Figure 5. The number of 6 devices diluted in 8×10^4, 8×10^5 and 8×10^6.

Discussion

Figure 1 demonstrated that under 5uM Fluorescein, the standard curve showed a similar polynomial shape, but the whole curve didn't. Therefore, we speculated that 10uM Fluorescein might beyond the range of the equipment, which meant the concentration of fluorescein was too high to acquire reliable data. According to Figure 2, the log graph of fluorescein standard curve showed a highly similar linear shape.

Particle standard curve (Figure 3 and Figure 4) demonstrated the same problem above (beyond the scale). According to figure 4, particle count under 5×10^7 showed a highly similar linear shape.

Table 1 demonstrated values of Net Fluorescein a.u. From data of 6 hours. We could notice that after 6-hour growth, Device 2 (BBa_J364001) showed a series of high values which were even higher than Positive control. Furthermore, according to Table 2, the Net Abs600 values of them just had little difference, which meant within the range of the errors allowed, the growth of Positive control and Device 2 are similar. Additionally, Device 4 also showed higher value, however, the 0-hour Net fluorescein a.u. values of Device 4 were higher than any other Devices and replicates of colony 1 of Device4 were not very good. Thus, we only compared colony 2 of Device 2 and Device 4, although the initial fluorescein values of device 4 are much higher, they became lower after 6 hours.

Conclusion

Both Device 2 and Device 4 showed higher fluorescence values than other Devices, and Device 2 is the best. The fluorescence result of Device 3 is the lowest, still higher than negative control though.