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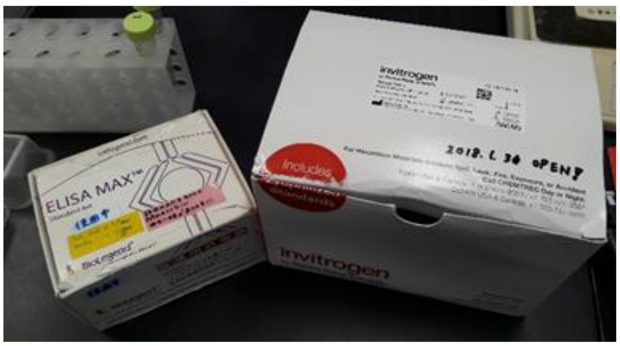
APPENDICES

Appendix 1

Several materials used in this study

1.1. ELISA kits and reagents



Mouse IL-6 ELISA MAX Standard Set (BioLegend) and Mouse TNF-α ELISA Ready-SET-Go! (Invitrogen)

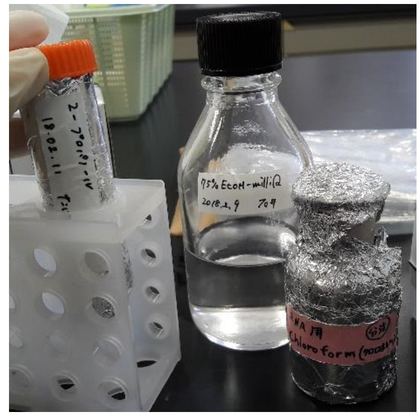
5× ELISA/ELISPOT diluent (TNF-α assay diluent)

Capture antibody for coating step: IL-6 (left), TNF-α (middle), and 10× coating buffer for TNF-α capture

Detection antibody: IL-6 (left) and TNF-α (right)

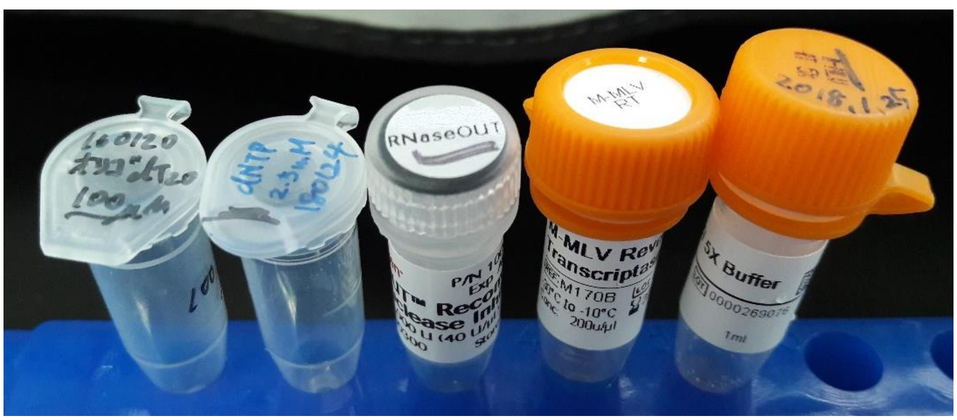
1.3. RNA extraction reagents

Sepasol-RNA 1 Super G (Nacalai Tesque)



2-propanol (left), 75% ethanol (middle), and chloroform (right)

1.4. Reverse transcription reagents



From left to right: Oligo-(dT) 20 primer (Toyobo), 10 mM dNTP, RNase Inhibitor, MMLV-Reverse Transcriptase (Promega), and 5 x Buffer

<u>Appendix 2</u>

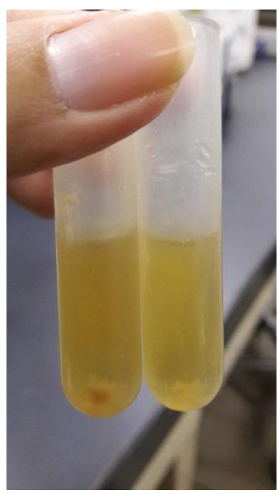
OWE preparation

Oncom (cut into cubes) was freeze-dried

Freeze-dried oncom was ground with food processor

Oncom powder was then suspended in DW to a concentration of 0.1 g/mL

After 24 hours of mixing with rotator, the suspension was centrifuged and supernatant was collected



The supernatant was centrifuged with higher g force to gain a clearer supernatant, OWE was transferred into microtubes

Appendix 3

Phagocytosis activity assay

3.1. Flow cytometry setups

Flow cytometry

Set up

1. Tune on Transformer, FACS, PC and Pressure valve.
2. PC name → Administrator PC pass → BDIS
3. Start up BD Cell Quest pro.

 Acquire → connect to cytometer → Browser : directory (change)

 (Put) commend ⌘+1~4 → Detectors, Threshold, Compensation, Status

 Acquire → Counters

 Graph creation → Dot plot

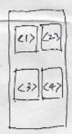
 <1>

 Plot type : Acquisition

 Parameter : X=FSC, Y=SSC

 No Gate

 <2>



 Plot type : Acquisition

 Parameter : X=FSC, Y=SSC

 Gate : G1=R1 → Make gate (hexagon in the center)

 <3>

 Plot type : Acquisition

 Parameter : X=FL2·H, Y=FL1·H

 No Gate

 Graph creation → Histogram

 <4>

 Plot type : Acquisition

 Parameter : X=FL2·H

 Gate : G1=R1

 Condition setting → Detectors

 SSC : change voltage → 490

 FL : change voltage and mode→log — FL 1 : 480 (log) / FL 2 : 480 (log)

4. Washing

 Slide support arm and remove the tube → Push HI+PRIME

 Once again Push PRIME

 Insert the tube containing D.W. → Push LO

Measurement

Push RUN

Acuire → acquisition & storage

Number of cells ; 10000

Change to the tube containing the control → Push Acquire

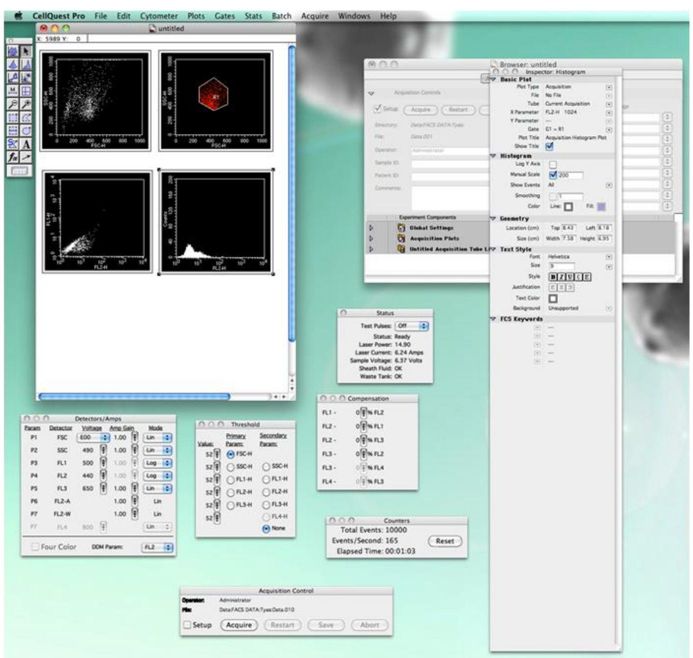
Change Voltage or AmpGain of Detectors

Click Set up → Acquire → Set up voltage FL1 & FL2 until the cell dots within the FL1 & FL2 range.

→ For the next samples, uncheck Setup.

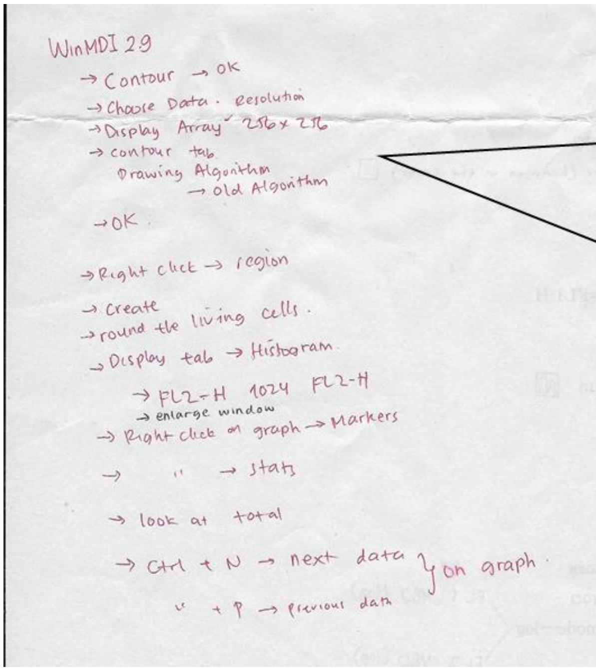
Finish → Washinh

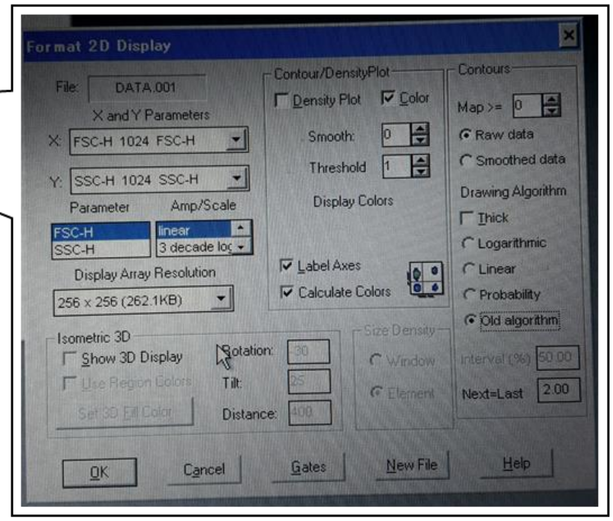
3.2. CellQuest software interface



1. Dot plot graph (Plot type: acquisition, parameters: X=FSC, Y=SSC)
2. Dot plot graph (Plot type: acquisition, parameters: X=FSC, Y=SSC, gate: G1=R1)
3. Dot plot graph (Plot type: acquisition, parameters: X=FL2-H, Y= FL1-H)
4. Histogram graph (Plot type: acquisition, parameters: X=FL2-H, gate: G1=R1)

3.3. WinMDI 2.9 steps to make histograms





Appendix 4
ELISA

4.1. RAW 264.7

4.1.1. Raw data

IL-6

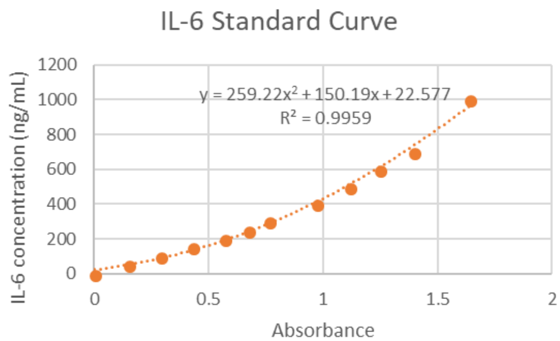
Sample		Absorbance									IL-6 concentration										SD
		1	2	3	4	5	6	7	8	9	1	2	3	4	5	6	7	8	9	Average	
Control		-0.012	-0.013	-0.015	-0.020	-0.020	-0.020	-0.032	-0.036	-0.031	20.812	20.668	20.382	17.326	17.326	17.326	17.319	16.320	17.571	18.339	1.750041
OWE (ng/mL)	24	0.156	0.144	0.083	0.150	0.137	0.080	0.154	0.147	0.095	52.315	49.580	36.829	65.632	60.486	40.762	77.631	74.870	55.564	57.074	14.05894
	98	0.484	0.515	0.527	0.342	0.292	0.305	0.522	0.316	0.404	155.993	168.676	173.720	169.632	137.498	145.511	276.825	152.246	201.396	175.722	42.25809
	391	0.668	0.687	0.675	0.454	0.403	0.403	0.547	0.626	0.501	238.574	248.101	242.062	254.518	213.652	213.652	294.207	352.350	262.604	257.747	43.16907
	1563	0.927	0.909	0.819	0.589	0.558	0.598	0.688	0.631	0.633	384.558	373.288	319.457	380.557	349.321	389.881	401.407	356.195	357.738	368.045	25.0114

TNF-α

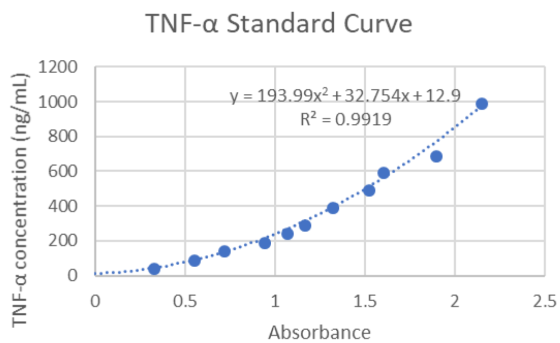
Sample		Absorbance									TNF-α concentration										SD
		1	2	3	4	5	6	7	8	9	1	2	3	4	5	6	7	8	9	Average	
Control		0.154	0.279	0.142	0.067	0.077	0.087	0.118	0.164	0.163	22.545	37.139	21.463	30.048	30.125	30.245	27.168	27.831	27.796	28.262	4.608487
OWE (ng/mL)	24	0.700	0.724	0.528	0.635	0.674	0.657	0.565	0.381	0.538	130.883	138.299	84.275	102.025	112.016	107.582	115.054	56.889	104.593	105.735	24.19711
	98	0.665	0.772	0.569	0.744	0.732	0.607	0.592	0.542	0.518	120.469	153.801	94.343	131.577	128.075	95.252	126.178	106.101	97.271	117.008	20.24854
	391	0.788	0.905	0.913	0.698	0.858	0.769	0.571	0.655	0.696	159.167	201.425	204.508	118.487	167.907	139.069	117.469	154.709	175.215	159.773	31.60206
	1563	0.933	1.063	0.916	1.064	0.913	0.939	0.741	0.734	0.766	212.326	266.920	205.671	247.615	187.417	197.089	199.479	195.584	213.755	213.984	26.32824

4.1.2. Measurement I

IL-6 Conc. (ng/mL)	Absorbance 1	Absorbance 2	Average
0	0.009	-0.009	0
50	0.156	0.142	0.149
100	0.286	0.29	0.288
150	0.441	0.415	0.428
200	0.571	0.56	0.5655
250	0.694	0.652	0.673
300	0.794	0.729	0.7615
400	1.024	0.918	0.971
500	1.146	1.085	1.1155
600	1.263	1.228	1.2455
700	1.398	1.385	1.3915
1000	1.627	1.652	1.6395

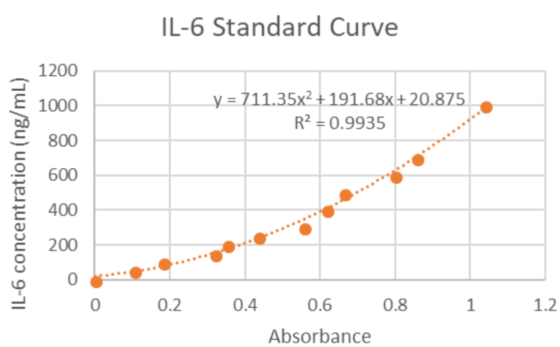


TNF-α Conc. (ng/mL)	Absorbance 1	Absorbance 2	Average
0	0.01	-0.011	-0.0005
50	0.322	0.311	0.3165
100	0.52	0.559	0.5395
150	0.722	0.69	0.706
200	0.956	0.908	0.932
250	1.092	1.03	1.061
300	1.193	1.124	1.1585
400	1.385	1.239	1.312
500	1.568	1.46	1.514
600	1.641	1.545	1.593
700	1.86	1.905	1.8825
1000	2.189	2.086	2.1375

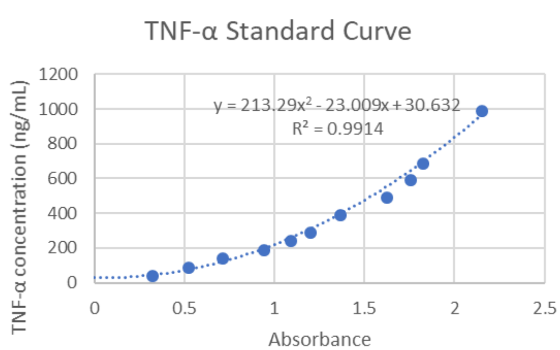


4.1.3. Measurement II

IL-6 Conc. (ng/mL)	Absorbance 1	Absorbance 2	Average
0	0.02	-0.02	0
50	0.105	0.1	0.1025
100	0.172	0.187	0.1795
150	0.284	0.351	0.3175
200	0.361	0.342	0.3515
250	0.443	0.427	0.435
300	0.579	0.531	0.555
400	0.561	0.671	0.616
500	0.681	0.647	0.664
600	0.836	0.761	0.7985
700	0.776	0.936	0.856
1000	1.091	0.982	1.0365

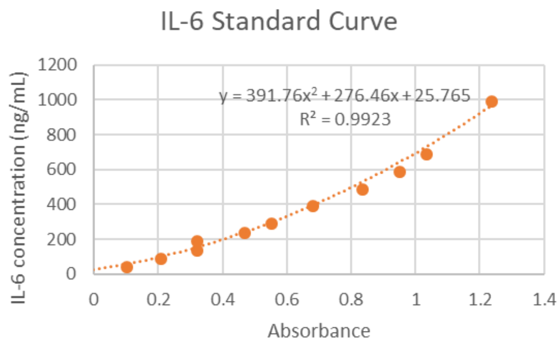


TNF-α Conc. (ng/mL)	Absorbance 1	Absorbance 2	Average
0	0.008	-0.009	-0.0005
50	0.332	0.291	0.3115
100	0.514	0.503	0.5085
150	0.691	0.71	0.7005
200	0.971	0.886	0.9285
250	1.096	1.061	1.0785
300	1.255	1.127	1.191
400	1.38	1.326	1.353
500	1.656	1.568	1.612
600	1.758	1.736	1.747
700	1.906	1.72	1.813
1000	2.189	2.095	2.142

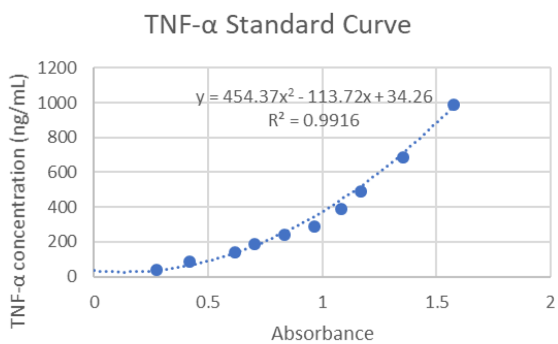


4.1.4 Measurement III

IL-6 Conc. (ng/mL)	Absorbance 1	Absorbance 2	Average
0	0.022	-0.023	-0.0005
50	0.104	0.088	0.096
100	0.216	0.189	0.2025
150	0.339	0.293	0.316
200	0.283	0.345	0.314
250	0.524	0.4	0.462
300	0.586	0.507	0.5465
400	0.688	0.664	0.676
500	0.868	0.792	0.83
600	0.977	0.914	0.9455
700	1.038	1.016	1.027
1000	1.266	1.195	1.2305



TNF-α Conc. (ng/mL)	Absorbance 1	Absorbance 2	Average
0	0.003	-0.004	-0.0005
50	0.281	0.245	0.263
100	0.437	0.38	0.4085
150	0.641	0.575	0.608
200	0.711	0.672	0.6915
250	0.868	0.777	0.8225
300	0.92	0.989	0.9545
400	1.104	1.045	1.0745
500	1.167	1.151	1.159
600	1.172	1.498	1.335
700	1.29	1.4	1.345
1000	1.555	1.576	1.5655



4.1.5. Statistical analysis

ELISA RAW 264.7 IL-6
Anova: Single Factor

SUMMARY

Groups	Count	Sum	Average	Variance
Control	9	165.0515	18.33906	3.062642
24	9	513.6693	57.07437	197.6537
98	9	1581.498	175.722	1785.746
391	9	2319.72	257.7467	1863.569
1563	9	3312.403	368.0448	625.5701

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	743059.6	4	185764.9	207.5307	3.57E-26	2.605975
Within Groups	35804.81	40	895.1202			
Total	778864.4	44				

Tukey Test

Group 1	Group 2	Difference	n1	n2	SE	q
Control	24	38.73531	9	9	9.972853	3.884075
Control	98	157.3829	9	9	9.972853	15.78113
Control	391	239.4076	9	9	9.972853	24.00593
Control	1563	349.7057	9	9	9.972853	35.06576

ELISA RAW 264.7 TNF-α
Anova: Single Factor

SUMMARY

Groups	Count	Sum	Average	Variance
Control	9	254.3577	28.26197	21.23815
24	9	951.6175	105.7353	585.5001
98	9	1053.068	117.0076	410.0036
391	9	1437.958	159.7731	998.69
1563	9	1925.856	213.984	693.1763

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	170285.1	4	42571.27	78.58514	2.12E-18	2.605975
Within Groups	21668.86	40	541.7216			
Total	191953.9	44				

Tukey Test

Group 1	Group 2	Difference	n1	n2	SE	q
Control	24	77.47331	9	9	9.972853	7.768419
Control	98	88.74561	9	9	9.972853	8.898718
Control	391	131.5111	9	9	9.972853	13.18691
Control	1563	185.722	9	9	9.972853	18.62276

The critical value obtained from Tukey's test was compared to the critical value (q) in the table below:

Number of means (k)	5
df error	44

Critical Values of the Studentized Range

Significance level (α)	Critical value (q)
0.05	4.023
0.01	4.906
0.001	6.068

If the critical value obtained from Tukey test is higher compared to critical value of the studentized range, the treatment group was statistically different compared to control group

4.2. P-Mac

4.2.1. Raw data

IL-6

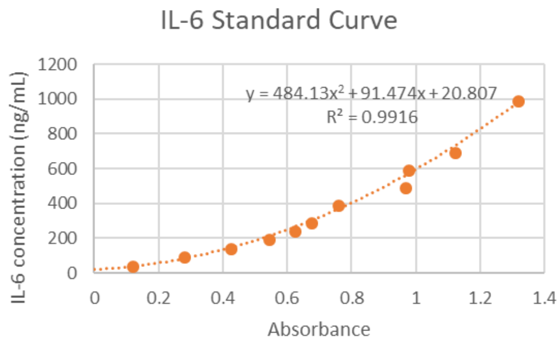
Sample		Absorbance									IL-6 concentration										SD
		1	2	3	4	5	6	7	8	9	1	2	3	4	5	6	7	8	9	Average	
Control		-0.011	-0.002	-0.014	-0.002	0.005	-0.003	0.060	0.009	0.018	19.859	23.332	22.147	23.332	24.070	23.229	32.722	12.289	15.799	21.864	5.717356
OWE (ng/mL)	24	0.044	0.090	0.063	0.081	0.088	0.092	0.074	0.076	0.093	28.819	35.812	31.525	34.325	35.476	36.150	38.561	39.403	46.642	36.301	5.065815
	98	0.114	0.150	0.153	0.157	0.153	0.168	0.086	0.085	0.107	40.057	47.192	47.829	48.687	47.829	51.106	43.644	43.217	52.714	46.919	3.991899
	391	0.099	0.241	0.171	0.150	0.253	0.178	0.096	0.106	0.123	37.355	69.338	51.780	47.192	72.697	53.379	47.935	52.277	59.773	54.636	11.08471
	1563	0.161	0.275	0.427	0.195	0.261	0.381	0.114	0.129	0.227	49.556	79.122	132.916	57.408	74.994	114.903	55.786	62.454	108.806	81.772	29.95214

TNF-α

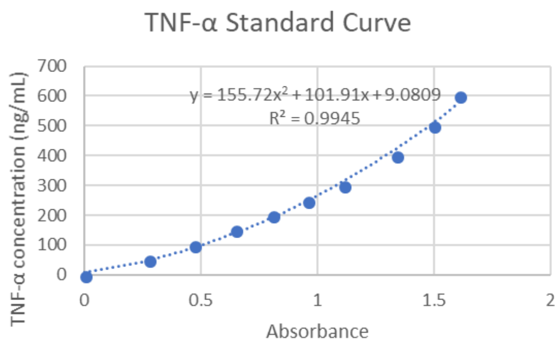
Sample		Absorbance									TNF-α concentration										SD
		1	2	3	4	5	6	7	8	9	1	2	3	4	5	6	7	8	9	Average	
Control		-0.018	-0.018	0.038	-0.018	-0.018	0.038	-0.022	-0.022	0.041	7.297	8.135	7.712	7.297	8.135	7.712	6.914	7.707	8.598	7.723	0.515
OWE (ng/mL)	24	0.109	0.094	0.099	0.141	0.101	0.087	0.153	0.123	0.104	22.039	22.232	27.386	26.546	23.248	23.241	28.318	26.547	29.153	25.412	2.734
	98	0.157	0.149	0.124	0.193	0.188	0.088	0.103	0.099	0.147	28.919	30.649	36.456	34.550	37.214	23.581	21.230	22.956	45.316	31.208	7.947
	391	0.215	0.195	0.135	0.203	0.187	0.126	0.203	0.203	0.138	38.190	38.445	40.631	36.186	37.040	37.206	36.186	39.871	41.790	38.394	1.992
	1563	0.155	0.163	0.164	0.268	0.257	0.141	0.262	0.282	0.187	28.618	32.949	52.183	47.577	50.042	42.957	46.471	55.072	61.903	46.419	10.444

4.2.2. Measurement I

IL-6 Conc. (ng/mL)	Absorbance 1	Absorbance 2	Average
0	0.001	-0.002	-0.0005
50	0.123	0.106	0.1145
100	0.3	0.25	0.275
150	0.415	0.427	0.421
200	0.551	0.524	0.5375
250	0.64	0.598	0.619
300	0.656	0.682	0.669
400	0.754	0.752	0.753
500	0.953	0.972	0.9625
600	0.992	0.951	0.9715
700	1.116	1.121	1.1185
1000	1.34	1.286	1.313

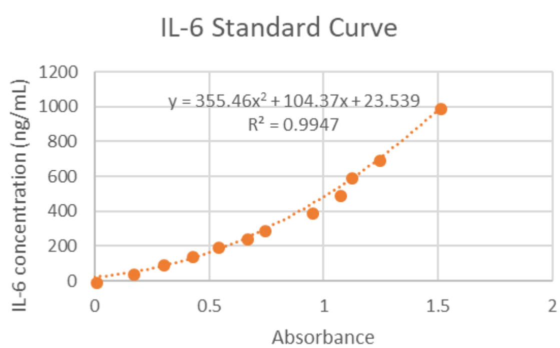


TNF-α Conc. (ng/mL)	Absorbance 1	Absorbance 2	Average
0	0.005	-0.005	0
50	0.281	0.268	0.2745
100	0.474	0.467	0.4705
150	0.589	0.702	0.6455
200	0.829	0.778	0.8035
250	0.949	0.961	0.955
300	1.156	1.064	1.11
400	1.427	1.247	1.337
500	1.495	1.496	1.4955
600	1.671	1.538	1.6045
700	1.765	1.845	1.805
1000	1.939	1.963	1.951

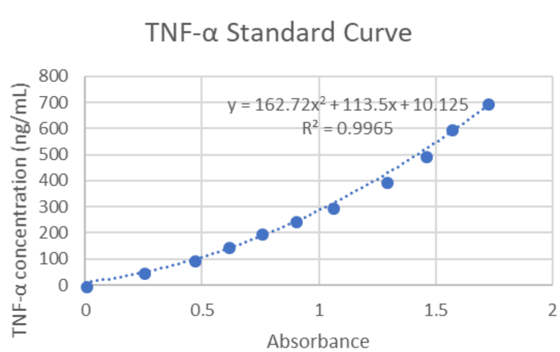


4.2.3. Measurement II

IL-6 Conc. (ng/mL)	Absorbance 1	Absorbance 2	Average
0	0	0	0
50	0.163	0.164	0.1635
100	0.28	0.309	0.2945
150	0.409	0.433	0.421
200	0.505	0.559	0.532
250	0.652	0.668	0.66
300	0.72	0.75	0.735
400	0.905	0.982	0.9435
500	1.057	1.076	1.0665
600	1.141	1.095	1.118
700	1.266	1.207	1.2365
1000	1.474	1.532	1.503

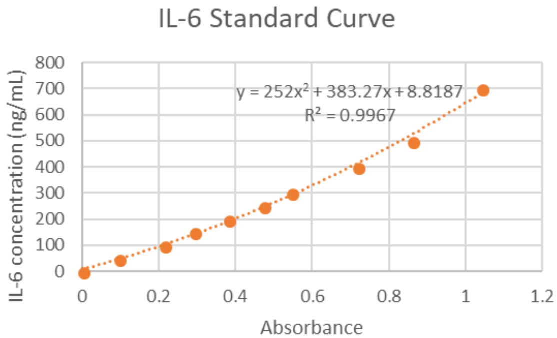


TNF-α Conc. (ng/mL)	Absorbance 1	Absorbance 2	Average
0	0.009	-0.009	0
50	0.232	0.258	0.245
100	0.466	0.457	0.4615
150	0.609	0.606	0.6075
200	0.727	0.775	0.751
250	0.845	0.947	0.896
300	1.101	1.006	1.0535
400	1.395	1.174	1.2845
500	1.448	1.458	1.453
600	1.571	1.559	1.565
700	1.656	1.784	1.72
1000	1.854	1.931	1.8925

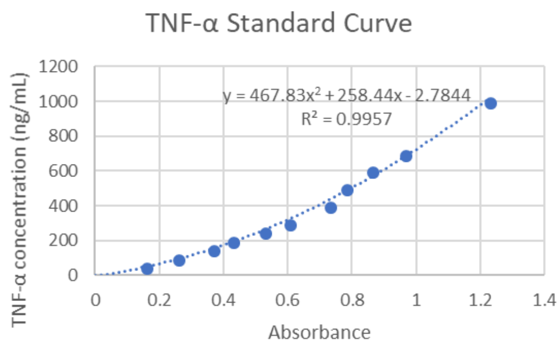


4.2.4. Measurement III

IL-6 Conc. (ng/mL)	Absorbance 1	Absorbance 2	Average
0	-0.006	0.006	0
50	0.089	0.103	0.096
100	0.207	0.221	0.214
150	0.28	0.303	0.2915
200	0.403	0.359	0.381
250	0.56	0.383	0.4715
300	0.575	0.515	0.545
400	0.73	0.704	0.717
500	0.867	0.852	0.8595
600	0.767	0.939	0.853
700	1.003	1.081	1.042
1000	1.168	1.238	1.203



TNF-α Conc. (ng/mL)	Absorbance 1	Absorbance 2	Average
0	0.003	-0.004	-0.0005
50	0.173	0.138	0.1555
100	0.25	0.259	0.2545
150	0.366	0.367	0.3665
200	0.447	0.405	0.426
250	0.539	0.511	0.525
300	0.601	0.607	0.604
400	0.719	0.737	0.728
500	0.788	0.769	0.7785
600	0.879	0.84	0.8595
700	1.004	0.922	0.963
1000	1.223	1.23	1.2265



4.2.5. Statistical analysis

ELISA P-Mac IL-6
Anova: Single Factor

SUMMARY

Groups	Count	Sum	Average	Variance
Control	9	196.7789	21.86432	32.68816
24	9	326.7129	36.30143	25.66248
98	9	422.2735	46.91928	15.93526
391	9	491.7275	54.63639	122.8709
1563	9	735.9456	81.77174	897.1309

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	18047.02	4	4511.754	20.61503	2.76E-09	2.605975
Within Groups	8754.301	40	218.8575			
Total	26801.32	44				

Tukey Test

Group 1	Group 2	Difference	n1	n2	SE	q
Control	24	14.43711254	9	9	4.931278	2.927661
Control	98	25.05495842	9	9	4.931278	5.080824
Control	391	32.77207024	9	9	4.931278	6.645756
Control	1563	59.9074145	9	9	4.931278	12.14846

ELISA P-Mac TNF-α
Anova: Single Factor

SUMMARY

Groups	Count	Sum	Average	Variance
Control	9	69.50619	7.72291	0.265294
24	9	228.7118	25.41242	7.475335
98	9	280.8706	31.20784	63.14763
391	9	345.5439	38.39377	3.969962
1563	9	417.7714	46.41904	109.0798

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	7728.041	4	1932.01	52.51796	2.14E-15	2.605975
Within Groups	1471.504	40	36.78761			
Total	9199.545	44				

Tukey Test

Group 1	Group 2	Difference	n1	n2	SE	q
Control	24	17.68951296	9	9	2.02176	8.749563
Control	98	23.48493332	9	9	2.02176	11.61609
Control	391	30.67085624	9	9	2.02176	15.17038
Control	1563	38.69612978	9	9	2.02176	19.13983

The critical value obtained from Tukey's test was compared to the critical value (q) in the table below:

Number of means (k)	5
df error	44

Critical Values of the Studentized Range

Significance level (α)	Critical value (q)
0.05	4.023
0.01	4.906
0.001	6.068

If the critical value obtained from Tukey test is higher compared to critical value of the studentized range, the treatment group was statistically different compared to control group

Appendix 5

Real-time RT-qPCR

5.1. RAW 264.7

5.1.1. Raw data

Cytokine	Sample		2^—⊿⊿Ct									Average	SD
IL-6	OWE conc. (ng/mL)	Control	1.24	0.92	0.87	1.01	1.30	0.76	0.82	1.40	0.87	1.02	0.232591
		24	6.36	8.40	4.50	19.12	16.19	16.07	6.53	5.57	5.38	9.79	5.667642
		98	33.28	42.13	36.93	64.89	37.79	51.63	41.45	36.34	41.74	42.91	9.754643
		391	81.95	30.63	50.80	80.82	68.44	75.93	66.41	62.39	50.33	63.08	16.74447
		1563	106.64	79.71	116.70	160.53	91.56	111.95	130.99	83.48	73.18	106.08	27.91092
TNF-α	OWE conc. (ng/mL)	Control	1.52	0.49	1.34	1.04	0.82	1.16	0.83	1.12	1.08	1.05	0.304874
		24	5.22	3.59	3.42	2.73	3.12	2.93	3.94	3.43	4.59	3.66	0.805192
		98	5.86	7.16	4.26	2.96	3.69	3.15	3.07	5.09	9.96	5.02	2.336329
		391	20.68	9.00	10.27	2.85	4.32	4.00	9.30	7.19	12.61	8.91	5.466677
		1563	15.35	17.03	11.63	7.73	6.36	5.46	10.17	11.13	9.76	10.51	3.850831

5.1.2. Statistical analysis

RT-PCR RAW 264.7 IL-6
Anova: Single Factor

SUMMARY

Groups	Count	Sum	Average	Variance
Control	9	9.200218	1.022246	0.054099
24	9	88.10843	9.789826	32.12216
98	9	386.1739	42.90821	95.15306
391	9	567.6961	63.07734	280.3772
1563	9	954.7363	106.0818	779.0196

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	65115.48	4	16278.87	68.58731	2.29E-17	2.605975
Within Groups	9493.809	40	237.3452			
Total	74609.29	44				

Tukey Test

Group 1	Group 2	Difference	n1	n2	SE	q
Control	24	8.767579375	9	9	5.135337	1.707303
Control	98	41.88596797	9	9	5.135337	8.15642
Control	391	62.05509562	9	9	5.135337	12.08394
Control	1563	105.0595626	9	9	5.135337	20.45816

RT-PCR RAW 264.7 TNF-α
Anova: Single Factor

SUMMARY

Groups	Count	Sum	Average	Variance
Control	9	9.412067	1.045785	0.092948
24	9	32.97511	3.663902	0.648335
98	9	45.18097	5.020108	5.458434
391	9	80.21175	8.912417	29.88456
1563	9	94.62957	10.5144	14.8289

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	537.1333	4	134.2833	13.18748	6.13E-07	2.605975
Within Groups	407.3054	40	10.18264			
Total	944.4387	44				

Tukey Test

Group 1	Group 2	Difference	n1	n2	SE	q
Control	24	2.618116475	9	9	1.063675	2.461388
Control	98	3.974323045	9	9	1.063675	3.736408
Control	391	7.866631633	9	9	1.063675	7.395712
Control	1563	9.468611906	9	9	1.063675	8.901793

5.2. P-Mac

5.2.1. Raw data

Cytokine	Sample		2^—⊿⊿Ct									Average	SD
IL-6	OWE conc. (ng/mL)	Control	1.52	1.01	0.65	1.34	0.83	0.90	0.54	1.39	1.34	1.06	0.353443
		24	6.45	5.54	6.23	9.87	4.73	1.90	6.70	3.59	4.01	5.45	2.275263
		98	9.96	5.27	8.67	10.27	7.01	4.11	10.48	7.21	8.88	7.99	2.251954
		391	6.25	15.49	7.80	8.32	11.77	16.76	7.52	9.71	12.55	10.68	3.694844
		1563	16.72	19.61	10.80	11.69	16.64	15.53	13.03	18.04	13.67	15.08	2.970825
TNF-α	OWE conc. (ng/mL)	Control	1.02	0.95	1.03	1.02	0.91	1.07	0.59	1.16	1.45	1.02	0.225762
		24	3.04	5.40	2.59	2.94	3.55	3.50	3.07	3.29	3.76	3.46	0.809753
		98	10.34	5.62	4.14	4.33	4.30	7.03	7.82	5.76	2.47	5.76	2.356998
		391	17.11	5.27	4.55	7.31	7.31	9.00	6.01	7.76	16.87	9.02	4.712422
		1563	21.51	12.97	17.59	10.27	19.29	17.75	9.51	26.17	16.56	16.85	5.341395

5.2.2. Statistical analysis

RT-PCR P-Mac IL-6
Anova: Single Factor

SUMMARY IL-6

Groups	Count	Sum	Average	Variance
Control	9	9.517739	1.057527	0.124922
24	9	49.02401	5.447112	5.176822
98	9	71.86568	7.985075	5.071298
391	9	96.16491	10.68499	13.65187
1563	9	135.7239	15.08043	8.825799

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	1008.397	4	252.0992	38.37043	3.43E-13	2.605975
Within Groups	262.8057	40	6.570142			
Total	1271.202	44				

Tukey Test

Group 1	Group 2	Difference	n1	n2	SE	q
Control	24	4.389585	9	9	0.85441	5.137566
Control	98	6.927549	9	9	0.85441	8.107995
Control	391	9.627464	9	9	0.85441	11.26797
Control	1563	14.02291	9	9	0.85441	16.41239

RT-PCR P-Mac TNF-α
Anova: Single Factor

SUMMARY

Groups	Count	Sum	Average	Variance
Control	9	9.21511	1.023901	0.050968
24	9	31.14397	3.460441	0.6557
98	9	51.804	5.756	5.555442
391	9	81.19258	9.021398	22.20692
1563	9	151.626	16.84734	28.5305

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	1355.399	4	338.8497	29.7239	1.67E-11	2.605975
Within Groups	455.9963	40	11.39991			
Total	1811.395	44				

Tukey Test

Group 1	Group 2	Difference	n1	n2	SE	q
Control	24	2.436539	9	9	1.125458	2.164931
Control	98	4.732099	9	9	1.125458	4.204597
Control	391	7.997496	9	9	1.125458	7.105991
Control	1563	15.82344	9	9	1.125458	14.05955

The critical value obtained from Tukey's test was compared to the critical value (q) in the table below:

Number of means (k)	5
df error	44

Critical Values of the Studentized Range

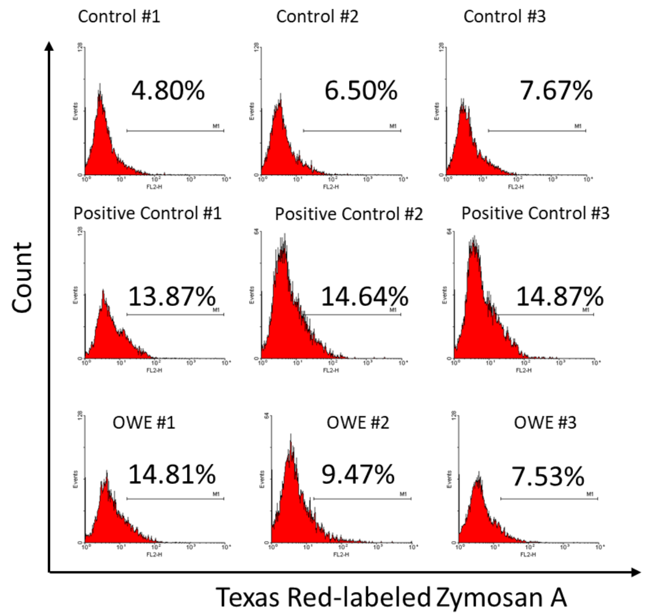
Significance level (α)	Critical value (q)
0.05	4.023
0.01	4.906
0.001	6.068

If the critical value obtained from Tukey test is higher compared to critical value of the studentized range, the treatment group was statistically different compared to control group

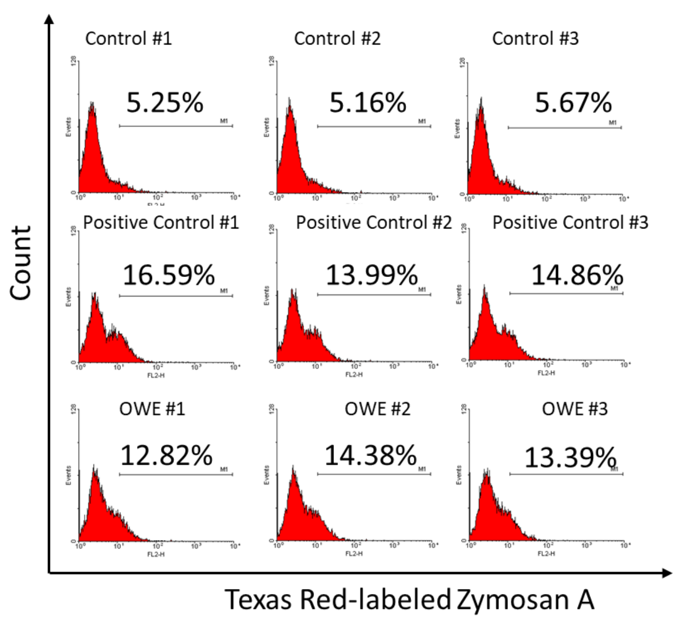
<u>Appendix 6</u>

Phagocytosis activity assay histograms

6.1. Measurement II



6.2. Measurement III



Appendix 7
E-OWE and H-OWE ELISA (RAW 264.7 cells)

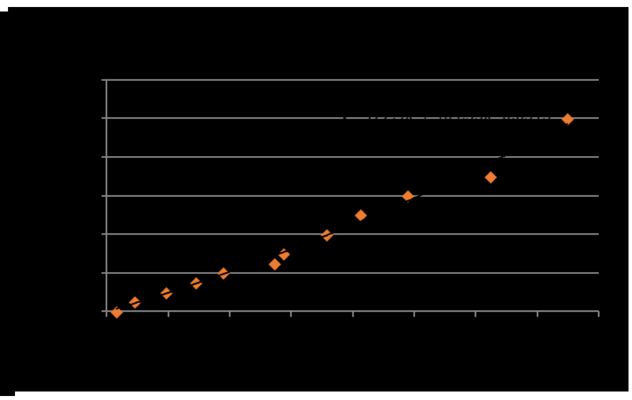
7.1. IL-6

7.1.1. Raw data

[redacted]

7.1.2. Standard curve

[redacted]



7.1.3. Statistical analysis (Two way ANOVA)

Anova: Two-Factor With Replication						
SUMMARY	U	E	Total			
98						
Count	3	3	6			
Sum	598.2003	462.2601	1060.46			
Average	199.4001	154.0867	176.7434			
Variance	451.4059	871.5527	1145.175			
391						
Count	3	3	6			
Sum	845.5053	673.1074	1518.613			
Average	281.8351	224.3691	253.1021			
Variance	1388.338	1529.446	2157.814			
Total						
Count	6	6				
Sum	1443.706	1135.368				
Average	240.6176	189.2279				
Variance	2774.555	2442.286				
ANOVA						
ce of Varia	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	17491.96	1	17491.96	16.49896	0.003624	5.317655
Columns	7922.696	1	7922.696	7.472932	0.0257	5.317655
Interactior	110.7629	1	110.7629	0.104475	0.754811	5.317655
Within	8481.486	8	1060.186			
Total	34006.9	11				

Anova: Two-Factor With Replication						
SUMMARY	U	H	Total			
98						
Count	3	3	6			
Sum	598.2029	344.3439	942.5468			
Average	199.401	114.7813	157.0911			
Variance	451.4157	859.6312	2672.566			
391						
Count	3	3	6			
Sum	845.5053	587.9794	1433.485			
Average	281.8351	195.9931	238.9141			
Variance	1388.338	354.599	2907.826			
Total						
Count	6	6				
Sum	1443.708	932.3233				
Average	240.618	155.3872				
Variance	2774.516	2464.302				
ANOVA						
ce of Varia	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	20085	1	20085	26.30663	0.000897	5.317655
Columns	21792.87	1	21792.87	28.54354	0.000692	5.317655
Interactior	1.12042	1	1.12042	0.001467	0.970381	5.317655
Within	6107.968	8	763.496			
Total	47986.97	11				

Tukey						
G1	G2	Diff	n1	n2	SE	q
U98	E98	45.31341	3	3	18.79881	2.410441
U391	E391	57.46594	3	3	18.79881	3.056892

G1	G2	Diff	n1	n2	SE	q
U98	H98	84.61969	3	3	15.95301	5.304307
U391	H391	85.84194	3	3	15.95301	5.380923

Note:

U98, U391 = untreated OWE or OWE 98 µg/mL, 391 µg/mL

E98, E391= enzyme-treated OWE 98 µg/mL, 391 µg/mL

H98, H391=heat-treated OWE 98 µg/mL, 391 µg/mL

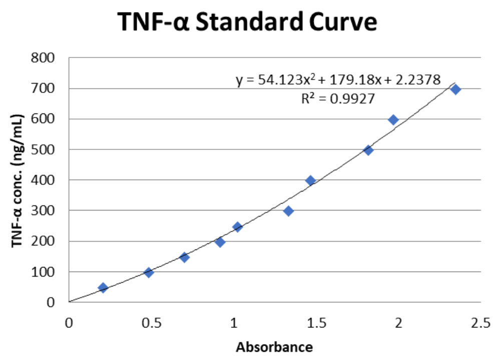
7.2. TNF-α

7.2.1. Raw data

Samples		Absorbance			TNF-α conc. (ng/mL)				SD
		#1	#2	#3	#1	#2	#3	Average	
Control		0.233	0.191	0.133	46.93	38.44	27.03	37.46	9.99
U-OWE	24	0.497	0.587	0.471	104.66	126.07	98.64	109.79	14.41
	98	0.786	0.940	0.885	176.51	218.49	203.20	199.40	21.25
	391	1.027	1.165	1.272	243.34	284.44	317.72	281.84	37.26
E-OWE	24	0.352	0.474	0.440	72.02	99.33	91.56	87.63	14.07
	98	0.564	0.784	0.745	120.51	175.98	165.77	154.09	29.52
	391	0.797	1.052	1.026	179.42	250.63	243.05	224.37	39.11
H-OWE	24	0.194	0.332	0.455	39.04	67.69	94.97	67.23	27.97
	98	0.404	0.559	0.650	83.46	119.31	141.57	114.78	29.32
	391	0.778	0.890	0.906	174.40	204.58	209.00	195.99	18.83

7.2.2. Standard curve

TNF-α conc. (ng/mL)	Absorbance		Average
0	0	-0.001	-0.0005
50	0.19	0.213	0.2015
100	0.464	0.49	0.477
150	0.682	0.711	0.6965
200	0.922	0.902	0.912
250	1.025	1.011	1.018
300	0.89	0.65	0.77
400	1.365	1.294	1.3295
500	1.456	1.465	1.4605
600	1.811	1.815	1.813
700	1.981	1.945	1.963
1000	2.389	2.299	2.344



7.2.3. Statistical analysis

ELISA TNF-α RAW 264.7 (Enzyme- & Heat-treated)
Anova: Single
Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Control	3	112.3868	37.46226	99.70217
U-OWE 24	3	329.363	109.7877	207.7904
U-OWE 98	3	598.2029	199.401	451.4157
U-OWE 391	3	845.5053	281.8351	1388.338
E-OWE 24	3	262.8997	87.63323	198.0507
E-OWE 98	3	462.2601	154.0867	871.5527
E-OWE 391	3	673.1074	224.3691	1529.446
H-OWE 24	3	201.6964	67.23214	782.3061
H-OWE 98	3	344.3439	114.7813	859.6312
H-OWE 391	3	587.9794	195.9931	354.599

Tukey Test

Group 1	*Group 2*	*Difference*	*n1*	*n2*	*SE*	*q*
Control	U-OWE 24	72.3254	3	3	14.99203	4.824255
Control	U-OWE 98	161.9387	3	3	14.99203	10.80165
Control	U-OWE 391	244.3728	3	3	14.99203	16.30018
Control	E-OWE 24	50.17097	3	3	14.99203	3.346508
Control	E-OWE 98	116.6244	3	3	14.99203	7.779094
Control	E-OWE 391	186.9069	3	3	14.99203	12.46708
Control	H-OWE 24	29.76988	3	3	14.99203	1.985713
Control	H-OWE 98	77.31902	3	3	14.99203	5.157341
Control	H-OWE 391	158.5309	3	3	14.99203	10.57434

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	161012.6	9	17890.29	26.53231	3.22E-09	2.392814
Within Groups	13485.66	20	674.2832			
Total	174498.3	29				

The critical value obtained from Tukey's test was compared to the critical value (q) in the table below:

Number of means (k)	10
df error	29

Critical Values of the Studentized Range

Significance level (α)	Critical value (q)
0.05	4.838
0.01	5.782
0.001	7.086

If the critical value obtained from Tukey test is higher compared to critical value of the studentized range, the treatment group was statistically different compared to control group