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Lampiran 1. Keterangan Lolos Kaji Etik

UNIVERSITAS AIRLANGGA FACULTY OF DENTAL MEDICINE
HEALTH RESEARCH ETHICAL CLEARANCE COMMISSION

ETHICAL CLEARANCE CERTIFICATE
Number : 320/HRECC.FODM/VII/2020

Universitas Airlangga Faculty Of Dental Medicine Health Research Ethical Clearance Commission has studied the proposed research design carefully, Declared to be ethically appropriate in accordance to 7 (seven) WHO 2011, and therefore, shall herewith certify that the research entitled :

"The Role of Interleukin 1β (IL-1 β) and Interleukin 6 (IL-6) on Diabetes Mellitus and its correlation with circadian rhythm in mice (Mus Musculus)"

Principal Researcher : ALYA SAHILLA FAHRI

Unit/Institution/Place of Research :
- *Research Center, Faculty of Dental Medicine, Universitas Airlangga, Surabaya*
- *Stem Cell Research and Development Center, Universitas Airlangga, Surabaya*

CERTIFIED TO BE ETHICALLY CLEARED

Surabaya, July 10, 2020
Chairman,

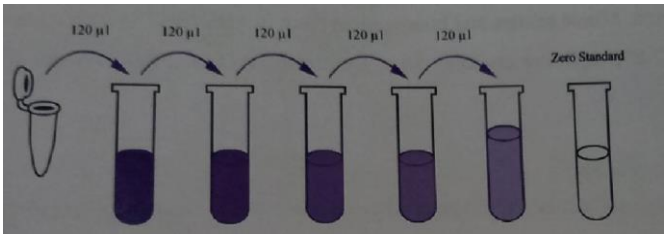
Prof. Dr. M. Rubianto, drg.,MS.,Sp.Perio(K)
Official No.195009081978021001

Lampiran 2. Prosedur Penelitian ELISA sesuai aturan pabrik

Persiapan Reagen Interleukin 1 β

4. Semua reagen harus disimpan dalam suhu kamar sebelum digunakan.
5. **Standar,** Original standar yang digunakan adalah 120 µl (9600pg / ml). Larutan yang tersisa harus dibekukan pada suhu -20 ºC dan digunakan dalam satu bulan. Pengenceran larutan standar yang disarankan adalah sebagai berikut:

4800 pg/ml	Standard No. 5	120 µl Original standard + 120 µl Standard diluent
2400 pg/ml	Standard No. 4	120 µl Standard No. 5 + 120 µl Standard diluent
1200 pg/ml	Standard No. 3	120 µl Standard No. 4 + 120 µl Standard diluent
600 pg/ml	Standard No. 2	120 µl Standard No. 3 + 120 µl Standard diluent
300 pg/ml	Standard No. 1	120 µl Standard No. 2 + 120 µl Standard diluent



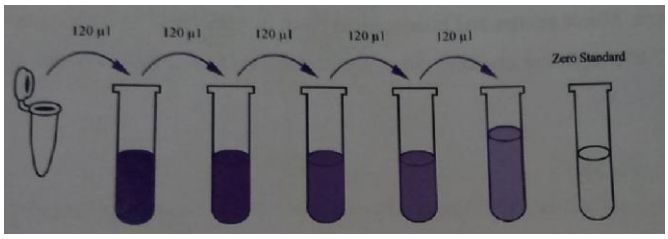
S. Concentration	S. 5	S. 4	S. 3	S. 2	S. 1
9600 pg/ml	4800 pg/ml	2400 pg/ml	1200 pg/ml	600 pg/ml	300 pg/ml

6. **Wash Buffer**, encerkan 200 ml konsentrat Wash Buffer (25x) dengan air suling untuk menghasilkan 500 ml buffer pencuci 1x. Jika kristal sudah terbentuk di dalam konsentrat, aduk perlahan sampai kristal benar-benar larut.

Persiapan Reagen Interleukin 6

1. Semua reagen harus disimpan dalam suhu kamar sebelum digunakan.
2. **Standar,** Original standar yang digunakan adalah 120 µl (48 ng/L). Larutan yang tersisa harus dibekukan pada suhu -20 ^{o}C dan digunakan dalam satu bulan. Pengenceran larutan standar yang disarankan adalah sebagai berikut:

24 ng/L	Standard No. 5	120 µl Original standard + 120 µl Standard diluent
12 ng/L	Standard No. 4	120 µl Standard No. 5 + 120 µl Standard diluent
6 ng/L	Standard No. 3	120 µl Standard No. 4 + 120 µl Standard diluent
3 ng/L	Standard No. 2	120 µl Standard No. 3 + 120 µl Standard diluent
1,5 ng/L	Standard No. 1	120 µl Standard No. 2 + 120 µl Standard diluent



S. Concentration	S. 5	S. 4	S. 3	S. 2	S. 1
48 ng/L	24 ng/L	12 ng/L	6 ng/L	3 ng/L	1,5 ng/L

3. **Wash Buffer**, encerkan 200 ml konsentrat Wash Buffer (25x) dengan air suling untuk menghasilkan 500 ml buffer pencuci 1x. Jika kristal sudah terbentuk di dalam konsentrat, aduk perlahan sampai kristal benar-benar larut.

Prosedur Assay

1. Siapkan semua reagen, larutan standar, dan sampel seperti yang diinstruksikan. Bawa semua reagen ke suhu kamar sebelum digunakan. Pengujian dilakukan pada suhu kamar.
2. Tentukan jumlah strip yang diperlukan untuk pengujian. Sisipkan strip di bingkai untuk digunakan. Strip yang tidak digunakan harus disimpan pada 2-8 ^{o}C.
3. Tambahkan standar 50 µl ke well standar. Catatan: Jangan tambahkan antibodi ke well standar karena larutan standar mengandung antibodi terbiotinilasi.
4. Tambahkan 40 µl sampel ke well sampel lalu tambahkan 10 µl antibodi anti-IL-1β ke well sampel, lalu tambahkan 50 µl streptavidin-HRP ke well sampel dan well standar (bukan well kontrol kosong). Campuran cairan di dalam well. Tutupi pelat dengan sealer. Inkubasi 60 menit pada 37 ^{o}C.
5. Lepas penutup dan cuci plate sebanyak 5 kali dengan wash buffer. Rendam wadah dalam wash buffer selama 30 detik sampai 1 menit untuk setiap pencucian. Untuk pencucian otomatis, dilakukan di semua well dan cuci 5 kali dengan wash buffer. Tepuk microplate ke atas handuk kertas atau bahan penyerap lainnya.
6. Tambahkan larutan media A 50 µl ke masing-masing wadah lalu tambahkan larutan substrat B 50 µl ke setiap wadah. Pelat inkubasi ditutup dengan sealer baru selama 10 menit pada suhu 37 ^{o}C dalam gelap
7. Tambahkan 50 µl stop solution ke tiap well, warna biru akan langsung berubah menjadi kuning.

8. Tentukan densitas optik (nilai OD) setiap well segera menggunakan *microplate reader* yang disetel ke 450 nm dalam 10 menit setelah menambahkan stop solution

Lampiran 3. Dokumentasi Penelitian

Lampu neon di ruang terang 24 jam

Lampu neon di ruang normal

Pemasangan karton

Pemasangan Lampu neon di ruang normal

Lux meter

Pemberian tanda warna di ekor tikus

Pemberian label pada tiap box tikus

Proses pembuatan minum

Melarutkan fruktosa 10-15 % dengan air

Minum tikus yang telah dilarutkan dengan fruktosa

Pembuatan pelet pakan modifikasi yang ditambah dengan bubuk glukosa

Penimbangan berat badan tikus

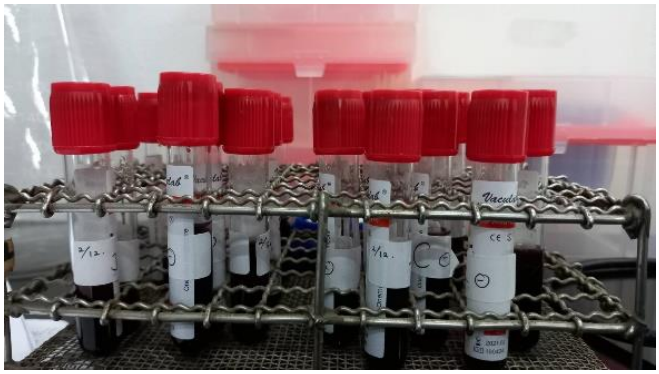
Membuat sayatan di ekor

Meneteskan darah ke strip yang telah terpasang di glukometer

Pengambilan Ketamin

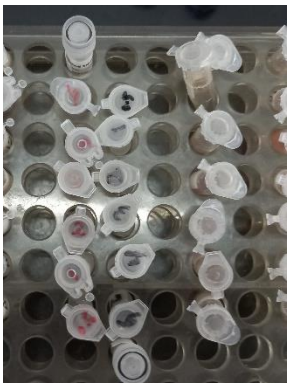
Pengambilan Xylacin

Anastesi pada tikus



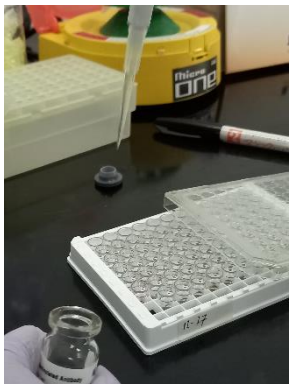
Darah ditampung di vacutainer

Sampel darah disentrifugasi



Serum hasil sentrifugasi

Memasukkan streptavidin-HRP ke well sampel

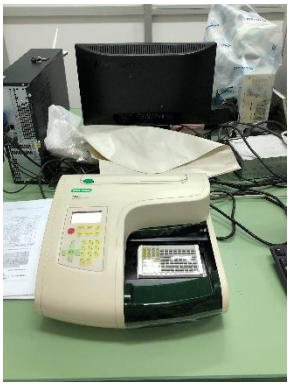


Memasukkan standar diluent ke well standar

Proses mencampurkan serum, antibody, dan HRP

Proses inkubasi elisa

Proses buffer washing

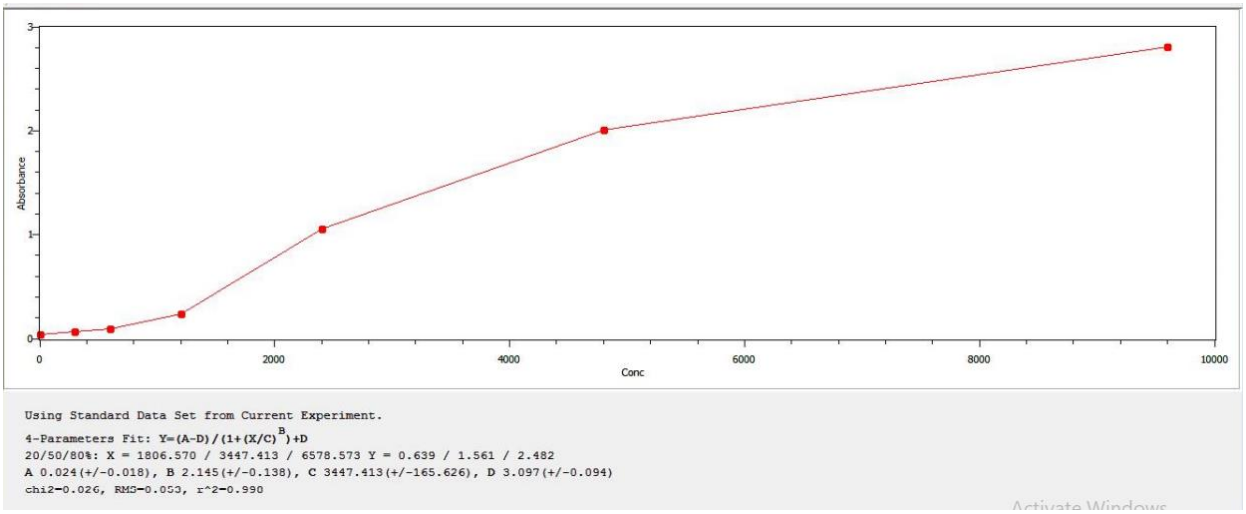


Pembacaan hasil elisa

Lampiran 4. Data Hasil Pengukuran ELISA Interleukin1β

Tabel Hasil Pengamatan Pengukuran Absorbansi untuk Kurva Standard Interleukin1β

Standar	Absorbance/OD
1	0,375
2	0,066
3	0,0905
4	0,485
5	1,0535
6	2,007
7	2,804

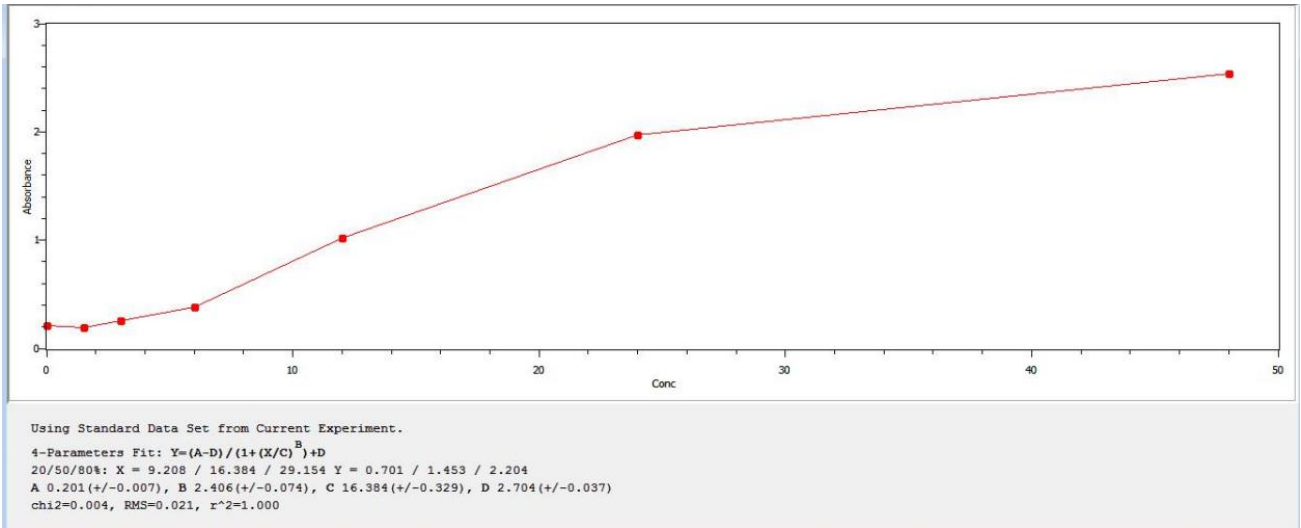


Gambar kurva standar Interleukin 1 β

Lampiran 5. Data Hasil Pengukuran ELISA Interleukin 6

Tabel Hasil Pengamatan Pengukuran Absorbansi untuk Kurva Standard Interleukin 6

Standar	Absorbance/OD
1	0,213
2	0,194
3	0,260
4	0,381
5	1,019
6	1,971
7	2,537



Gambar kurva standar Interleukin 6

Lampiran 6. Tabel Hasil SPSS

Hasil Uji Beda Repeated Measure Anova Kelompok Normal

Pairwise Comparisons

Measure: MEASURE_1

(I) time	(J) time	Mean Difference (I-J)	Std. Error	Sig.[a]	95% Confidence Interval for Difference[a]	
					Lower Bound	Upper Bound
1	2	-18.500	7.593	.589	-54.743	17.743
	3	-8.333	3.955	.890	-27.213	10.547
	4	-14.000	4.274	.221	-34.401	6.401
	5	-11.833	3.816	.268	-30.048	6.381
2	1	18.500	7.593	.589	-17.743	54.743
	3	10.167	6.988	1.000	-23.188	43.521
	4	4.500	6.869	1.000	-28.288	37.288
	5	6.667	8.628	1.000	-34.518	47.852
3	1	8.333	3.955	.890	-10.547	27.213
	2	-10.167	6.988	1.000	-43.521	23.188
	4	-5.667	3.748	1.000	-23.555	12.222
	5	-3.500	4.493	1.000	-24.945	17.945
4	1	14.000	4.274	.221	-6.401	34.401
	2	-4.500	6.869	1.000	-37.288	28.288
	3	5.667	3.748	1.000	-12.222	23.555
	5	2.167	4.206	1.000	-17.912	22.246
5	1	11.833	3.816	.268	-6.381	30.048
	2	-6.667	8.628	1.000	-47.852	34.518
	3	3.500	4.493	1.000	-17.945	24.945
	4	-2.167	4.206	1.000	-22.246	17.912

Based on estimated marginal means

a. Adjustment for multiple comparisons: Bonferroni.

Hasil Uji Beda Repeated Measure Anova Kelompok Gelap

Pairwise Comparisons

Measure: GD

(I) time	(J) time	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
1	2	-4.000	4.297	1.000	-24.512	16.512
	3	2.667	4.216	1.000	-17.460	22.793
	4	-16.000*	3.162	.039	-31.095	-.905
	5	-49.667*	5.207	.002	-74.521	-24.813
2	1	4.000	4.297	1.000	-16.512	24.512
	3	6.667	1.585	.084	-.897	14.231
	4	-12.000	5.007	.619	-35.899	11.899
	5	-45.667*	3.232	.000	-61.093	-30.240
3	1	-2.667	4.216	1.000	-22.793	17.460
	2	-6.667	1.585	.084	-14.231	.897
	4	-18.667	5.426	.184	-44.568	7.235
	5	-52.333*	3.730	.000	-70.137	-34.530
4	1	16.000*	3.162	.039	.905	31.095
	2	12.000	5.007	.619	-11.899	35.899
	3	18.667	5.426	.184	-7.235	44.568
	5	-33.667*	4.349	.006	-54.424	-12.909
5	1	49.667*	5.207	.002	24.813	74.521
	2	45.667*	3.232	.000	30.240	61.093
	3	52.333*	3.730	.000	34.530	70.137
	4	33.667*	4.349	.006	12.909	54.424

Based on estimated marginal means

*. The mean difference is significant at the ,05 level.

b. Adjustment for multiple comparisons: Bonferroni.

Hasil Uji Beda Repeated Measure Anova Kelompok Terang

Pairwise Comparisons

Measure: GD

(I) time	(J) time	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
1	2	-4.333	3.565	1.000	-21.352	12.685
	3	-2.333	2.917	1.000	-16.259	11.592
	4	-6.500	3.263	1.000	-22.077	9.077
	5	-22.667*	1.202	.000	-28.404	-16.930
2	1	4.333	3.565	1.000	-12.685	21.352
	3	2.000	3.759	1.000	-15.945	19.945
	4	-2.167	2.272	1.000	-13.011	8.677
	5	-18.333	3.972	.058	-37.294	.627
3	1	2.333	2.917	1.000	-11.592	16.259
	2	-2.000	3.759	1.000	-19.945	15.945
	4	-4.167	2.414	1.000	-15.690	7.357
	5	-20.333*	2.951	.010	-34.422	-6.245
4	1	6.500	3.263	1.000	-9.077	22.077
	2	2.167	2.272	1.000	-8.677	13.011
	3	4.167	2.414	1.000	-7.357	15.690
	5	-16.167*	3.135	.036	-31.131	-1.203
5	1	22.667*	1.202	.000	16.930	28.404
	2	18.333	3.972	.058	-.627	37.294
	3	20.333*	2.951	.010	6.245	34.422
	4	16.167*	3.135	.036	1.203	31.131

Based on estimated marginal means

*. The mean difference is significant at the ,05 level.

b. Adjustment for multiple comparisons: Bonferroni.

Hasil Uji Beda One-way Anova Kadar Glukosa Darah

ANOVA

GD

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4550.111	2	2275.056	55.913	.000
Within Groups	610.333	15	40.689		
Total	5160.444	17			

Multiple Comparisons

Dependent Variable: GD

Tukey HSD

(I) Kelompok	(J) Kelompok	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Normal	Gelap	-38.500*	3.683	.000	-48.07	-28.93
	Terang	-14.167*	3.683	.004	-23.73	-4.60
Gelap	Normal	38.500*	3.683	.000	28.93	48.07
	Terang	24.333*	3.683	.000	14.77	33.90
Terang	Normal	14.167*	3.683	.004	4.60	23.73
	Gelap	-24.333*	3.683	.000	-33.90	-14.77

*. The mean difference is significant at the 0.05 level.

Hasil Uji Korelasi Pearson Interleukin 1β dan kadar glukosa darah

Correlations

		GD	IL-1B
GD	Pearson Correlation	1	.599**
	Sig. (2-tailed)		.009
	N	18	18
IL-1B	Pearson Correlation	.599**	1
	Sig. (2-tailed)	.009	
	N	18	18

**. Correlation is significant at the 0.01 level (2-tailed).

Hasil Uji Korelasi Pearson Interleukin 6 dan kadar glukosa darah

Correlations

		GD	IL-6
GD	Pearson Correlation	1	.652**
	Sig. (2-tailed)		.003
	N	18	18
IL-6	Pearson Correlation	.652**	1
	Sig. (2-tailed)	.003	
	N	18	18

**. Correlation is significant at the 0.01 level (2-tailed).