LAMPIRAN A

HARGA NORMAL PARAMETER PATOLOGI KLINIK PADA HEWAN COBA TIKUS

Parameter Patologi Klinik	Satuan	Jantan	Betina
Hemoglobin	g/dl	13,0 – 17,0	11,0 – 17,0
Hitung Jumlah Platelet	x 10^3 / µl	700 - 1500	700 – 1500
Hitung Jumlah Leukosit	x 10^3 / µl	3,0 – 14,5	2,0 – 11,5
Hitung Jenis Leukosit			
Neutrofil Segmen	x 10^3 / µl	0,3 – 3,0	0,1 – 2,0
Neutrofil band	x 10^3 / µl	0,0 – 0,0	0,0 – 0,0
Limfosit	x 10^3 / µl	3,0 – 12,0	1,0 – 10,0
Monosit	x 10^3 / µl	0,0 – 0,5	0,0 – 0,3
Eosinofil	x 10^3 / µl	0,0 – 0,5	0,0 – 0,3
Basofil	x 10^3 / µl	0,0 – 0,0	0,0 – 0,0
BUN	mg/dl	10 - 16	10 – 19
Kreatinin	mg/dl	0,5 – 0,8	0,5 – 0,8
SGOT	IU/l	60 - 300	80 – 250
SGPT	IU/l	25 - 55	25 - 50

Dikutip dari : Hall, Robert L., 1992, Clinical Pathology of Laboratory Animals, In: **Animal Model in Toxicology**, J.M. Andress (Ed), Marcell Dekker Inc, New York, 791.

LAMPIRAN B

VOLUME MAKSIMUM OBAT YANG DIBERIKAN PADA BINATANG PERCOBAAN

Binatang	Volume maksimum (ml)				
	i.v	i.m	i.p	Subkutan	p.o
Mencit (20–30 g)	0,5	0,05	1,0	0,5 – 1,0	1,0
Tikus (200 g)	1,0	0,1	2,0 – 5,0	2,0 – 5,0	5,0
Tupai (50 g)	-	0,1	1,0 – 2,0	2,5	2,5
Marmut (250 g)	-	0,25	2,0 – 5,0	5,0	10,0
Merpati (300 g)	2,0	0,5	2,0	2,0	10,0
Kelinci (2,5 kg)	5,0 – 10,0	0,5	2,0	2,0	10,0
Kucing (3 kg)	5,0 – 10,0	0,5	10,0 – 20,0	5,0 – 10,0	20,0
Anjing (5 kg)	10,0 – 20,0	5,0	20,0 – 50,0	10,0	100,0

Keterangan:
i.v : Intravena
i.m : Intramuskular
i.p : Intraperitoneal
p.o : Per oral

LAMPIRAN C
ANALISIS ONE WAY ANOVA DATA SGPT HARI KE-0

Oneway

Descriptives

SGPT_0

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Min	Max	Between-Component Variance
						Lower Bound	Upper Bound			
kontrol		10	50.00	6.464	2.044	45.38	54.62	40	59	
pembanding		10	54.80	6.356	2.010	50.25	59.35	43	64	
zat uji		10	50.70	7.009	2.216	45.69	55.71	42	60	
Total		30	51.83	6.737	1.230	49.32	54.35	40	64	
Model	Fixed Effects			6.616	1.208	49.36	54.31			
	Random Effects				1.497	45.39	58.27			2.347

Test of Homogeneity of Variances

SGPT_0

Levene Statistic	df1	df2	Sig.
.416	2	27	.664

ANOVA

SGPT_0

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	134.467	2	67.233	1.536	.233
Within Groups	1181.700	27	43.767		
Total	1316.167	29			

Robust Tests of Equality of Means

SGPT_0

	Statistic(a)	df1	df2	Sig.
Welch	1.561	2	17.969	.237
Brown-Forsythe	1.536	2	26.795	.234

a Asymptotically F distributed.

Post Hoc Tests

Multiple Comparisons

Dependent Variable: SGPT_0

Tukey HSD

(I) Kelompok	(J) Kelompok	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
kontrol	pembanding	-4.800	2.959	.254	-12.14	2.54
	zat uji	-.700	2.959	.970	-8.04	6.64
pembanding	kontrol	4.800	2.959	.254	-2.54	12.14
	zat uji	4.100	2.959	.362	-3.24	11.44
zat uji	kontrol	.700	2.959	.970	-6.64	8.04
	pembanding	-4.100	2.959	.362	-11.44	3.24

Homogeneous Subsets

SGPT_0

Tukey HSD

Kelompok	N	Subset for alpha = .05
	1	1
kontrol	10	50.00
zat uji	10	50.70
pembanding	10	54.80
Sig.		.254

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 10.000

LAMPIRAN D
ANALISIS ONE WAY ANOVA DATA SGOT HARI KE-0

Oneway

Descriptives

SGOT_0

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Min	Max	Between-Component Variance
						Lower Bound	Upper Bound			
kontrol		10	122.50	13.802	4.365	112.63	132.37	103	143	
pembanding		10	124.80	17.894	5.658	112.00	137.60	100	154	
zat uji		10	119.70	10.904	3.448	111.90	127.50	95	131	
Total		30	122.33	14.138	2.581	117.05	127.61	95	154	
Model	Fixed Effects		14.487	2.645	116.91	127.76				
	Random Effects			2.645	110.95	133.71				-14.463

Test of Homogeneity of Variances

SGOT_0

Levene Statistic	df1	df2	Sig.
1.562	2	27	.228

ANOVA

SGOT_0

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	130.467	2	65.233	.311	.735
Within Groups	5666.200	27	209.859		
Total	5796.667	29			

Robust Tests of Equality of Means

SGOT_0

	Statistic(a)	df1	df2	Sig.
Welch	.320	2	17.346	.730
Brown-Forsythe	.311	2	23.325	.736

a Asymptotically F distributed.

Post Hoc Tests

Multiple Comparisons

Dependent Variable: SGOT_0

Tukey HSD

(I) Kelompok	(J) Kelompok	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
kontrol	pembanding	-2.300	6.479	.933	-18.36	13.76
	zat uji	2.800	6.479	.903	-13.26	18.86
pembanding	kontrol	2.300	6.479	.933	-13.76	18.36
	zat uji	5.100	6.479	.714	-10.96	21.16
zat uji	kontrol	-2.800	6.479	.903	-18.86	13.26
	pembanding	-5.100	6.479	.714	-21.16	10.96

Homogeneous Subsets

SGOT_0

Tukey HSD

	N	Subset for alpha = .05
Kelompok	1	1
kontrol	10	119.70
zat uji	10	122.50
pembanding	10	124.80
Sig.		.714

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 10.000

LAMPIRAN E
ANALISIS ONE WAY ANOVA DATA SGPT HARI KE-1

Oneway

Descriptives

SGPT_1

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Min	Max	Between-Component Variance
						Lower Bound	Upper Bound			
kontrol		10	53.90	6.082	1.923	49.55	58.25	45	62	
pembanding		10	119.40	21.991	6.954	103.67	135.13	77	147	
zat uji		10	53.00	10.760	3.403	45.30	60.70	41	74	
Total		30	75.43	34.605	6.318	62.51	88.35	41	147	
Model	Fixed Effects		14.564	2.659	69.98	80.89				
	Random Effects			21.985	-19.16	170.03				1428.791

Test of Homogeneity of Variances

SGPT_1

Levene Statistic	df1	df2	Sig.
4.162	2	27	.027

ANOVA

SGPT_1

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	29000.067	2	14500.033	68.357	.000
Within Groups	5727.300	27	212.122		
Total	34727.367	29			

Robust Tests of Equality of Means

SGPT_1

	Statistic(a)	df1	df2	Sig.
Welch	40.470	2	15.301	.000
Brown-Forsythe	68.357	2	14.658	.000

a Asymptotically F distributed.

Post Hoc Tests

Multiple Comparisons

Dependent Variable: SGPT_1

Tukey HSD

(I) Kelompok	(J) Kelompok	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
kontrol	pembanding	-65.500(*)	6.513	.000	-81.65	-49.35
	zat uji	.900	6.513	.990	-15.25	17.05
pembanding	kontrol	65.500(*)	6.513	.000	49.35	81.65
	zat uji	66.400(*)	6.513	.000	50.25	82.55
zat uji	kontrol	-.900	6.513	.990	-17.05	15.25
	pembanding	-66.400(*)	6.513	.000	-82.55	-50.25

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

Homogeneous Subsets

SGPT_1

Tukey HSD

	N	Subset for alpha = .05
Kelompok	1	1
kontrol	10	53.00
zat uji	10	53.90
pembanding	10	
Sig.		.990

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 10.000

LAMPIRAN F
ANALISIS ONE WAY ANOVA DATA SGOT HARI KE-1

Oneway

Descriptives

SGOT_1

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Min	Max	Between-Component Variance
						Lower Bound	Upper Bound			
kontrol		10	129.00	17.670	5.588	116.36	141.64	110	166	
pembanding		10	183.90	66.316	20.971	136.46	231.34	110	306	
zat uji		10	143.80	20.917	6.614	128.84	158.76	120	171	
Total		30	152.23	46.411	8.473	134.90	169.56	110	306	
Model	Fixed Effects		41.423	7.563	136.72	167.75				
	Random Effects			16.400	81.67	222.80				635.256

Test of Homogeneity of Variances

SGOT_1

Levene Statistic	df1	df2	Sig.
6.301	2	27	.006

ANOVA

SGOT_1

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	16136.867	2	8068.433	4.702	.018
Within Groups	46328.500	27	1715.870		
Total	62465.367	29			

Robust Tests of Equality of Means

SGOT_1

	Statistic(a)	df1	df2	Sig.
Welch	3.894	2	16.266	.042
Brown-Forsythe	4.702	2	12.149	.031

a Asymptotically F distributed.

Post Hoc Tests

Multiple Comparisons

Dependent Variable: SGOT_1

Tukey HSD

(I) Kelompok	(J) Kelompok	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
kontrol	pembanding	-54.900(*)	18.525	.017	-100.83	-8.97
	zat uji	-14.800	18.525	.707	-60.73	31.13
pembanding	kontrol	54.900(*)	18.525	.017	8.97	100.83
	zat uji	40.100	18.525	.096	-5.83	86.03
zat uji	kontrol	14.800	18.525	.707	-31.13	60.73
	pembanding	-40.100	18.525	.096	-86.03	5.83

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

Homogeneous Subsets

SGOT_1

Tukey HSD

	N	Subset for alpha = .05
Kelompok	1	1
kontrol	10	129.00
zat uji	10	143.80
pembanding	10	
Sig.		.707

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 10.000

LAMPIRAN G
ANALISIS ONE WAY ANOVA DATA SGPT HARI KE-3

Oneway

Descriptives

SGPT_3

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Min	Max	Between-Component Variance
						Lower Bound	Upper Bound			
kontrol		10	63.40	5.337	1.688	59.58	67.22	56	71	
pembanding		10	258.00	65.264	20.638	211.31	304.69	183	359	
zat uji		10	78.20	13.895	4.394	68.26	88.14	58	99	
Total		30	133.20	97.388	17.781	96.83	169.57	56	359	
Model	Fixed Effects		38.648	7.056	118.72	147.68				
	Random Effects			62.546	-135.91	402.31				11586.677

Test of Homogeneity of Variances

SGPT_3

Levene Statistic	df1	df2	Sig.
17.354	2	27	.000

ANOVA

SGPT_3

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	234720.800	2	117360.400	78.574	.000
Within Groups	40328.000	27	1493.630		
Total	275048.800	29			

Robust Tests of Equality of Means

SGPT_3

	Statistic(a)	df1	df2	Sig.
Welch	46.021	2	13.583	.000
Brown-Forsythe	78.574	2	9.940	.000

a Asymptotically F distributed.

Post Hoc Tests

Multiple Comparisons

Dependent Variable: SGPT_3

Tukey HSD

(I) Kelompok	(J) Kelompok	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
kontrol	pembanding	-194.600(*)	17.284	.000	-237.45	-151.75
	zat uji	-14.800	17.284	.672	-57.65	28.05
pembanding	kontrol	194.600(*)	17.284	.000	151.75	237.45
	zat uji	179.800(*)	17.284	.000	136.95	222.65
zat uji	kontrol	14.800	17.284	.672	-28.05	57.65
	pembanding	-179.800(*)	17.284	.000	-222.65	-136.95

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

Homogeneous Subsets

SGPT_3

Tukey HSD

	N	Subset for alpha = .05
Kelompok	1	1
kontrol	10	63.40
zat uji	10	78.20
pembanding	10	
Sig.		.672

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 10.000

LAMPIRAN H
ANALISIS ONE WAY ANOVA DATA SGOT HARI KE-3

Oneway

Descriptives

SGOT_3

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Min	Max	Between-Component Variance
						Lower Bound	Upper Bound			
kontrol		10	130.20	18.085	5.719	117.26	143.14	106	164	
pembanding		10	252.30	83.549	26.421	192.53	312.07	161	377	
zat uji		10	176.60	45.889	14.511	143.77	209.43	111	258	
Total		30	186.37	74.439	13.591	158.57	214.16	106	377	
Model	Fixed Effects		56.016	10.227	165.38	207.35				
	Random Effects			35.584	33.26	339.47				3484.865

Test of Homogeneity of Variances

SGOT_3

Levene Statistic	df1	df2	Sig.
13.718	2	27	.000

ANOVA

SGOT_3

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	75972.867	2	37986.433	12.106	.000
Within Groups	84720.100	27	3137.781		
Total	160692.967	29			

Robust Tests of Equality of Means

SGOT_3

	Statistic(a)	df1	df2	Sig.
Welch	13.046	2	14.034	.001
Brown-Forsythe	12.106	2	14.971	.001

a Asymptotically F distributed.

Post Hoc Tests

Multiple Comparisons

Dependent Variable: SGOT_3

Tukey HSD

(I) Kelompok	(J) Kelompok	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
kontrol	pembanding	-122.100(*)	25.051	.000	-184.21	-59.99
	zat uji	-46.400	25.051	.172	-108.51	15.71
pembanding	kontrol	122.100(*)	25.051	.000	59.99	184.21
	zat uji	75.700(*)	25.051	.015	13.59	137.81
zat uji	kontrol	46.400	25.051	.172	-15.71	108.51
	pembanding	-75.700(*)	25.051	.015	-137.81	-13.59

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

Homogeneous Subsets

SGOT_3

Tukey HSD

	N	Subset for alpha = .05
Kelompok	1	1
kontrol	10	130.20
zat uji	10	176.60
pembanding	10	
Sig.		.172

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 10.000

LAMPIRAN I

ANALISIS ONE WAY ANOVA DATA PERSEN BERAT HATI TERHADAP BERAT BADAN TIKUS

Oneway

Descriptives

Berat_hati

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Min	Max	Between-Component Variance
						Lower Bound	Upper Bound			
Control		10	3.6160	.45668	.14442	3.2893	3.9427	3.09	4.25	
pembanding		10	3.9990	.27642	.08741	3.8013	4.1967	3.69	4.41	
zat uji		10	3.6880	.41247	.13043	3.3929	3.9831	2.97	4.24	
Total		30	3.7677	.41208	.07524	3.6138	3.9215	2.97	4.41	
Model	Fixed Effects		.38949	.07111	3.6218	3.9136				
	Random Effects			.11752	3.2620	4.2733				.02626

Test of Homogeneity of Variances

Berat_hati

Levene Statistic	df1	df2	Sig.
1.897	2	27	.169

ANOVA

Berat_hati

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.829	2	.414	2.731	.083
Within Groups	4.096	27	.152		
Total	4.925	29			

Robust Tests of Equality of Means

Berat_hati

	Statistic(a)	df1	df2	Sig.
Welch	3.401	2	17.078	.057
Brown-Forsythe	2.731	2	23.813	.086

a Asymptotically F distributed.

Post Hoc Tests

Multiple Comparisons

Dependent Variable: Berat_hati

Tukey HSD

(I) Kelompok	(J) Kelompok	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
kontrol	pembanding	-.38300	.17418	.090	-.8149	.0489
	zat uji	-.07200	.17418	.910	-.5039	.3599
pembanding	kontrol	.38300	.17418	.090	-.0489	.8149
	zat uji	.31100	.17418	.193	-.1209	.7429
zat uji	kontrol	.07200	.17418	.910	-.3599	.5039
	pembanding	-.31100	.17418	.193	-.7429	.1209

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

Homogeneous Subsets

Berat_hati

Tukey HSD

	N	Subset for alpha = .05
Kelompok	1	1
kontrol	10	3.6160
zat uji	10	3.6880
pembanding	10	3.9990
Sig.		.090

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 10.000

LAMPIRAN J

ANALISIS KRUSKAL –WALLIS DATA HISTOPATOLOGI HATI

NPar Tests

Descriptive Statistics

	N	Mean	Std. Deviation	Minimum	Maximum
Skor	15	0.9333	1.27988	.00	3.00
Kelompok	15	2.0000	.84515	1.00	3.00

Kruskal-Wallis Test

Ranks

	Kelompok	N	Mean Rank
Skor	Kontrol	5	5.00
	Pembanding	5	13.00
	Zat uji	5	6.00
	Total	15	

Test Statistics(a,b)

	Skor
Chi-Square	12.230
df	2
Asymp. Sig.	.002

a Kruskal Wallis Test

b Grouping Variable: Kelompok

LAMPIRAN K

UJI Z 5 % TERHADAP DATA HISTOPATOLOGI HATI

$$|Ri - Rj| \leq Z \sqrt{\frac{K\,(N\,(N^2-1)) - \sum(t^3-t)}{6\,N\,(N-1)}}$$

Keterangan : Ri : Rata – rata kelompok perlakuan ke- i
Rj : Rata – rata kelompok perlakuan ke- j
Z : Nilai Z tabel
K : Banyaknya perlakuan
N : Jumlah seluruh preparat hati
t : Banyaknya nilai pengamatan yang sama dalam kelompok skor

Penentuan Z_{tabel}

$$Z = \frac{\alpha}{k\,(k-1)}$$

Keterangan : α : Taraf nyata
k : Banyaknya perlakuan

Sehingga diperoleh : $Z = \frac{0{,}05}{3(3-1)} = 0{,}0083$

Maka $Z_{tabel} = 2{,}39$

Penentuan $\sum t$

T_0 : $9^3 - 9 = 720$
T_1 : $1^3 - 1 = 0$
T_2 : $2^3 - 2 = 6$
T_3 : $3^3 - 3 = 24$
$\sum T$ = 690

Uji Z 5%

$$|Ri - Rj| \leq 2{,}39 \sqrt{\frac{3(15(15^2-1)) - 690}{6 \times 15(15-1)}}$$

$$\leq 6{,}52$$

Maka nilai $Z_{hitung} = 6{,}52$

LAMPIRAN L

DATA SGOT DAN SGPT TIKUS PERCOBAAN

LABORATORIUM KLINIK Harapan Mulia

Jl. Raya Siwalankerto No. 180 Telp. (031) 8481711, Fax. (031) 8481711, Surabaya

Kelompok	I		II		III	
	SGOT	SGPT	SGOT	SGPT	SGOT	SGPT
K1	103	56	113	57	124	67
K2	143	48	166	50	160	58
K3	140	58	145	62	125	71
K4	113	46	113	51	120	61
K5	126	40	126	46	125	63
K6	131	43	143	45	164	67
K7	129	59	130	61	131	71
K8	104	46	110	52	106	56
K9	116	51	120	55	119	59
K10	120	53	124	60	128	61
P1	100	54	168	124	317	256
P2	154	52	199	125	377	359
P3	113	48	110	114	162	290
P4	146	60	164	77	260	348
P5	141	54	295	126	315	262
P6	130	57	306	147	356	296
P7	109	54	177	86	215	186
P8	123	64	131	124	182	198
P9	124	62	140	138	161	202
P10	108	43	149	133	178	183
Z1	128	51	171	74	187	89
Z2	123	44	170	52	227	99
Z3	129	57	134	47	111	61
Z4	95	46	120	42	117	58
Z5	114	56	166	41	175	68
Z6	111	60	163	55	258	69
Z7	131	47	134	44	209	92
Z8	127	60	128	66	163	87
Z9	118	42	124	50	159	76
Z10	121	44	128	59	160	83

Surabaya, 31 Maret 2011

Laboratorium Klinik Harapan Mulia

LAMPIRAN M

REAGEN YANG DIGUNAKAN UNTUK PEMERIKSAAN SGPT DALAM SERUM DARAH TIKUS PERCOBAAN

CE

DiaSys Diagnostic Systems International

ALAT (GPT) FS* (IFCC mod.)

with/without pyridoxal-5-phosphate

Diagnostic reagent for quantitative in vitro determination of ALAT (GPT) in serum or plasma on photometric systems

Order Information

Cat. No.	Kit size					
1 2701 99 10 021	R1	5 x	20 mL	+ R2	1 x	25 mL
1 2701 99 10 076	R1	5 x	80 mL	+ R2	1 x	100 mL
1 2701 99 10 023	R1	1 x	800 mL	+ R2	1 x	200 mL
1 2701 99 10 704	R1	8 x	50 mL	+ R2	8 x	12,5 mL
1 2701 99 10 917	R1	8 x	60 mL	+ R2	8 x	15 mL
1 2701 99 10 191	R1	4 x	36 mL	+ R2	4 x	9 mL
1 2701 99 90 314	R1	10 x	20 mL	+ R2	2 x	30 mL
1 2701 99 10 950	4500 Tests on ADVIA 1650/1800					

For determination with pyridoxal-5-phosphate activation additionally required:

2 5010 99 10 030	6 x	3 mL

Summary [1,2]

Alanine Aminotransferase (ALAT/ALT), formerly called Glutamic Pyruvic Transaminase (GPT) and Aspartate Aminotransferase (ASAT/AST), formerly called Glutamic Oxalacetic Transaminase (GOT) are the most important representatives of a group of enzymes, the amino-transferases or transaminases, which catalyze the conversion of α-keto acids into amino acids by transfer of amino groups.

As a liver specific enzyme, ALAT is only significantly elevated in hepatobiliary diseases. Increased ASAT levels, however, can occur in connection with damages of heart or skeletal muscle as well as of liver parenchyma. Parallel measurement of ALAT and ASAT is, therefore, applied to distinguish liver from heart or skeletal muscle damages. The ASAT/ALAT ratio is used for differential diagnosis in liver diseases. While ratios < 1 indicate mild liver damage, ratios >1 are associated with severe, often chronic liver diseases.

Method

Optimized UV-test according to IFCC (International Federation of Clinical Chemistry and Laboratory Medicine)[modified]

Principle

L-Alanine + 2-Oxoglutarate <—ALAT—> L-Glutamate + Pyruvate

Pyruvate + NADH + H^+ <—LDH—> D-Lactate + NAD^+

Addition of pyridoxal-5-phosphate (P-5-P) stabilizes the transaminases and avoids falsely low values in samples containing insufficient endogenous P-5-P, e.g. from patients with myocardial infarction, liver disease and intensive care patients [1].

Reagents

Components and Concentrations

R1:	TRIS	pH 7.15	140 mmol/L
	L-Alanine		700 mmol/L
	LDH (lactate dehydrogenase)		≥ 2300 U/L
R2:	2-Oxoglutarate		85 mmol/L
	NADH		1 mmol/L
Pyridoxal-5-Phosphate FS			
	Good's buffer	pH 9.6	100 mmol/L
	Pyridoxal-5-phosphate		13 mmol/L

Storage Instructions and Reagent Stability

The reagents are stable up to the end of the indicated month of expiry, if stored at 2 - 8 °C, protected from light and contamination is avoided. Do not freeze the reagents!

Warnings and Precautions

1. The reagents contain sodium azide (0.95 g/L) as preservative. Do not swallow! Avoid contact with skin and mucous membranes.
2. Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents.

Waste Management

Please refer to local legal requirements.

Reagent Preparation

Substrate Start

The reagents are ready to use.

For the determination with pyridoxal-5-phosphate (P-5-P) mix 1 part of P-5-P with 100 parts of reagent 1, e.g. 100 µL P-5-P + 10 mL R1

Stability after mixing: 6 days at 2 - 8 °C
24 hours at 15 - 25 °C

Sample Start

(without pyridoxal-5-phosphate)

Mix 4 parts of R1 + 1 part of R2
(e.g. 20 mL R1 + 5 mL R2) = monoreagent

Stability: 4 weeks at 2 - 8° C
5 days at 15 - 25° C

The monoreagent must be protected from light!

Materials required but not provided

DiaSys Pyridoxal-5-Phosphate FS in case of determination with P-5-P activation (Cat. No. 2 5010 99 10 030)
NaCl solution 9 g/L
General laboratory equipment

Specimen

Serum, heparin plasma or EDTA plasma

Stability [4]:

3 days	at	20 - 25 °C
7 days	at	4 - 8 °C
7 days	at	-20 °C

Discard contaminated specimens!

Assay Procedure

Application sheets for automated systems are available on request.

Wavelength	340 nm, Hg 365 nm, Hg 334 nm
Optical path	1 cm
Temperature	37°C
Measurement	Against air

Substrate Start

Sample or calibrator	100 µL
Reagent 1	1000 µL
Mix, incubate for 5 min., then add:	
Reagent 2	250 µL
Mix, read absorbance after 1 min. and start stopwatch. Read absorbance again 1, 2 and 3 min thereafter.	

* fluid stable

Sample Start

Do not use sample start with pyridoxal-5-phosphate!

Sample or calibrator	100 µL
Monoreagent	1000 µL

Mix, read absorbance after 1 min. and start stopwatch.
Read absorbance again 1, 2 and 3 min thereafter.

Calculation

With factor

From absorbance readings calculate ΔA/min and multiply by the corresponding factor from table below:

ΔA/min x factor = ALAT activity [U/L]

	Substrate Start	Sample Start
340 nm	2143	1745
334 nm	2184	1780
365 nm	3971	3235

With calibrator

$$\text{ALAT [U/L]} = \frac{\Delta A/\text{min Sample}}{\Delta A/\text{min Calibrator}} \times \text{Conc. Calibrator [U/L]}$$

Calibrators and Controls

For the calibration of automated photometric systems the DiaSys TruCal U calibrator is recommended. For internal quality control DiaSys TruLab N and P controls should be assayed with each batch of samples.

	Cat. No.	Kit size
TruCal U	5 9100 99 10 063	20 x 3 mL
	5 9100 99 10 064	6 x 3 mL
TruLab N	5 9000 99 10 062	20 x 5 mL
	5 9000 99 10 061	6 x 5 mL
TruLab P	5 9050 99 10 062	20 x 5 mL
	5 9050 99 10 061	6 x 5 mL

Performance Characteristics

Measuring range

On automated systems the test is suitable for the determination of ALAT activities up to 600 U/L.
In case of a manual procedure, the test is suitable for ALAT activities which correspond to a maximum of ΔA/min of 0.16 at 340 and 334 nm or 0.08 at 365 nm.
If such values are exceeded the samples should be diluted 1 + 9 with NaCl solution (9 g/L) and results multiplied by 10.

Specificity/Interferences

No interference was observed by ascorbic acid up to 30 mg/dL, bilirubin up to 40 mg/dL, hemoglobin up to 400 mg/dL and lipemia up to 2,000 mg/dL triglycerides.

Sensitivity/Limit of Detection

The lower limit of detection is 4 U/L.

Precision

Without P-5-P

Intra-assay precision n = 20	Mean [U/L]	SD [U/L]	CV [%]
Sample 1	22.2	1.38	6.22
Sample 2	44.8	1.17	2.62
Sample 3	101	1.02	1.00

Inter-assay precision n = 20	Mean [U/L]	SD [U/L]	CV [%]
Sample 1	22.8	0.70	3.08
Sample 2	42.6	0.68	1.60
Sample 3	99.3	0.92	0.92

With P-5-P

Intra-assay precision n = 20	Mean [U/L]	SD [U/L]	CV [%]
Sample 1	33.8	1.25	3.71
Sample 2	72.0	2.04	2.83
Sample 3	128	2.77	2.16

Inter-assay precision n = 20	Mean [U/L]	SD [U/L]	CV [%]
Sample 1	33.3	0.99	2.96
Sample 2	72.1	1.36	1.88
Sample 3	133	1.76	1.32

Method Comparison

With P-5-P

A comparison of DiaSys ALAT (GPT) FS with P-5-P (y) and the IFCC reference reagent (x) using 51 samples gave following results:
y = 1.000 x – 0.200 U/L; r = 0.999.

A comparison between DiaSys ALAT (GPT) FS with P-5-P (y) and a commercially available test (x) using 51 samples gave following results:
y = 0,970 x + 0.531 U/L; r = 1.000.

Without P-5-P

A comparison of DiaSys ALAT (GPT) FS without P-5-P (y) with a commercially available test (x) using 51 samples gave following results:
y =0.971 x + 0.047 U/L; r =1.000.

Reference Range

With pyridoxal-5-phosphate activation

Women [3]		< 34 U/L
Men [3]		< 45 U/L
Children [1]	1 – 30 day(s)	< 25 U/L
	2 – 12 months	< 25 U/L
	1 – 3 year(s)	< 30 U/L
	4 – 6 years	< 25 U/L
	7 – 9 years	< 25 U/L
	10 – 18 years	< 30 U/L

Without pyridoxal-5-phosphate activation

Women	< 31 U/L
Men	< 41 U/L

Each laboratory should check if the reference ranges are transferable to its own patient population and determine own reference ranges if necessary.

Literature

1. Thomas L. Alanine aminotransferase (ALT), Aspartate aminotransferase (AST). In: Thomas L, editor. Clinical Laboratory Diagnostics. 1st ed. Frankfurt: TH-Books Verlagsgesellschaft; 1998. p. 55-65.
2. Moss DW, Henderson AR. Clinical enzymology. In: Burtis CA, Ashwood ER, editors. Tietz Textbook of Clinical Chemistry. 3rd ed. Philadelphia: W.B Saunders Company; 1999. p. 617-721.
3. Schumann G, Bonora R, Ceriotti F, Férard G et al. IFCC primary reference procedure for the measurement of catalytic activity concentrations of enzymes at 37 °C. Part 5: Reference procedure for the measurement of catalytic concentration of alanine aminotransferase. Clin Chem Lab Med 2002;40:718-24.
4. Guder WG, Zawta B et al. The Quality of Diagnostic Samples. 1st ed. Darmstadt: GIT Verlag; 2001; p. 14-5.

Manufacturer

DiaSys Diagnostic Systems GmbH
Alte Strasse 9 65558 Holzheim Germany

LAMPIRAN N

REAGEN YANG DIGUNAKAN UNTUK PEMERIKSAAN SGOT DALAM SERUM DARAH TIKUS PERCOBAAN

CE

DiaSys Diagnostic Systems International

ASAT (GOT) FS* (IFCC mod.)

with/without pyridoxal-5-phosphate

Diagnostic reagent for quantitative in vitro determination of ASAT(GOT) in serum or plasma on photometric systems

Order Information

Cat. No.		Kit size					
1 2601 99 10 021	R1	5 x	20 mL	+	R2	1 x	25 mL
1 2601 99 10 026	R1	5 x	80 mL	+	R2	1 x	100 mL
1 2601 99 10 022	R1	1 x	800 mL	+	R2	1 x	200 mL
1 2601 99 10 704	R1	8 x	50 mL	+	R2	8 x	12.5 mL
1 2601 99 10 917	R1	8 x	60 mL	+	R2	8 x	15 mL
1 2601 99 10 191	R1	4 x	36 mL	+	R2	4 x	9 mL
1 2601 99 90 314	R1	10 x	20 mL	+	R2	2 x	30 mL
1 2601 99 10 950	4500 Tests on ADVIA 1650/1800						

For determination with pyridoxal-5-phosphate activation additionally required:

2 5010 99 10 030	6 x	3 mL

Summary [1,2]

Alanine Aminotransferase (ALAT/ALT), formerly called Glutamic Pyruvic Transaminase (GPT) and Aspartate Aminotransferase (ASAT/AST), formerly called Glutamic Oxalacetic Transaminase (GOT) are the most important representatives of a group of enzymes, the amino-transferases or transaminases, which catalyze the conversion of α-keto acids into amino acids by transfer of amino groups.

As a liver specific enzyme ALAT is only significantly elevated in hepatobiliary diseases. Increased ASAT levels, however, can occur in connection with damages of heart or skeletal muscle as well as of liver parenchyma. Parallel measurement of ALAT and ASAT is therefore applied to distinguish liver from heart or skeletal muscle damages. The ASAT/ALAT ratio is used for differential diagnosis in liver diseases. While ratios < 1 indicate mild liver damage, ratios >1 are associated with severe, often chronic liver diseases.

Method

Optimized UV-test according to IFCC (International Federation of Clinical Chemistry and Laboratory Medicine) [modified]

Principle

L-Aspartate + 2-Oxoglutarate $\xrightarrow{ASAT}$ L-Glutamate + Oxalacetate

Oxalacetate + NADH + H^+ $\xrightarrow{MDH}$ L-Malate + NAD^+

Addition of pyridoxal-5-phosphate (P-5-P) stabilizes the transaminases and avoids falsely low values in samples containing insufficient endogenous P-5-P, e.g. from patients with myocardial infarction, liver disease and intensive care patients [1].

Reagents

Components and Concentrations

R1:	TRIS	pH 7.65	110 mmol/L
	L-Aspartate		320 mmol/L
	MDH (malate dehydrogenase)		≥ 800 U/L
	LDH (lactate dehydrogenase)		≥ 1200 U/L
R2:	2-Oxoglutarate		65 mmol/L
	NADH		1 mmol/L
Pyridoxal-5-phosphate FS			
	Good's buffer	pH 9.6	100 mmol/L
	Pyridoxal-5-phosphate		13 mmol/L

Storage Instructions and Reagent Stability

The reagents are stable up to the end of the indicated month of expiry, if stored at 2 – 8 °C, protected from light and contamination is avoided. Do not freeze the reagents!

Warnings and Precautions

1. The reagents contain sodium azide (0.95 g/L) as preservative. Do not swallow! Avoid contact with skin and mucous membranes.
2. Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents.

Waste Management

Please refer to local legal requirements.

Reagent Preparation

Substrate Start

The reagents are ready to use.

For the determination with pyridoxal-5-phosphate mix 1 part of P-5-P with 100 parts of reagent 1,
e.g. 100 µL P-5-P + 10 mL R1

Stability after mixing: 6 days at 2 - 8 °C
24 hours at 15 - 25 °C

Sample Start

(without pyridoxal-5-phosphate)

Mix 4 parts of R1 + 1 part of R2
(e.g. 20 mL R1 + 5 mL R2) = monoreagent

Stability: 4 weeks at 2 – 8 °C
5 days at 15 – 25 °C

The monoreagent must be protected from light!

Materials required but not provided

DiaSys Pyridoxal-5-Phosphate FS in case of determination with P-5-P activation (Cat.-no. 2 5010 99 10 030)
NaCl solution 9 g/L
General laboratory equipment

Specimen

Serum, heparin plasma or EDTA plasma

Stability [4]:

4 days	at	20 – 25 °C
7 days	at	4 – 8 °C
3 months	at	-20 °C

Discard contaminated specimens.

Assay Procedure

Application sheets for automated systems are available on request.

Wavelength	340 nm, Hg 365 nm, Hg 334 nm
Optical path	1 cm
Temperature	37 °C
Measurement	Against air

Substrate Start

Sample/Calibrator	100 µL
Reagent 1	1000 µL
Mix, incubate for 5 min., then add:	
Reagent 2	250 µL
Mix, read absorbance after 1 min. and start stopwatch.	
Read absorbance again 1, 2 and 3 min thereafter.	

Sample Start

Don't use sample start with pyridoxal-5-phosphate !

Sample/Calibrator	100 µL
Monoreagent	1000 µL
Mix, read absorbance after 1 min. and start stopwatch.	
Read absorbance again 1, 2 and 3 min thereafter.	

* fluid stable

Calculation

With factor

From absorbance readings calculate ΔA/min and multiply by the corresponding factor from table below:

ΔA/min x factor = ASAT activity [U/L]

Substrate Start	
340 nm	2143
334 nm	2184
365 nm	3971
Sample Start	
340 nm	1745
334 nm	1780
365 nm	3235

With calibrator

$$\text{ASAT [U/L]} = \frac{\Delta A/\text{min Sample}}{\Delta A/\text{min Calibrator}} \times \text{Conc. Calibrator [U/L]}$$

Calibrators and Controls

For the calibration of automated photometric systems the DiaSys TruCal U calibrator is recommended. For internal quality control DiaSys TruLab N and P controls should be assayed with each batch of samples.

	Cat. No.	Kit size
TruCal U	5 9100 99 10 063	20 x 3 mL
	5 9100 99 10 064	6 x 3 mL
TruLab N	5 9000 99 10 062	20 x 5 mL
	5 9000 99 10 061	6 x 5 mL
TruLab P	5 9050 99 10 062	20 x 5 mL
	5 9050 99 10 061	6 x 5 mL

Performance Characteristics

Measuring range

On automated systems the test is suitable for the determination of ASAT activities up to 700 U/L.

In case of a manual procedure, the test is suitable for ASAT activities which correspond to a maximum of ΔA/min of 0.16 at 340 and 334 nm or 0.08 at 365 nm.

If such values are exceeded the samples should be diluted 1 + 9 with NaCl solution (9 g/L) and results multiplied by 10.

Specificity/Interferences

No interference was observed by ascorbic acid up to 30 mg/dL, bilirubin up to 40 mg/dL and lipemia up to 2,000 mg/dL triglycerides. The presence of hemoglobin in serum indicates destruction of erythrocytes with release of ASAT, thus producing high interference.

Sensitivity/Limit of Detection

The lower limit of detection is 2 U/L.

Precision

Without P-5-P

Intra-assay precision n = 20	Mean [U/L]	SD [U/L]	CV [%]
Sample 1	25.1	0.82	3.25
Sample 2	51.3	1.57	3.06
Sample 3	116	0.90	0.77

Inter-assay precision n = 20	Mean [U/L]	SD [U/L]	CV [%]
Sample 1	25.7	1.13	4.40
Sample 2	48.6	0.67	1.38
Sample 3	115	0.80	0.69

With P-5-P

Intra-assay precision n = 20	Mean [U/L]	SD [U/L]	CV [%]
Sample 1	43.6	1.10	2.51
Sample 2	74.5	1.79	2.41
Sample 3	174	3.18	1.83

Inter-assay precision n = 20	Mean [U/L]	SD [U/L]	CV [%]
Sample 1	44.0	1.59	3.61
Sample 2	77.0	3.05	3.97
Sample 3	187	3.37	1.80

Method Comparison

With P-5-P

A comparison of DiaSys ASAT (GOT) FS with P-5-P (y) with the IFCC reference reagent (x) using 51 samples gave following results: y = 1.000 x – 0.200 U/L; r = 1.000.

A comparison of DiaSys ASAT (GOT) FS (y) with P-5-P and a commercially available test (x) using 51 samples gave following results:
y = 0.970 x + 0.350 U/L; r= 0.999.

Without P-5-P

A comparison of DiaSys ASAT (GOT) FS without P-5-P (y) and a commercially available test (x) using 51 samples gave following results:
y = 0.997 x + 0.621 U/L; r= 1.000.

Reference Range

With pyridoxal-5-phosphate activation [3]

Women [3]		< 31 U/L
Men [3]		< 35 U/L
Children [1]	1 – 3 years	< 50 U/L
	4 – 6 years	< 45 U/L
	7 – 9 years	< 40 U/L
	10 – 12 years	< 40 U/L
	13 – 15 years	< 35 U/L
	16 – 18 years	< 35 U/L

Without pyridoxal-5-phosphate activation

Women	< 31 U/L
Men	< 35 U/L

Each laboratory should check if the reference ranges are transferable to its own patient population and determine own reference ranges if necessary.

Literature

1. Thomas L. Alanine aminotransferase (ALT), Aspartate aminotransferase (AST). In: Thomas L, editor. Clinical Laboratory Diagnostics. 1st ed. Frankfurt: TH-Books Verlagsgesellschaft; 1998. p. 55-65.
2. Moss DW, Henderson AR. Clinical enzymology. In: Burtis CA, Ashwood ER, editors. Tietz Textbook of Clinical Chemistry. 3rd ed. Philadelphia: W.B Saunders Company; 1999. p. 617-721.
3. Schumann G, Bonora R, Ceriotti F, Férard G et al. IFCC primary reference procedure for the measurement of catalytic activity concentrations of enzymes at 37 °C. Part 5: Reference procedure for the measurement of catalytic concentration of aspartate aminotransferase. Clin Chem Lab Med 2002;40:725-33.
4. Guder WG, Zawta B et al. The Quality of Diagnostic Samples. 1st ed. Darmstadt: GIT Verlag; 2001; p. 18-9.

Manufacturer

DiaSys Diagnostic Systems GmbH
Alte Strasse 9 65558 Holzheim Germany

LAMPIRAN O

TABEL Z

z	.00	.01	.02	.03	.04	.05	.06	.07	.08	.09
.0	.5000	.4960	.4920	.4880	.4840	.4801	.4761	.4721	.4681	.4641
.1	.4602	.4562	.4522	.4483	.4443	.4404	.4364	.4325	.4286	.4247
.2	.4207	.4168	.4129	.4090	.4052	.4013	.3974	.3936	.3897	.3859
.3	.3821	.3783	.3745	.3707	.3669	.3632	.3594	.3557	.3520	.3483
.4	.3446	.3409	.3372	.3336	.3300	.3264	.3228	.3192	.3156	.3121
.5	.3085	.3050	.3015	.2981	.2946	.2912	.2877	.2843	.2810	.2776
.6	.2743	.2709	.2676	.2643	.2611	.2578	.2546	.2514	.2483	.2451
.7	.2420	.2389	.2358	.2327	.2296	.2266	.2236	.2206	.2177	.2148
.8	.2119	.2090	.2061	.2033	.2005	.1977	.1949	.1922	.1894	.1867
.9	.1841	.1814	.1788	.1762	.1736	.1711	.1685	.1660	.1635	.1611
1.0	.1587	.1562	.1539	.1515	.1492	.1469	.1446	.1423	.1401	.1379
1.1	.1357	.1335	.1314	.1292	.1271	.1251	.1230	.1210	.1190	.1170
1.2	.1151	.1131	.1112	.1093	.1075	.1056	.1038	.1020	.1003	.0985
1.3	.0968	.0951	.0934	.0918	.0901	.0885	.0869	.0853	.0838	.0823
1.4	.0808	.0793	.0778	.0764	.0749	.0735	.0721	.0708	.0694	.0681
1.5	.0668	.0655	.0643	.0630	.0618	.0606	.0594	.0582	.0571	.0559
1.6	.0548	.0537	.0526	.0516	.0505	.0495	.0485	.0475	.0465	.0455
1.7	.0446	.0436	.0427	.0418	.0409	.0401	.0392	.0384	.0375	.0367
1.8	.0359	.0351	.0344	.0336	.0329	.0322	.0314	.0307	.0301	.0294
1.9	.0287	.0281	.0274	.0268	.0262	.0256	.0250	.0244	.0239	.0233
2.0	.0228	.0222	.0217	.0212	.0207	.0202	.0197	.0192	.0188	.0183
2.1	.0179	.0174	.0170	.0166	.0162	.0158	.0154	.0150	.0146	.0143
2.2	.0139	.0136	.0132	.0129	.0125	.0122	.0119	.0116	.0113	.0110
2.3	.0107	.0104	.0102	.0099	.0096	.0094	.0091	.0089	.0087	.0084
2.4	.0082	.0080	.0078	.0075	.0073	.0071	.0069	.0068	.0066	.0064
2.5	.0062	.0060	.0059	.0057	.0055	.0054	.0052	.0051	.0049	.0048
2.6	.0047	.0045	.0044	.0043	.0041	.0040	.0039	.0038	.0037	.0036
2.7	.0035	.0034	.0033	.0032	.0031	.0030	.0029	.0028	.0027	.0026
2.8	.0026	.0025	.0024	.0023	.0023	.0022	.0021	.0021	.0020	.0019
2.9	.0019	.0018	.0018	.0017	.0016	.0016	.0015	.0015	.0014	.0014
3.0	.0013	.0013	.0013	.0012	.0012	.0011	.0011	.0011	.0010	.0010
3.1	.0010	.0009	.0009	.0009	.0008	.0008	.0008	.0008	.0007	.0007
3.2	.0007									
3.3	.0005									
3.4	.0003									
3.5	.00023									
3.6	.00016									
3.7	.00011									
3.8	.00007									
3.9	.00005									
4.0	.00003									

LAMPIRAN P

PEMBUATAN PREPARAT HISTOPATOLOGI

Cara Pembuatan Preparat Histopatologi (Suntoro, 1983; Asviah, 2008) :

1. Fiksasi dan pencucian

   Tujuan:

   - Mencegah degenerasi post mortem
   - Mematikan kuman atau bakteri
   - Meningkatkan afinitas jaringan terhadap bermacam – macam zat warna
   - Menjadikan jaringan lebih keras sehingga mengawetkan bentuk sebenarnya dan mudah dipotong
   - Meningkatkan indeks refraksi berbagai komponen jaringan

   Reagen: Formalin 10%

   Cara kerja:

   Organ hati dimasukkan ke dalam wadah yang telah diisi oleh larutan formalin 10% dan dibiarkan 24 jam. Organ hati harus tenggelam dalam larutan formalin 10%, kemudian organ dicuci dengan air mengalir selama 30 menit untuk menghilangkan larutan fiksasi dari jaringan.

2. Dehidrasi dan clearing

Tujuan:

- Menarik air yang terdapat dalam jaringan agar nantinya seluruh ruangan antar sel dalam jaringan dapat diisi oleh molekul – molekul paraffin.
- Membersihkan dan menjernihkan jaringan

Reagen:

- Alkohol bertingkat: Alkohol 70%, 80%, 90%
- Xylol I dan II

Cara kerja:

Organ hati yang telah dicuci dengan air mengalir selama 30 menit dimasukkan ke dalam reagen dengan urutan alkohol 70%, 80%, 90%, 96%, alkohol absolut. Kemudian dilakukan proses penjernihan yang bertujuan untuk menarik molekul alkohol dari dalam jaringan agar nantinya dapat digantikan oleh molekul paraffin. Organ kemudian dimasukkan ke dalam xylol I, xylol II, dan xylol III masing – masing selama 30 menit.

3. Infiltrasi

Tujuan : Jaringan lebih tahan terhadap pemotongan

Reagen : Parafin I dan II

Cara kerja:

Setelah dilakukan dehidrasi dan clearing, organ hati dimasukkan ke dalam zat penjernih-paraffin murni untuk menghindari perubahan lingkungan yang sangat mendadak. Setelah itu organ hati dimasukkan ke dalam paraffin I, paraffin II, dan kemudian

paraffin III masing – masing 30 – 60 menit, selanjutnya dioven dengan suhu 50 - 60°C selama 60 menit.

4. Pembuatan blok paraffin

   Tujuan : Jaringan lebih mudah dipotong

   Reagen : Parafin cair

   Cara kerja:

   Disiapkan beberapa cetakan besi yang sebelumnya telah diolesi gliserin dengan maksud untuk mencegah melekatnya paraffin pada cetakan besi tersebut, kemudian cetakan besi tersebut diisi dengan paraffin cair panas. Organ selanjutnya dimasukkan dengan menggunakan pinset ke dalam cetakan besi dengan posisi diatur, dan ditunggu sampai paraffin tersebut membeku atau mengeras.

5. Pengirisan dengan mikrotom

   Tujuan : Mendapatkan jaringan setipis mungkin agar mudah dilihat dengan mikroskop

   Alat : Mikrotom

   Cara kerja:

   Blok paraffin yang sudah mengeras disiapkan. Mikrotom dibersihkan, digosok dengan kertas tisu pada relnya hingga bersih, kemudian rel tersebut diberi minyak pelican. Mata pisau dipasang pada mikrotom. Blok sediaan dipasang pada mikrotom, diatur tinggi rendahnya permukaan blok pada skala 10 – 15. Sudut permukaan organ diatur dengan arah potongan pisau harus membentuk 45°, dan tebal tipisnya potongan diatur, biasanya 3□m, sedangkan untuk organ yang keras dipotong dengan ketebalan 5□m. Pemotongan diambil secara random. Setelah itu

jaringan diletakkan pada gelas obyek yang sebelumnya telah diolesi putih telur dan dikeringkan di atas hotplate dengan suhu 60°C.

6. Pewarnaan

   Tujuan : Memudahkan pengamatan perubahan jaringan

   Cara kerja:

   Jaringan diwarnai dengan menggunakan *Hematoxylin Eosin* (HE), sehingga terlihat jelas bagian – bagian selnya, sitoplasma berwarna merah dan inti berwarna biru. Proses pewarnaan dilakukan dengan memasukkan irisan jaringan yang terletak pada gelas obyek ke dalam reagen dengan urutan: xylol I (5 menit), xylol II (5 menit), xylol III (3 menit), alkohol absolut I (3 menit), alkohol absolut II (2 menit), alkohol absolut III (3 menit), alkohol 96% (2 menit), alkohol 90% (2 menit), alkohol 80% (1 menit), alkohol 70% (1 menit), dan dicuci dengan air mengalir selama 5 menit. Jaringan yang telah dicuci kemudian direndam dalam *Hematoxylin* selama 4 sampai 10 menit, lalu dicuci lagi dengan air mengalir selama 10 menit. Selanjutnya jaringan direndam dalam *Eosin* selama 3 – 8 menit, dilanjutkan dengan perendaman alkohol 70% (1 menit), alkohol 80% (2 menit), alkohol 90% (3 menit), alkohol absolut I, II, III, masing – masing 3 menit, xylol I (3 menit), xylol II (4 menit), xylol III (5 menit).

7. Mounting

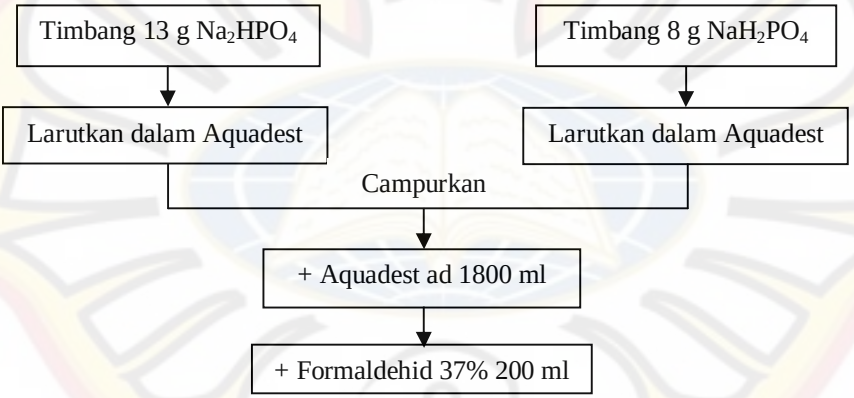
   Bila pewarnaan telah kering, maka gelas obyek yang terdapat sayatan jaringan tersebut ditutup dengan menggunakan gelas penutup yang sebelumnya telah diolesi dengan Canada balsam.

LAMPIRAN Q

PEMBUATAN BUFFER FORMALIN FOSFAT 10%

R/	Formaldehid 37%200 ml	
	Na_2HPO_4	13 g
	NaH_2PO_4	8 g
	Aquadest	ad 2 liter

Cara pembuatan :



LAMPIRAN R
SURAT ANALISIS PARASETAMOL

HENGSHUI JIHENG PHARMACY CO., LTD.

No. 368 Jianshe Street,Hengshui City,Hebei Province, 053000 P.R. China

CERTIFICATE OF ANALYSIS

Name of Product	PARACETAMOL		
Lot No.	*1006049*	Report No.	*06072*
Quantity	*6000kg*	Test Date	*2010/06/18*
Mfg date	*2010/06/09*	Exp. date	*2014/06/08*
Quality Standard	USP31/ BP2008		

Tests	Standards	Results
Appearance	A white crystalline powder	*A white crystalline powder*
Identification	Positive	*Positive*
Melting range	168~172℃	*169.0~170.1℃*
Water	Not more than0.5%	*0.1%*
Related substance	ImpurityJ(chloroacetanilide)not more than 10 ppm	*Not detected*
	Impurity K(4-aminophenol)not more than 50 ppm	*3ppm*
	Impurity F(4-nitrophenol)not more than 0.05%	*Not detected*
	any other impurity not more than 0.05%	*0.02%*
	Total of other impurity not more than 0.1%	*0.05%*
Residue on ignition	Not more than 0.1%	*0.08%*
Chloride	Not more than 0.014%	*Less than 0.014%*
Sulfate	Not more than 0.02%	*Less than 0.02%*
Sulfide	Conforms	*Conforms*
Heavy metals	Not more than 0.001%	*Less than 0.001%*
Free *p* —aminophenol	Not more than 0.005%	*Less than 0.005%*
Limit of P — chloroacetanilide	Not more than 0.001%	*Less than 0.001%*
Readily carbonizable substances	Conforms	*Conforms*
Residual solvent	Residual content of acetic acid Not more than0.5%	0.1%
Assay(anhydrous basis)	99.0~101.0%	*99.5%*
Conclusion: Complies with USP31/ BP2008		
Rechecker by 吴芳芳	Reported by	陈春荣

LAMPIRAN S

SURAT KETERANGAN
SENYAWA O-(4-NITROBENZOIL)PARASETAMOL

Laporan Hasil Pemeriksaan Senyawa

6. Nama senyawa : O-(4-Nitrobenzoil)parasetamol
7. Dibuat oleh : Siswandono
8. Tanggal dibuat : 10 Juli 2010
9. Rendemen : 63%
10. Pemeriksaan :

No.	Jenis Pemeriksaan	Hasil Pemeriksaan
1. 2. 3. 4.	Pemerian/Organoleptis Jarak lebur Kelarutan Uji KLT(3 eluen)	Serbuk, warna kuning, tidak berbau. 213-215°C. etanol, aseton, dimetilsulfoksid. 1 noda.
5.	Spektrum UV: λ maks. (nm) Dalam pelarut etanol	246
6.	Spektrum IR : ν (cm^{-1}) Dalam pelet KBr	3325 (–NH, amida); 1736 (–C=O, ester); 1678 (–C=O, amida); 1524 (–C=C- aromatis); 1271(-C-O, ester)
7.	Spektrum ^{1H}NMR : δ (ppm) Dalam pelarut DMSO-D_6	10,02, s, (-CONH); 7,25-8,38, m, (8H dari 2 -C_6H_4); 2,06, s, (CH_3)

Kesimpulan : Senyawa adalah O-(4-Nitrobenzoil)parasetamol.

Surabaya, 2 Februari 2011

Mengetahui:
Ketua Departemen Kimia Farmasi
Fakultas Farmasi Unair

Marcellino Rudyanto, MSi, Ph.D
NIP. 19660518 199203 1 002

Ketua Peneliti,

Prof. Dr. Siswandono, MS.

LAMPIRAN T

SURAT KETERANGAN HEWAN COBA

Departemen Pendidikan Nasional
Universitas Gadjah Mada
Fakultas Kedokteran Hewan
Bagian Ilmu Penyakit Dalam

Alamat :
Rumah Sakit Hewan FKH UGM
Jl. Asti Kuningan, Yogyakarta 55281

SURAT KETERANGAN

Yang bertanda tangan dibawah ini :

Nama : drh. Slamet Raharjo, MP
Alamat : Bagian Ilmu Penyakit Dalam Fakultas Kedokteran Hewan Universitas Gadjah Mada Yogyakarta
Jabatan : Dokter Hewan pada Rumah Sakit Hewan FKH UGM
Jabatan Lain : Dokter Hewan praktisi di Klinik hewan Calico Jl. Raya Tajem Stan Maguwoharjo

Dengan ini menerangkan bahwa :
Nama : UD. WISTAR
Nama Pemilik : Bpk. Suparno
Ternak Hewan: Tikus Putih *(Laboratory rat)*

Berdasarkan hasil identifikasi terhadap morfologi anatomi dapat kami simpulkan bahwa tikus putih *(Laboratory rat)* tersebut adalah galur **WISTAR.**

Demikian surat ini kami buat untuk dapat digunakan sebagaimana semestinya.

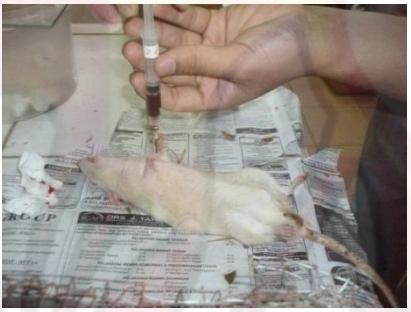
Yogyakarta, 1 Februari 2011

drh. Slamet Raharjo, MP
NIP. 132 230 583

LAMPIRAN U

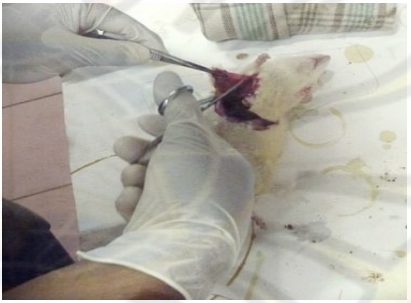
FOTO – FOTO SELAMA PENELITIAN BERLANGSUNG

A

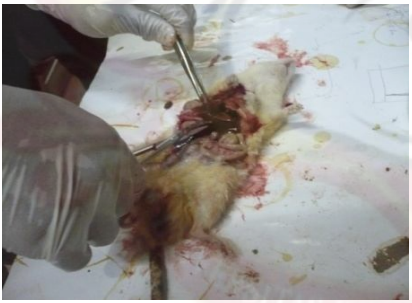


B

C



D



E

F

Keterangan Gambar :

A : Hewan coba tikus putih jantan galur wistar
B : Proses pengambilan darah intrakardial
C : Pemberian bahan uji per oral dengan alat sonde
D : Proses pembedahan
E : Pengambilan organ hati
F : Organ hati tikus coba