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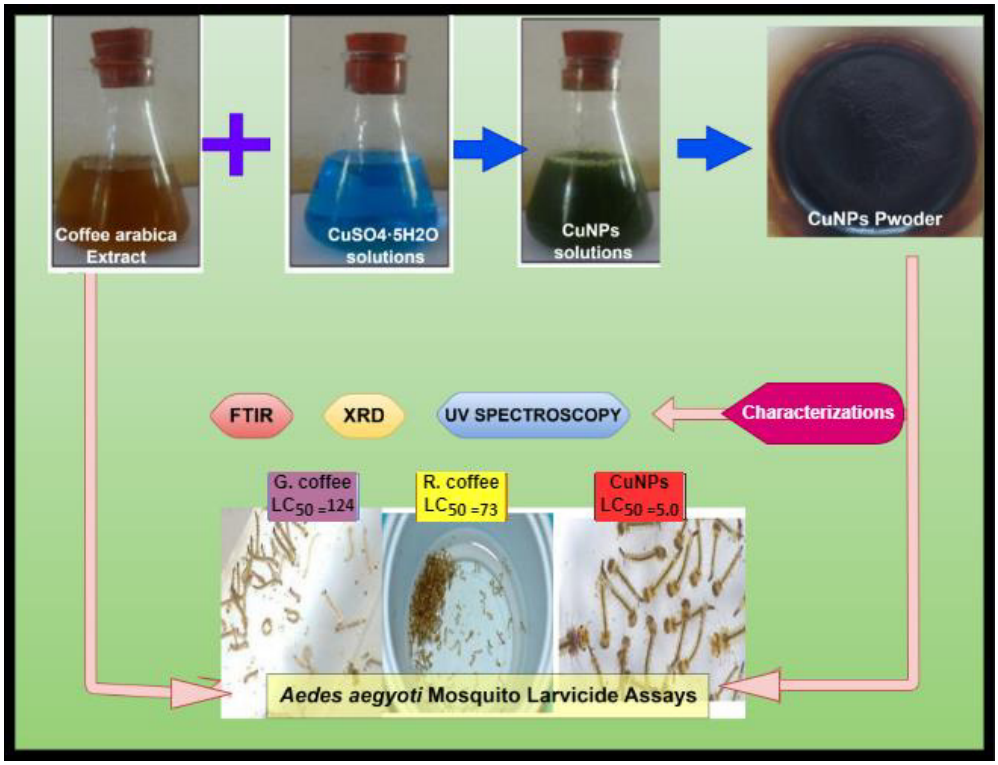
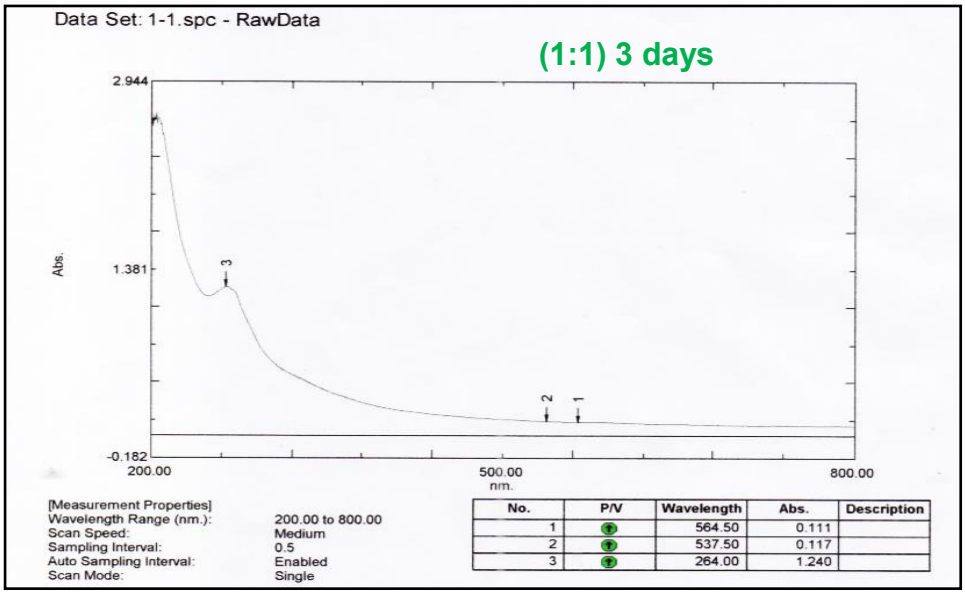
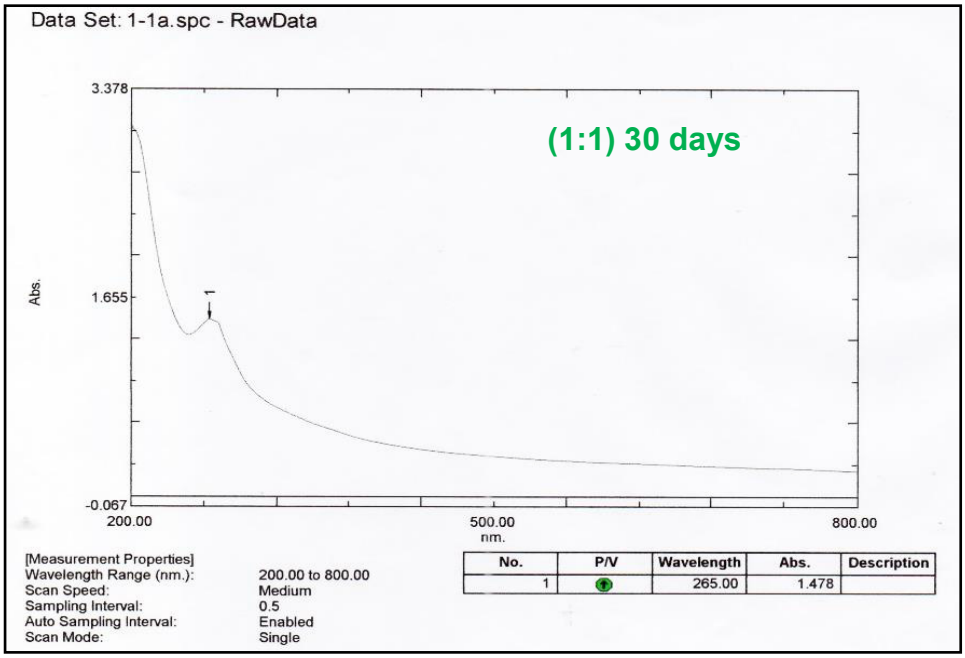
APPENDIXES

Appendix 1. Abstract Research

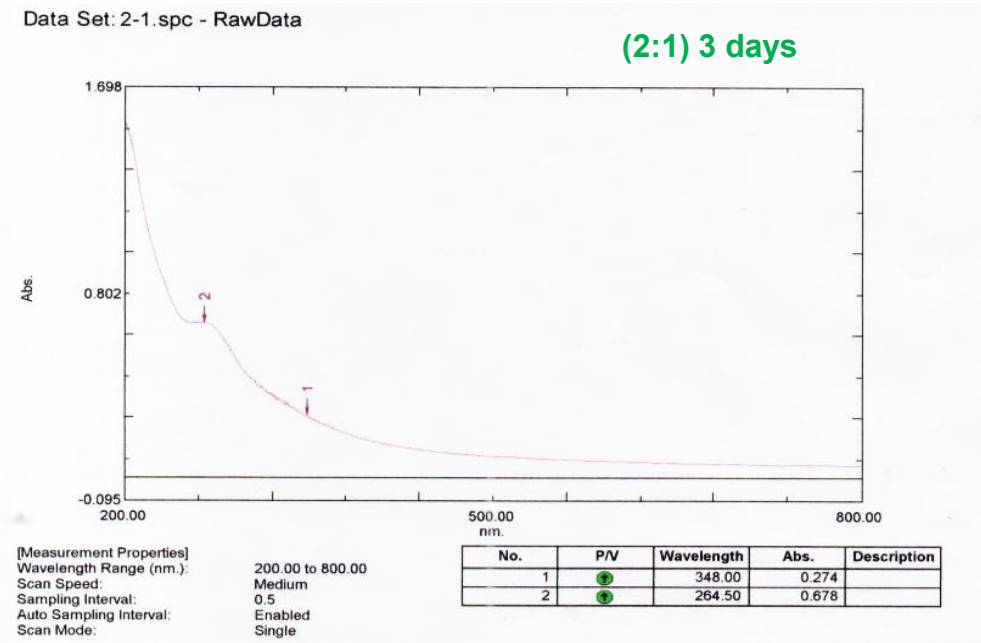


Appendix 2. U V Spectrophotometer CuNPs Synthesis Ratio (1:1)





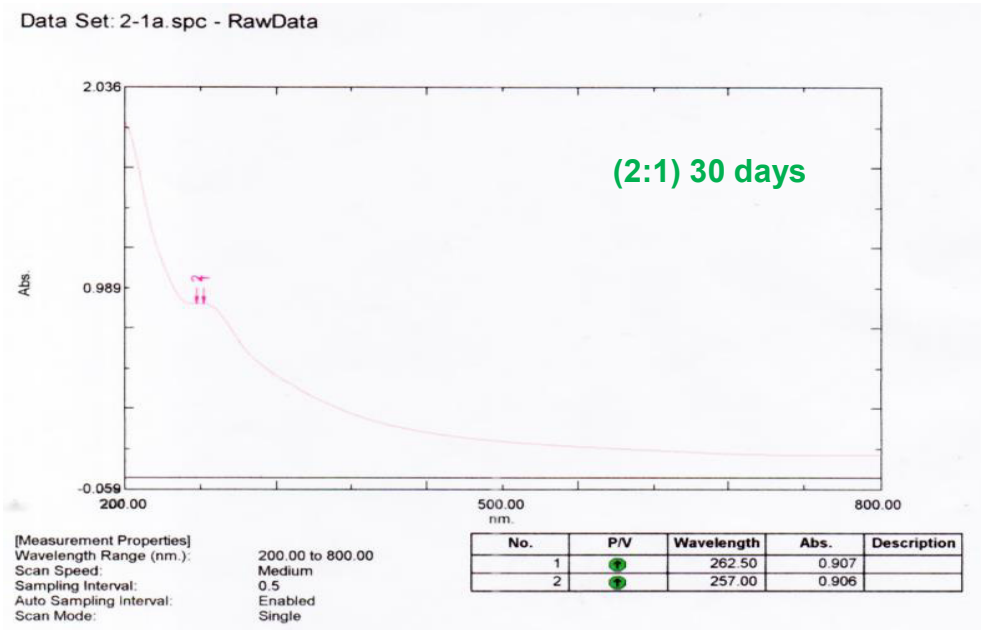
Appendix 3. U V Spectrophotometer CuNPs Synthesis Ratio (2:1)



[Measurement Properties]

Wavelength Range (nm.):	200.00 to 800.00
Scan Speed:	Medium
Sampling Interval:	0.5
Auto Sampling Interval:	Enabled
Scan Mode:	Single

No.	P/V	Wavelength	Abs.	Description
1	↑	348.00	0.274	
2	↑	264.50	0.678	

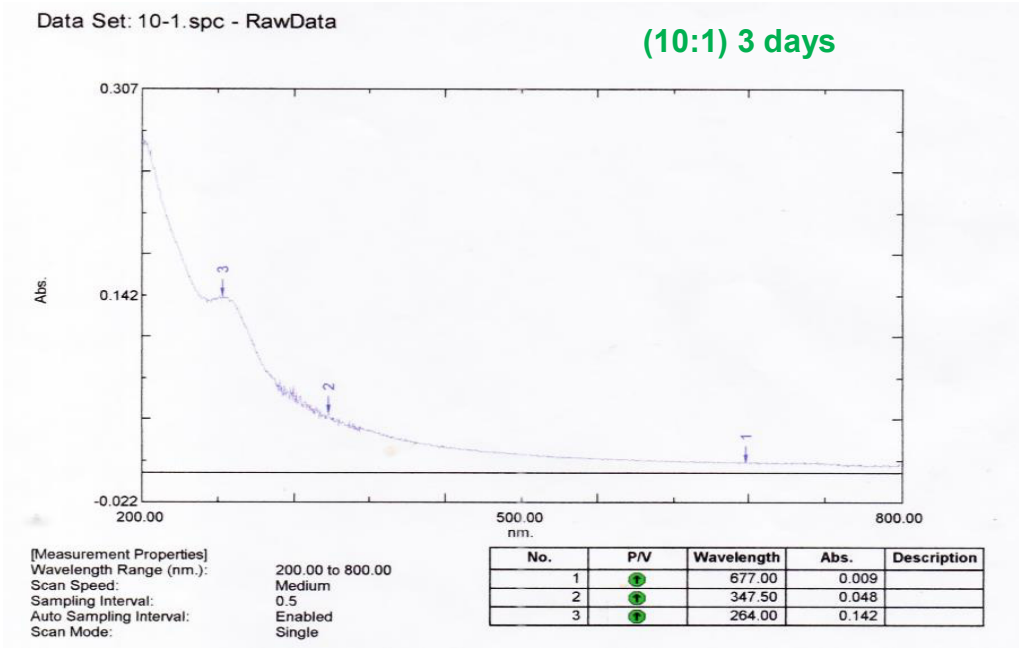


[Measurement Properties]

Wavelength Range (nm.):	200.00 to 800.00
Scan Speed:	Medium
Sampling Interval:	0.5
Auto Sampling Interval:	Enabled
Scan Mode:	Single

No.	P/V	Wavelength	Abs.	Description
1	↑	262.50	0.907	
2	↑	257.00	0.906	

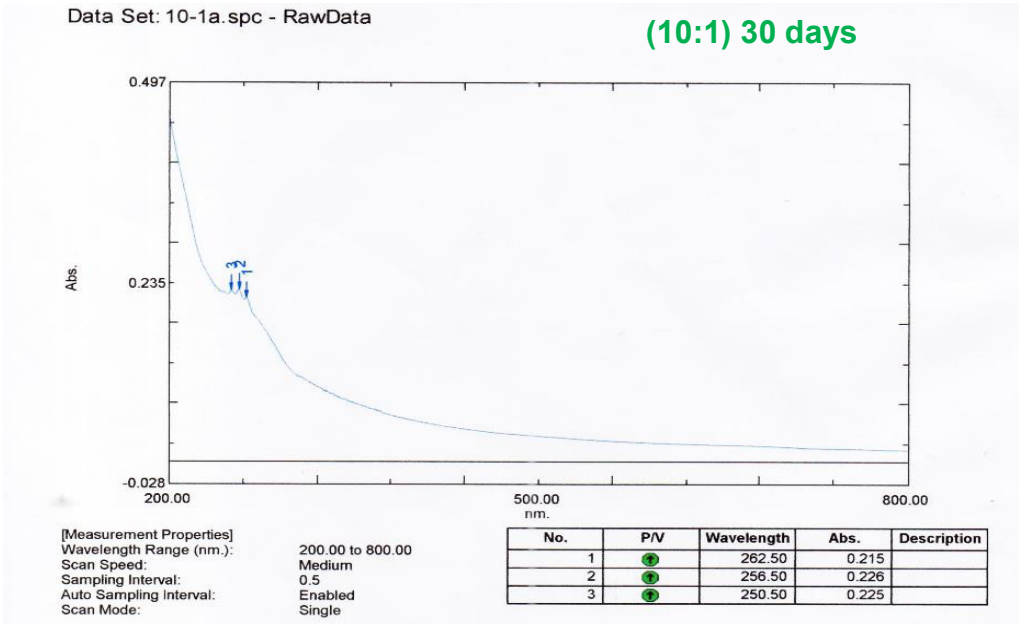
Appendix 4. U V Spectrophotometer CuNPs Synthesis Ratio (10:1)



[Measurement Properties]

Wavelength Range (nm.):	200.00 to 800.00
Scan Speed:	Medium
Sampling Interval:	0.5
Auto Sampling Interval:	Enabled
Scan Mode:	Single

No.	P/V	Wavelength	Abs.	Description
1	↑	677.00	0.009	
2	↑	347.50	0.048	
3	↑	264.00	0.142	



[Measurement Properties]

Wavelength Range (nm.):	200.00 to 800.00
Scan Speed:	Medium
Sampling Interval:	0.5
Auto Sampling Interval:	Enabled
Scan Mode:	Single

No.	P/V	Wavelength	Abs.	Description
1	↑	262.50	0.215	
2	↑	256.50	0.226	
3	↑	250.50	0.225	

Appendix 5. Data XRD

*** Basic Data Process ***

Group : Standard
Data : yousefAhmad

Strongest 3 peaks

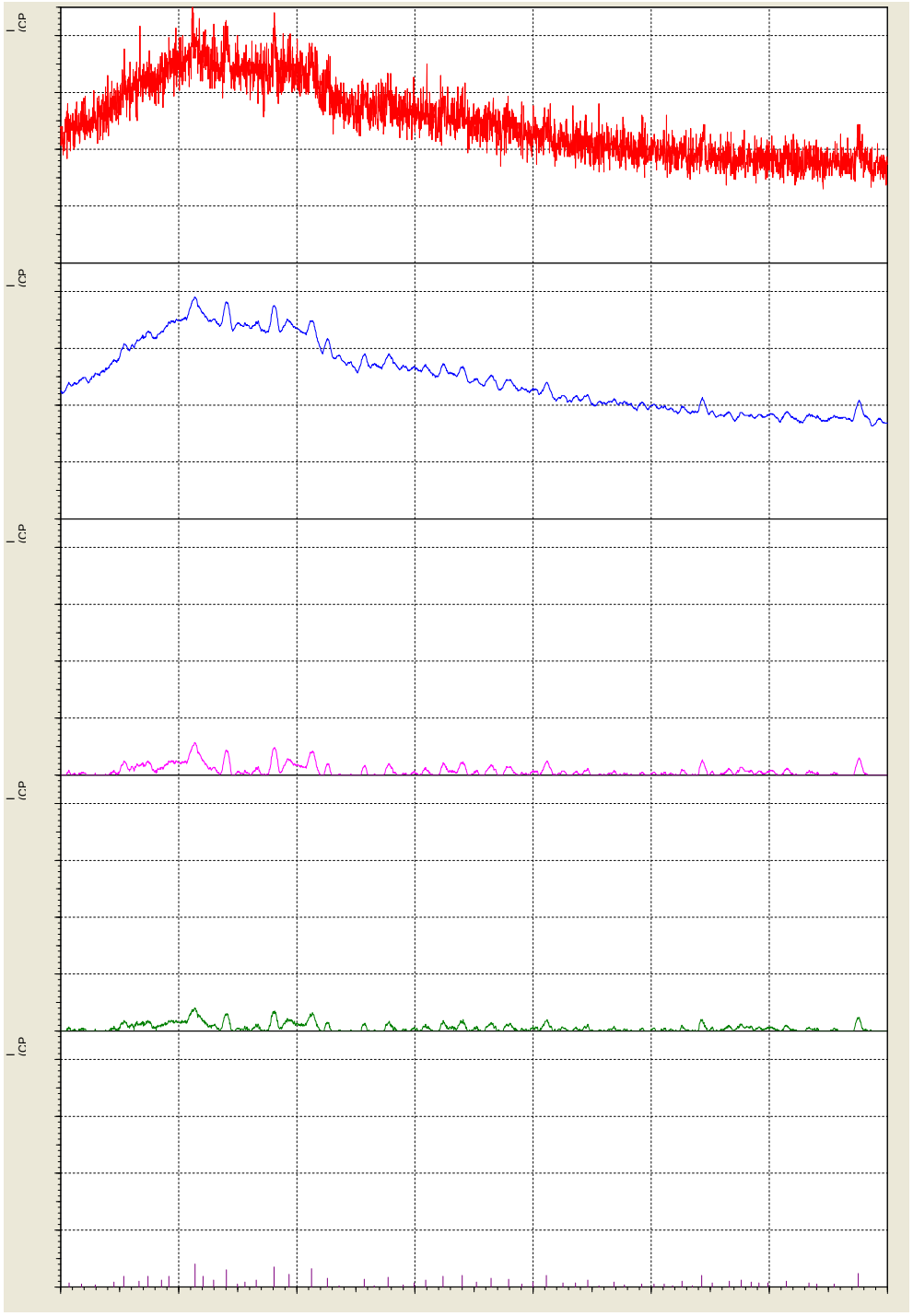
no.	peak no.	2Theta (deg)	d (A)	I/I1	FWHM (deg)	Intensity (Counts)	Integrated Int (Counts)
1	10	21.3900	4.15075	100	1.10000	24	1372
2	17	28.0700	3.17630	88	0.52000	21	602
3	19	31.2500	2.85995	79	0.70000	19	781

Peak Data List

peak no.	2Theta (deg)	d (A)	I/I1	FWHM (deg)	Intensity (Counts)	Integrated Int (Counts)
1	10.7200	8.24615	17	0.12000	4	43
2	11.7800	7.50641	13	0.04000	3	21
3	12.9400	6.83599	8	0.04000	2	4
4	14.5283	6.09203	21	0.04330	5	24
5	15.3550	5.76585	46	0.57000	11	579
6	16.6400	5.32337	25	0.00000	6	0
7	17.4100	5.08963	46	0.58000	11	567
8	18.5400	4.78189	29	0.08000	7	64
9	19.1800	4.62374	46	0.56000	11	582
10	21.3900	4.15075	100	1.10000	24	1372
11	22.0600	4.02618	46	0.60000	11	347
12	22.9633	3.86980	29	0.39330	7	191
13	24.0360	3.69947	75	0.55200	18	501
14	24.9700	3.56317	13	0.10000	3	47
15	25.6000	3.47689	21	0.08000	5	49
16	26.5600	3.35336	29	0.12000	7	133
17	28.0700	3.17630	88	0.52000	21	602
18	29.3500	3.04063	54	0.74000	13	709
19	31.2500	2.85995	79	0.70000	19	781
20	32.5816	2.74604	38	0.34330	9	165
21	33.5800	2.66665	4	0.00000	1	0
22	35.7100	2.51232	33	0.38000	8	137
23	36.5600	2.45584	4	0.00000	1	0
24	37.7300	2.38233	42	0.42000	10	243
25	39.0100	2.30705	8	0.10000	2	23
26	39.9300	2.25599	17	0.14000	4	58
27	40.9000	2.20470	29	0.08000	7	89
28	42.3850	2.13083	46	0.37000	11	256
29	44.0000	2.05629	50	0.52000	12	389
30	45.2150	2.00382	21	0.05000	5	38
31	46.4400	1.95377	38	0.44000	9	261
32	47.9300	1.89646	33	0.54000	8	254
33	49.0400	1.85610	13	0.04000	3	17
34	49.9900	1.82303	21	0.06000	5	42
35	51.1300	1.78502	50	0.42000	12	363
36	52.5300	1.74070	17	0.30000	4	95
37	53.6000	1.70845	17	0.16000	4	57
38	54.6300	1.67864	29	0.14000	7	103
39	55.5800	1.65218	4	0.00000	1	0
40	56.8500	1.61825	21	0.10000	5	49
41	57.7300	1.59566	8	0.14000	2	21
42	59.2000	1.55950	13	0.04000	3	26
43	60.2400	1.53503	13	0.16000	3	41
44	61.1000	1.51547	13	0.04000	3	22
45	61.8200	1.49954	4	0.00000	1	0
46	62.6000	1.48272	25	0.16000	6	83
47	63.4800	1.46427	4	0.00000	1	0
48	64.2850	1.44786	50	0.43000	12	264

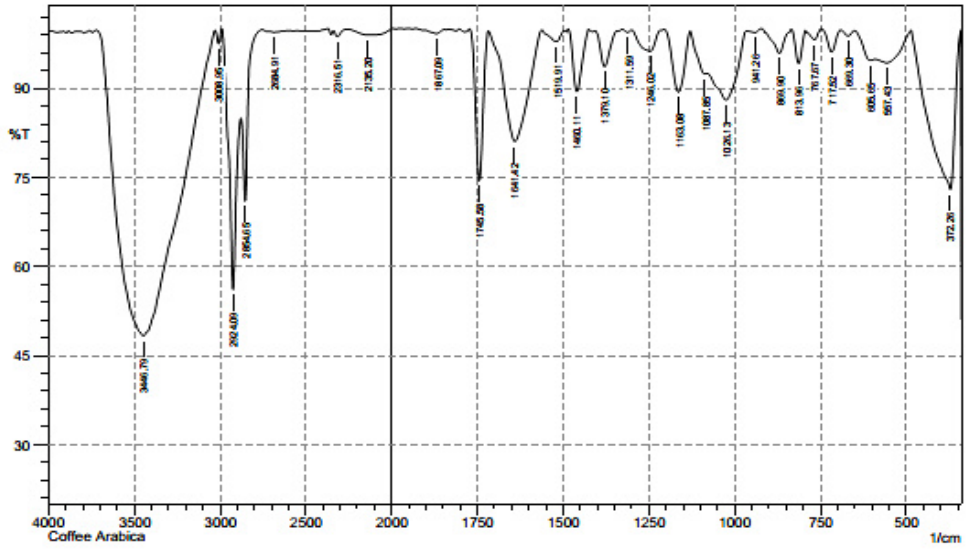
peak no.	2Theta (deg)	d (A)	I/I1	FWHM (deg)	Intensity (Counts)	Integrated Int (Counts)
50	66.6100	1.40285	25	0.26000	6	142
51	67.6200	1.38433	29	0.28000	7	209
52	68.4600	1.36938	21	0.20000	5	103
53	69.1300	1.35774	17	0.26000	4	90
54	69.8900	1.34482	17	0.22000	4	130
55	71.4400	1.31940	25	0.40000	6	165
56	73.3600	1.28954	17	0.20000	4	90
57	74.0250	1.27959	13	0.03000	3	16
58	75.4900	1.25836	13	0.10000	3	38
59	77.5350	1.23019	58	0.49000	14	346

Appendix 6. The curves XRD



Appendix 7. Data FTIR *Coffee arabica*

SHIMADZU



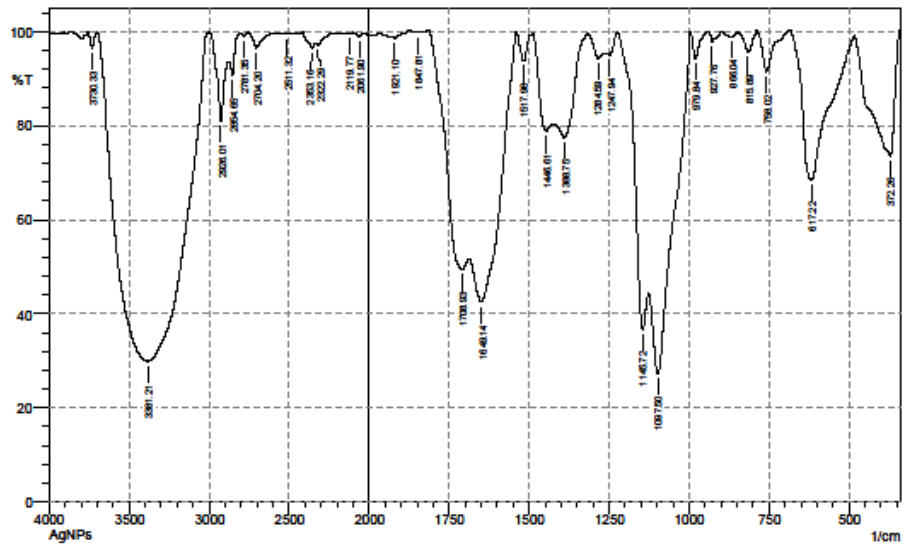
	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	372.26	72.975	26.49	486.06	343.33	10.846	10.603
2	557.43	94.343	2.055	588.29	487.99	1.827	0.653
3	605.65	94.756	1.415	650.01	590.22	0.902	0.196
4	669.3	98.868	0.953	692.44	650.01	0.118	0.086
5	717.52	96.129	3.841	748.38	692.44	0.43	0.425
6	767.67	98.244	1.741	794.67	748.38	0.19	0.183
7	813.96	94.214	5.714	835.18	794.67	0.53	0.518
8	869.9	95.931	3.806	910.4	835.18	0.659	0.565
9	941.26	99.442	0.482	964.41	921.97	0.063	0.045
10	1026.13	88.068	7.86	1080.14	966.34	4.279	2.316
11	1087.85	92.443	0.935	1130.29	1082.07	1.083	0.208
12	1163.08	89.405	10.286	1203.58	1132.21	1.842	1.753
13	1246.02	96.288	3.599	1300.02	1215.15	0.833	0.795
14	1311.59	99.465	0.484	1328.95	1300.02	0.036	0.031
15	1379.1	93.697	6.061	1406.11	1342.46	0.865	0.798
16	1460.11	89.461	10.565	1485.19	1427.32	1.381	1.386
17	1519.91	97.905	1.898	1562.34	1498.69	0.341	0.292
18	1641.42	81.076	18.73	1718.58	1562.34	6.83	6.699
19	1745.58	74.434	25.402	1772.58	1720.5	2.978	2.94
20	1867.09	99.251	0.135	1870.95	1845.88	0.045	0.004
21	2135.2	99.012	0.094	2250.93	2123.63	0.332	0.054
22	2316.51	98.738	1.081	2339.65	2250.93	0.195	0.145
23	2684.91	99.491	0.057	2706.13	2673.34	0.067	0.004
24	2854.65	71.034	16.788	2877.79	2756.28	4.998	1.885
25	2924.09	56.088	35.012	2989.66	2879.72	12.425	8.506
26	3008.95	97.492	2.488	3030.17	2991.59	0.209	0.203
27	3446.79	48.329	51.497	3716.83	3030.17	113.206	112.688

Comment;
Coffee Arabica

Date/Time; 9/13/2022 8:44:01 AM
No. of Scans;
Resolution;
Apodization;

Appendix 8. Data FTIR *CuNPs*

SHIMADZU



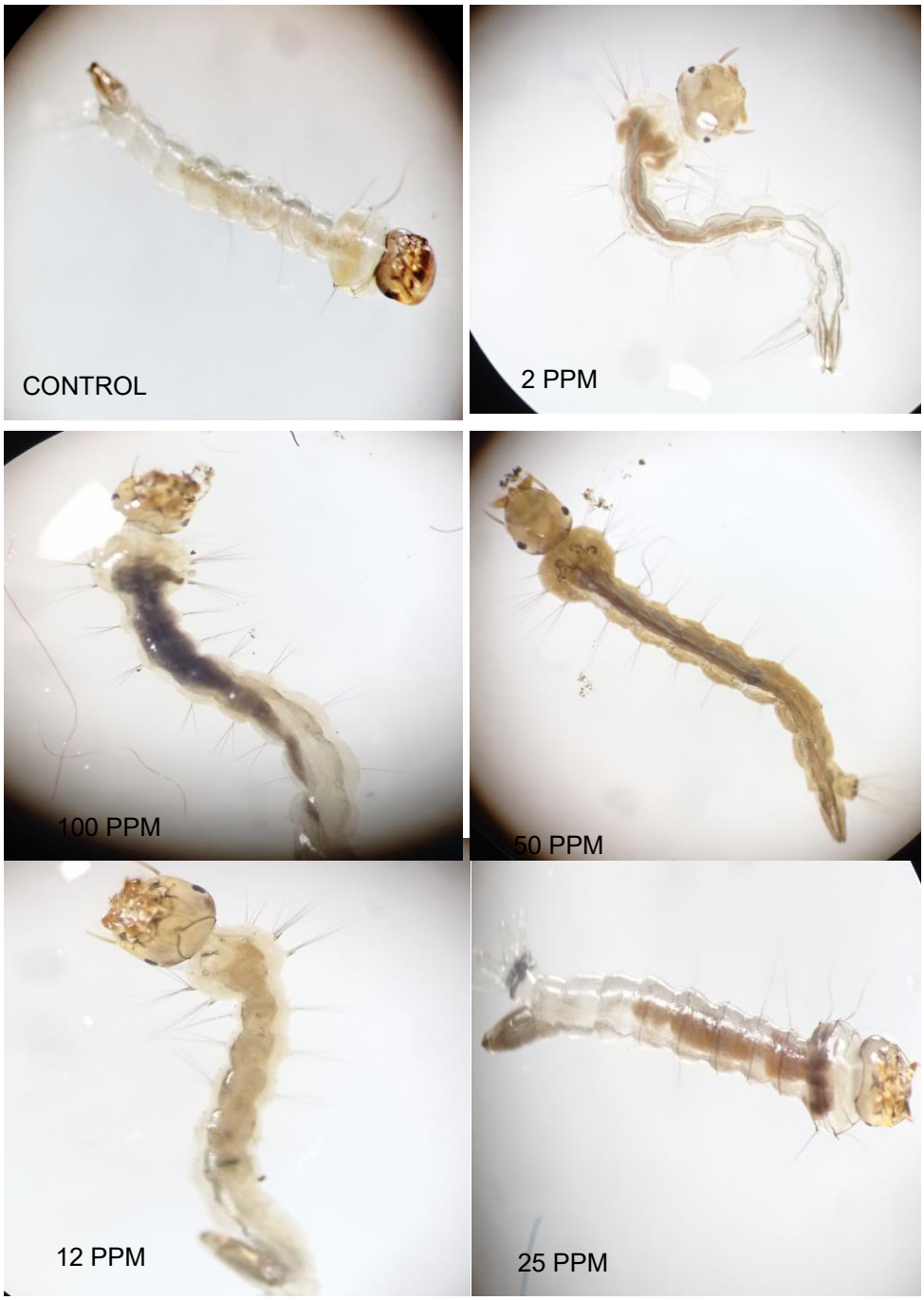
	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	372.26	73.523	26.261	482.2	343.33	10.946	10.688
2	617.22	68.491	31.362	684.73	484.13	14.357	14.01
3	758.02	91.507	8.652	786.96	686.66	1.41	1.504
4	815.89	95.761	4.034	839.03	788.89	0.504	0.466
5	866.04	98.937	1.013	898.83	846.75	0.16	0.147
6	927.76	97.896	2.123	943.19	898.83	0.176	0.175
7	979.84	94.235	5.706	997.2	943.19	0.566	0.56
8	1097.5	27.23	29.739	1126.43	999.13	37.419	15.229
9	1145.72	36.627	17.947	1222.87	1128.36	16.857	2.965
10	1247.94	95.083	1.894	1261.45	1222.87	0.579	0.178
11	1284.59	94.247	2.979	1315.45	1261.45	0.927	0.336
12	1388.75	77.44	9.194	1423.47	1317.38	6.994	2.094
13	1446.61	78.894	7.836	1489.05	1425.4	4.576	1.42
14	1517.98	93.79	5.804	1541.12	1494.83	0.654	0.572
15	1649.14	42.508	21.883	1687.71	1541.12	34.14	13.388
16	1708.93	49.383	9.645	1816.94	1689.64	21.284	3.79
17	1847.81	99.825	0.217	1857.45	1834.3	0.002	0.007
18	1921.1	98.606	1.298	1969.32	1872.88	0.292	0.251
19	2061.9	98.877	0.861	2098.55	2031.04	0.183	0.107
20	2119.77	99.539	0.16	2135.2	2098.55	0.059	0.013
21	2322.29	97.215	0.549	2337.72	2187.28	0.832	-0.071
22	2353.16	96.448	0.958	2368.59	2337.72	0.416	0.064
23	2511.32	99.491	0.219	2569.18	2438.02	0.222	0.058
24	2704.2	96.539	3.23	2746.63	2611.62	0.993	0.833
25	2781.35	99.009	0.664	2804.5	2746.63	0.132	0.056
26	2854.65	90.724	4.517	2873.94	2804.5	1.34	0.411
27	2926.01	81.097	15.096	2997.38	2875.86	4.964	3.167
28	3381.21	29.878	70.059	3701.4	2999.31	214.261	214.053
29	3730.33	96.336	3.608	3755.4	3701.4	0.392	0.379

Comment;
AgNPs

Date/Time; 8/31/2022 2:38:41 PM
No. of Scans;
Resolution;
Apodization;

Appendix 9. CuNPs synthesis

Appendix 10. Treated Aedes aegypti



Appendix 11 protocol DNA extraction

DNA EXTRACTION

Isolate the genomic DNA of an adult mosquito (whole body) using a modified phenol-chloroform protocol

Reagents and materials needed:

Phenol/chloroform isoamyl alcohol (24:24:1)
1X PBS Buffer
ATL Buffer (from Dneasy kit)
Absolute ethanol (ice-cold)
70% ethanol
Proteinase K
Chloroform
TE Buffer (pH 7.5 - 8)
3M sodium acetate (pH 5.2)
RnaseA (100mg/mL)
1.5 mL sterile tubes
Tube rack
Pipet tips and tip boxes
Plastic pestles
Kim wipes
Liquid waste container

Equipment needed:

Centrifuge	Vortex mixer	Water bath	Autoclave
Fume hood	Freezer	Micro-Pipettes	

I

Sample to be extracted:
Larvae mosquitos

Initial preparation:

1. Wear lab gown, masker, gloves and foot protection shoes for your safety.
2. Disinfect the hood and work table with 70% ethanol.
3. Disinfect with 70% ethanol all the laboratory materials needed and then expose all in the hood under the UV lamp for 15 minutes before and after using.
4. All tubes, pipette tips, and plastic pestles that will be used should had been be sterilized (autoclaved).

Procedures:

1. Add 200 µL 1X PBS into a 1.5 mL sterile tube and individual mosquito sample, then homogenized.

2. Digest 200 µL PBS-homogenized samples in 180 µL Buffer ATL* and 20 µl Proteinase K.
3. Incubate at 56°C overnight. Allow to cool down for 10 minutes.
4. Add 4 µL RNaseA (100mg/mL). Vortex to mix.
5. Incubate at 37°C for 1 hour.
6. Add an equal volume of phenol/chloroform/isoamyl alcohol (25:24:1). ➔ *"equal" means equal volume to the volume of 200 µL (1X PBS) + 180 µL (Buffer ATL*) + 20 µl (Proteinase K) ➔ 400 µL*
7. Vortex vigorously for 20 sec.
8. Centrifuge at 12, 000 RPM for 10 min.
9. Add 100 µL of chloroform. Vortex vigorously for 20 sec.
10. Centrifuge at 12, 000 RPM for 2 min. Carefully transfer aqueous phase to new tube.
11. Add 1/10 volume of 3M sodium acetate, pH 5.2. Gently invert 10x. ➔ *1/10 of aqueous phase (upper layer) collected in the previous step (10), see the volume in the pipet tip.*
12. Add 2 volumes (calculated after salt addition) of ice-cold 100% ethanol. Gently invert 10x. ➔ (*For Eg.: upper layer is 200µL + 1/10 of 200 is 20µL so the ice-cold 100% ethanol to be added is [200 + 20] µL x 2 = 440µL*)
13. Incubate in -20°C for 1 hour to overnight.
14. Centrifuge at 12,000 RPM for 15 min. Carefully decant supernatant.
15. Wash pellet with 1 mL 70% ethanol (RT). Vortex briefly for 5 sec.
16. Centrifuge at 12,000 RPM for 2 min. Carefully decant supernatant.
17. Air dry for 15 min or until excess ethanol is evaporated.
18. Resuspend pellet in 30 µL TE buffer (pH 8) or nuclease-free water.
19. Incubate in water bath for 10 min at 55°C. Briefly centrifuge to collect sample.

Appendix 12. Total protein

Total Protein Kit
CliniQuant - FSR
Biuret Method, End Point

Meril Diagnostics

For *in vitro* diagnostic use
Read this pack insert thoroughly before use

REF	Pack Size	R1 Total Protein Reagent	R2 Total Protein Standard
TPRFSR-01	4 x 50ml	4 x 50ml	1 x 5ml
TPRFSR-02	2 x 500ml	2 x 500ml	2 x 5ml

INTENDED USE

This reagent is intended for quantitative determination of proteins concentration in human serum or plasma.

CLINICAL SIGNIFICANCE

Serum Total Protein is useful in monitoring change in the protein levels due to diseases. Increased levels are found in dehydration due to inadequate water intake or to excessive water loss as in severe vomiting, diarrhoea, Addison' s disease and in multiple myeloma.

PRINCIPLE OF THE METHOD

Proteins peptidic bonds react with Cu(II) in alkaline solution to form blue-purple complex, the absorbance of which is measured at 546 nm. Each Cu(II) can complex up to 6 peptidic bonds.

$$\text{Proteins} \xrightarrow[\text{alkaline medium}]{Cu^{2+}} \text{Blue color complex}$$

KIT COMPONENTS

Composition

R1 - Total Protein Reagent : Cupric Sulphate 12.01 mmol/l, Potassium sodium tartarate 31.8 g/l, Potassium Iodide 54.21 mmol/l, Sodium Hydroxide 200 mmol/l

R2 - Total Protein Standard : 6.0 g/dl, BSA 60g/l, Sodium Azide 0.1%

MATERIALS REQUIRED BUT NOT PROVIDED

Laboratory instrumentation, Spectrophotometer UV/VIS with thermostatic cuvette holder or clinical chemistry analyzer: semi automated, calibrated micropipettes, glass or high quality polystyrene cuvettes, test tube/ rack, heating bath, controls, saline.

REAGENT PREPARATION, STORAGE & STABILITY

Reagent is ready to use. Keep away from direct light sources.

Stability: up to expiration date on labels at 2-8 °C.

Upon opening of kit, store Reagent R1 at 15 -30 °C and standard at 2-8 °C.

Stability since first opening of reagent bottle: preferable within 60 days at 15 -30 °C.

REAGENT DETERIORATION

1. Discard the reagent if absorbance exceeds 0.2 at 546 nm against distilled water.
2. Keep the Standard vial plugged after use, in order to avoid deterioration.

WARNINGS AND PRECAUTIONS

1. Reagent contains strong alkali. Do not mouth pipette. It is suggested to handle carefully, avoiding contact with skin and ingestion.
2. Specimens should be considered infectious and handled appropriately.
3. Perform the test according to the general "Good Laboratory Practice" (GLP) guidelines.

SPECIMEN

Use unhemolysed serum or plasma. Plasma specimens may obtain 0.4 g/dl high due to fibrinogen. Serum/ plasma is stable for 7 days at 2-8 °C and 1 month at -20 °C.

Programme Parameter for MERILYZER CliniQuant

Reading Mode	End Point
Standard Conc.	6 (g/dl)
Filter – 1 (nm)	546
Temperature	37 °C
Volume (µl)	500
Delay Time (Sec)	5
Reaction Direction	Increase
Reference Low	6.0
Reference High	8.3
Linearity Limit	15

CE
Meril Diagnostics Pvt. Ltd., Second Floor, D1-D3, Meril Park, Survey No. 135/2/B & 174/2, Muktanand Marg, Chala, Vapi-396191, Gujarat, India. T+91-260-3052100, F +91-260-3052125, W www.merillife.com
EC REP Obelis s.a., Bd General Wahis 53, 1030, Brussels, Belgium. T +(32) 2 732-59-54, F +(32) 2 732-60-03, E mail@obelis.net

Total protein

TEST PROCEDURE

Dispense	Blank	Standard	Sample
Reagent 1	1ml	1ml	1ml
Distilled water	20 µl	-	-
Standard	-	20 µl	-
Sample	-	-	20 µl
Mix, incubate for 5 min at 37°C. Read absorbance of standard (As) and samples (Ax) against reagent blank.			

RESULT CALCULATION

Serum/plasma:

Proteins g/dl = Ax/As x Concentration of Standard

SI conversion factor: 1 g/dl x 10 = 1 g/l

EXPECTED VALUES

6.0 – 8.3 g/dl OR 60 – 83 g/l

It is recommended that each laboratory verifies this range or derives reference interval for the population it serves.

QUALITY CONTROL AND CALIBRATION

It is recommended to perform internal quality control with assayed normal (BioNorm) and assayed abnormal (BioPath), to confirm the validity of the test and assure the accuracy of patient result.

Using the recommended Calibrator (BioCal) or the Standard included, calibrate the assay:

a. When using a new reagent or lot

b. When QC values are out of range

PERFORMANCE CHARACTERISTICS

1. Linearity

The linearity is up to 15.0 g/dl.

2. Sensitivity/ Limit of detection (LOD)

The limit of detection is 0.05 g/dl.

The limit of quantification is 0.1 g/dl.

3. Interferences

Gross hemolysis, lipaemia and icteric specimens may cause falsely elevated results, a sample blank be set by adding 20 µl sample in 1ml saline.

4. Precision

Intra-assay precision

	Mean	SD	CV
n = 20	g/dl	g/dl	%
sample 1	6.66	0.02	0.33
sample 2	4.91	0.03	0.62

Inter-assay precision

	Mean	SD	CV
n = 20	g/dl	g/dl	%
sample 1	6.52	0.24	3.70
sample 2	4.83	0.22	4.50

5. Methods Comparison

Comparison was done between reference Total Protein Reagent and CliniQuant - FSR Total Protein Reagent (test)

N = 36 $y = 0.973x + 0.267$

$r^2 = 0.963$

LIMITATIONS

Samples with values above 15 g/dl should be diluted with 0.9% saline, re-run and results multiplied by dilution factor.

WASTE DISPOSAL

This product is made to be used in professional laboratories. Please consult local regulations for correct waste disposal.

REFERENCES

1. Burtis, C.A., Ashwood, E.R., editors. Tietz Textbook of Clinical Chemistry. 2nd ed. Philadelphia, W.B. Saunders Company, 1994, p. 695 – 700.

2. Data on file: Meril Diagnostics.

IFU/TPRFSR01/00
06-11-2018

Symbols used on Meril Diagnostics labels:

REF	Catalogue No.		Attention See Instruction for Use
LOT	Batch No.	IVD	*In vitro* Diagnostics
	Expiry Date		Consult Instruction for Use
	Manufacturer		Storage Temperature
	Keep Dry		Keep Away from Sunlight
	Manufacturing Date		Do not use if package is damaged
EC REP	Authorized European Representative in the European Community		

Appendix 13 GOT

SGOT Kit

CliniQuant - FSR

IFCC Method, Kinetic

Meril Diagnostics

For *in vitro* diagnostic use
Read this pack insert thoroughly before use

REF	Pack Size	R1 SGOT Reagent	R2 SGOT Reagent
GOTFSR-01	4 x 20 / 4 x 5ml	4 x 20ml	4 x 5ml
GOTFSR-02	4 x 100 / 2 x 50ml	4 x 100ml	2 x 50ml

INTENDED USE

This reagent is intended for quantitative determination of SGOT level in human serum.

CLINICAL SIGNIFICANCE

The aminotransferases (transaminases) are widely distributed in animal tissues. Both AST & ALT are normally present in human plasma, bile, cerebrospinal fluid, and saliva. Elevated AST levels are observed in viral hepatitis and other liver disease, cirrhosis, myocardial infarction.

PRINCIPLE OF THE METHOD

$$\text{L-Aspartate} + \text{2-Oxoglutarate} \xrightarrow{\text{AST}} \text{Oxoloacetate} + \text{L-Glutamate}$$

$$\text{Oxoloacetate} + \text{NADH} \xrightarrow{\text{MDH}} \text{Malate} + \text{NAD}$$

$$\text{Pyruvate} + \text{NADH} \xrightarrow{\text{LDH}} \text{L-Lactate} + \text{NAD}$$

AST: Aspartate aminotransferase
MDH: Malate dehydrogenase
LDH: Lactate dehydrogenase

KIT COMPONENTS

Composition

R1 - SGOT Reagent : Tris buffer 20 mmol/l pH 7.8, L-Asparatic acid 231 mmol/l, MDH > 0.2 KU/l, LDH > 4 KU/l, 2-oxoglutarate 17.17 mmol/l

R2 - SGOT Reagent : NADH 0.18 mmol/l, 2-oxoglutarate 15 mmol/l

MATERIALS REQUIRED BUT NOT PROVIDED

Laboratory instrumentation, Spectrophotometer UV/VIS with thermostatic cuvette holder or clinical chemistry analyzer: semi automated, calibrated micropipettes, glass or high quality polystyrene cuvettes, test tube/ rack, heating bath, controls, saline.

REAGENT PREPARATION, STORAGE & STABILITY

Mix reagent 1 & reagent 2 in ratio 4:1. Keep away from direct light sources.
Stability: up to expiration date on labels at 2-8 °C.
Stability of working reagent: 30 days at 2-8 °C.

REAGENT DETERIORATION

Discard the working reagent if absorbance < 1.0 at 340 nm against distilled water.

WARNINGS AND PRECAUTIONS

1. Reagent may contain some non-reactive and preservative components. It is recommended to handle carefully, avoiding contact with skin and ingestion.
2. Specimens should be considered infectious and handled appropriately.
3. Perform the test according to the general "Good Laboratory Practice" (GLP) guidelines.

SPECIMEN

Use serum, plasma. SGOT is stable for 4 days at 2-8 °C or 1 month at -20°C.

Programme Parameter for MERILYZER CliniQuant

Procedure	Assay protocol1: Normal	Assay protocol2: High linearity
Reading Mode	Rate	Rate
Factor	1768	1768
Filter- 1(nm)	340	340
Temperature	37 °C	37 °C
Volume (µl)	450	450
Delay Time (Sec)	60	30
Read Time (Sec)	120	60
Unit	U/l	U/l
Reaction Direction	Decrease	Decrease
Reference Low	0	0
Reference High	45	45
Linearity Limit	450	1600

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. GOT

TEST PROCEDURE

Dispense in tube : working reagent	500 µl
Add Sample	50 µl
Assay Protocol1: Mix and incubate 60 seconds at 37°C, then record first reading of absorbance. Perform other 2 readings at 60 seconds intervals. Calculate the ΔA/min.	

Assay Protocol2: Mix and incubate 30 seconds at 37°C, then record first reading of absorbance. Perform other 2 readings at 30 seconds intervals. Calculate the ΔA/min.

RESULT CALCULATION

Perform calculations in units per litre, multiplying the ΔA/min by the factor.

Activity in U/l = ΔA/min x 1768

SI conversion factor: 1 U/l x 0.017= 1 µkat/l

EXPECTED VALUES

< 45 U/l at 37°C OR 0.8 µkat/l

It is recommended that each laboratory verifies this range or derives reference interval for the population it serves.

QUALITY CONTROL AND CALIBRATION

It is suggested to perform internal quality control with assayed normal (BioNorm) and assayed abnormal (BioPath), to confirm the validity of the test and assure the accuracy of patient result.

When using the recommended Calibrator (BioCal), calibrate the assay:

a. When using a new reagent or lot

b. When QC values are out of range

PERFORMANCE CHARACTERISTICS

1. Linearity

As per assay protocol1: Linearity is up to 450 U/l or 7.7 µkat/l.

As per assay protocol2: Linearity is up to 1600 U/l or 27.2 µkat/l

2. Sensitivity/ Limit of detection (LOD)

The limit of detection is 1 U/l

The limit of quantification is 4 U/l.

3. Interferences

No interference has been observed for the following

Hemoglobin up to 70 mg/dl; Bilirubin up to 40 mg/dl

Triglyceride up to 1000 mg/dl

4. Precision

Intra-assay precision

	Mean	SD	CV
n = 20	U/l	U/l	%
sample 1	46.94	0.43	0.92
sample 2	137.49	0.78	0.57

Inter-assay precision

	Mean	SD	CV
n = 20	U/l	U/l	%
sample 1	49.86	2.41	4.84
sample 2	143.33	2.36	1.65

5. Methods Comparison

Comparison was done between reference AST (SGOT) Reagent and CliniQuant - FSR SGOT Reagent (test)

N = 36 y = 0.967x + 6.082

r^2 = 0.946

LIMITATIONS

Samples with values above 1600 U/l should be diluted with 0.9% saline, re-run and results multiplied by dilution factor

WASTE DISPOSAL

This product is made to be used in professional laboratories. Please consult local regulations for correct waste disposal.

REFERENCES

1. Burtis, C.A., Ashwood, E.R., editors. Tietz Textbook of Clinical Chemistry. 2nd ed. Philadelphia, W.B. Saunders Company, 1994, p. 790 - 795.

2. Data on file: Meril Diagnostics.

IFU/GOTFSR01/00

06-11-2018

Symbols used on Meril Diagnostics labels:

REF	Catalogue No.		Attention See Instruction for Use
LOT	Batch No.	IVD	*In vitro* Diagnostics
	Expiry Date		Consult Instruction for Use
	Manufacturer		Storage Temperature
	Keep Dry		Keep Away from Sunlight
	Manufacturing Date		Do not use if package is damaged

EC REP Authorized European Representative in the European Community

Appendix 14. ALP

Alkaline Phosphatase Kit

CliniQuant - FSR

Modified IFCC, Kinetic

Meril Diagnostics

For *in vitro* diagnostic use
Read this pack insert thoroughly before use

REF	Pack Size	R1 ALP Reagent
ALPFSR-01	4 x 10ml	4 x 10ml
ALPFSR-02	5 x 20ml	5 x 20ml

INTENDED USE

This reagent is intended for quantitative determination of alkaline phosphatase in human serum.

CLINICAL SIGNIFICANCE

ALP occurs in high levels in liver, bone, intestine and placenta. Increased levels occur in hepatobiliary diseases and bone diseases. Elevated ALP occurs in pregnant women and growing children.

PRINCIPLE OF THE METHOD

The enzyme alkaline phosphatase hydrolizes the 4-NitroPhenolPhosphate to release 4-nitrophenol, under alkaline conditions. The 4-nitrophenol formed is detected spectrophotometrically at 405 nm to give a measurement of alkaline phosphatase activity in the sample.

2-amino-2-methyl-1-propanol + p-nitrophenylphosphate + H_2O
↓ ALP
4-nitrophenol + 2-amino-2-methyl-1-propanol phosphate

KIT COMPONENTS

Composition

R1 - Alkaline Phosphatase Reagent : AMP buffer 700 mmol/l , magnesium salt 2.7 mmol/l, zinc salt 1.36mmol/l, HEDTA 2 mmol/l : 2.69mmol/l, pNPP 19.51 mmol/l.

MATERIALS REQUIRED BUT NOT PROVIDED

Laboratory instrumentation, Spectrophotometer UV/VIS with thermostatic cuvette holder or clinical chemistry analyzer: semi automated, calibrated micropipettes, glass or high quality polystyrene cuvettes, test tubes/rack, heating bath, controls, saline.

REAGENT PREPARATION, STORAGE & STABILITY

Reagent is ready to use. Keep away from direct light sources.
Stability: up to expiration date on labels at 2-8 °C.
Stability since first opening of bottle: ≤ 60 days at 2-8 °C.

REAGENT DETERIORATION

Discard the reagent if absorbance exceeds 1.25 against distilled water at 405 nm.

WARNINGS AND PRECAUTIONS

1. Reagent may contain some non-reactive and preservative components. It is suggested to handle carefully, avoiding contact with skin and ingestion.
2. Specimens should be considered infectious and handled appropriately.
3. Perform the test according to the general "Good Laboratory Practice" (GLP) guidelines.

SPECIMEN

Serum, plasma (heparinate only). Sera kept at room temperatures usually show a slight increase in activity, which varies from 1% over a 6 hour period to 3 to 6% over a 1 to 4 days period. Even in sera stored at refrigerator temperature, activity increases slowly. In frozen sera, activity decreases but slowly recovers after thawing the serum. EDTA, Citrate, and Oxalate are not suitable because of inhibition of ALP activity.

Programme Parameter for MERILYZER CliniQuant

Reading Mode	Rate
Factor	2764
Filter – 1 (nm)	405
Temperature	37 °C
Volume (µl)	500
Delay Time (Sec)	60
Read Time (Sec)	120
Unit	U/L
Reaction Direction	Increase
Reference Low	42
Reference High	128
Linearity Limit	1200

TEST PROCEDURE

Dispense reagent in tube	1000 µl
Sample	20 µl
Mix, execute a first reading of absorbance after 1 minute, incubating at 37°C. Perform other 2 readings at 60 seconds intervals. Calculate the ΔA/min.	

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. ALP

RESULT CALCULATION

Serum/plasma: ALP U/l = ΔA/min x 2764

SI conversion factor: 1 U/l x 0.017 = 1 µkat/l

CONVERSION FACTOR

Following factors can be used for conversion of IU/l from one temperature to another.

Temperature of assay	Temperature factors		
	25°C	30°C	37°C
25°C	1.0	1.30	1.80
30°C	0.75	1.0	1.35
37°C	0.55	0.74	1.0

EXPECTED VALUES (in U/l at 37°C)

Age	Males	Females
Newborns (1-3 days)	95 – 368	95 – 368
2 – 24 months	115 – 460	115 – 460
2 – 5 years	115 – 391	115 – 391
6 – 7 years	115 – 460	115 – 460
8 – 9 years	115 – 345	115 – 345
10 – 11 years	115 – 336	115 – 437
12 – 13 years	127 – 403	92 – 336
14 – 15 years	79 – 446	78 – 212
16 – 18 years	58 – 331	35 – 124
Adults	41 – 137	39 - 118

It is recommended that each laboratory verifies this range or derives reference interval for the population it serves.

QUALITY CONTROL AND CALIBRATION

It is recommended to perform internal quality control with assayed normal (BioNorm) and assayed abnormal (BioPath), to confirm the validity of the test and assure the accuracy of patient result. When using the recommended Calibrator (BioCal), calibrate the assay:

a. When using a new reagent or lot

b. When QC values are out of range

Symbols used on Meril Diagnostics labels:

REF	Catalogue No.		Attention See Instruction for Use
LOT	Batch No.	IVD	*In vitro* Diagnostics
	Expiry Date		Consult Instruction for Use
	Manufacturer		Storage Temperature
	Keep Dry		Keep Away from Sunlight
	Manufacturing Date		Do not use if package is damaged
EC REP	Authorized European Representative in the European Community		

PERFORMANCE CHARCTERISTICS

1. Linearity

The linearity is up to 1200 U/l or 20.4 µkat/l.

2. Sensitivity/ Limit of detection (LOD)

The limit of detection is 1.8 U/l.

The limit of quantification is 5.5 U/l.

3. Interferences

No interference has been observed for the following

Hemoglobin up to 1200 mg/dl; Bilirubin up to 10 mg/dl

Triglycerides up to 1900 mg/dl; Ascorbate up to 30 mg/dl

4. Precision

Intra-assay precision

	Mean	SD	CV
n = 20	U/l	U/l	%
sample 1	81.7	1.08	1.32
sample 2	208.5	1.73	0.83

Inter-assay precision

	Mean	SD	CV
n = 20	U/l	U/l	%
sample 1	85.0	2.8	3.3
sample 2	207	2.89	1.4

5. Methods Comparison

Comparison was done between reference ALP Reagent and CliniQuant -FSR Alkaline Phosphatase Reagent (test)

N = 20 y = 0.962x – 3.622 U/l

r^2 = 0.999

LIMITATIONS

Samples with values above 1200 U/l should be diluted with 0.9% saline, re-run and results multiplied by dilution factor

WASTE DISPOSAL

This product is made to be used in professional laboratories. Please consult local regulations for correct waste disposal

REFERENCES

1. Burtis, C.A., Ashwood, E.R., editors. Tietz Textbook of Clinical Chemistry. 2nd ed. Philadelphia, W.B. Saunders Company, 1994, p. 830 - 843.

2. Data on file: Meril Diagnostics.

IFU/ALPFSR01/00

06-11-2018

Appendix 15. Glucose

Glucose Kit

CliniQuant – FSR

Trinder' s Method, End Point

Meril Diagnostics

For *in vitro* diagnostic use
Read this pack insert thoroughly before use

REF	Pack Size	R1 Glucose Reagent	R2 Glucose Standard
GLUFSR-01	4 x 50ml	4 x 50ml	1 x 5ml
GLUFSR-02	5 x 100ml	5 x 100ml	1 x 5ml
GLUFSR-03	4 x 250ml	4 x 250ml	2 x 5ml
GLUFSR-04	4 x 500ml	4 x 500ml	2 x 5ml

INTENDED USE

This reagent is intended for quantitative determination of glucose concentration in human serum or plasma.

CLINICAL SIGNIFICANCE

Glucose measurements are used in the diagnosis and treatment of carbohydrate metabolism disorders including diabetes mellitus, hypoglycaemia and various other conditions.

PRINCIPLE OF THE METHOD

Glucose in the sample is oxidised to yield gluconic acid and hydrogen peroxide in the presence of Glucose oxidase. The enzyme peroxidase catalyses the oxidative coupling of 4-aminoantipyrine with phenol to yield a coloured quinonemine complex, with absorbance proportional to the concentration of glucose in sample.

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{Glucose Oxidase}} \text{Gluconic acid} + H_2O_2$$

$$H_2O_2 + \text{phenol} + \text{4AAP} \xrightarrow{\text{Peroxidase}} \text{Quinonemine dye} + 2H_2O$$

KIT COMPONENTS

Composition

R1 - Glucose Reagent: Dipotassium hydrogen phosphate 21.7 g/l , Potassium dihydrogen phosphate 10.2 g/l ,4-AAP 0.081 g/l , Phenol 0.282 g/l , Peroxidase 0.8ku/l ,Glucose oxidase 15 ku/l, Sodium glutamate 15 g/l, p-Chloro meta cresol 0.01 % ,Sodium Azide 0.025 %

R2 - Glucose Standard : 100 mg/dl, Benzoic acid 0.2 %, Dextrose 1 g/l

MATERIALS REQUIRED BUT NOT PROVIDED

Laboratory instrumentation, Spectrophotometer UV/VIS with thermostatic cuvette holder or clinical chemistry analyzer: semi automated, calibrated micropipettes, glass or high quality polystyrene cuvettes, test tube/ rack, heating bath, controls, saline.

REAGENT PREPARATION, STORAGE & STABILITY

Reagent is ready to use. Keep away from direct light sources.

Stability: up to expiration date on labels at 2-8 °C.

Stability since first opening of bottle: preferable within 60 days at 2-8 °C.

REAGENT DETERIORATION

1. Discard the reagent if absorbance exceeds 0.3 against distilled water.
2. Keep the Standard vial plugged after use, in order to avoid deterioration.

WARNINGS AND PRECAUTIONS

1. Reagent may contain some non-reactive and preservative components. It is suggested to handle carefully, avoiding contact with skin and ingestion.
2. Specimens should be considered infectious and handled appropriately.
3. Perform the test according to the general "Good Laboratory Practice" (GLP) guidelines.

SPECIMEN

Use fresh unhaemolysed serum. The stability of glucose in specimen is reduced by bacterial contamination and by glycolysis. Serum or plasma should be separated from the cells, as soon as possible, to prevent glycolysis. The addition of sodium fluoride is recommended to inhibit glycolysis. Serum / plasma is stable for 3 days at 2-8 °C. It is recommended to perform the assay with freshly collected samples, as glycolysis decreases serum glucose by approximately 5 to 10 mg/dl in 1 hr in normal uncentrifuged coagulated blood at room temperature.

Programme Parameter for MERILYZER CliniQuant

Assay protocol1: Normal	
Reading Mode	End Point
Standard Conc.	100 (mg/dl)
Filter – 1 (nm)	505
Filter – 2 (nm)	620
Temperature	37 °C
Volume (µl)	500
Delay Time (Sec)	5
Reaction Direction	Increase
Reference Low	70
Reference High	110
Linearity Limit	500

Assay protocol2: High linearity	
Reading Mode	Fixed Time
Standard Conc.	100 (mg/dl)
Filter – 1 (nm)	505
Temperature	37 °C
Volume (µl)	500
Delay Time (Sec)	30
Read Time (Sec)	60
Reaction Direction	Increase
Reference Low	70
Reference High	110
Linearity Limit	800

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. Glucose

TEST PROCEDURE

Assay Protocol1:

Dispense	Blank	Standard	Sample
Reagent 1	1ml	1ml	1ml
Distilled water	10 µl	-	-
Standard	-	10 µl	-
Sample	-	-	10 µl
Mix, incubate for 10 min at 37°C. Read absorbance of standard (As) and samples (Ax) against reagent blank.			

Assay Protocol2:

Dispense	Standard	Sample
Reagent 1	1000 µl	1000 µl
Standard	10 µl	-
Sample	-	10 µl
Mix, incubate 30 seconds at 37°C, then record absorbance as A1. After exactly 60 seconds, record again absorbance as A2.		

RESULT CALCULATION

Serum/plasma:

Glucose mg/dl = Ax/As x Concentration of Standard

or

Glucose mg/dl = A2-A1(sample)/A2-A1(standard) x concentration of Standard.

SI conversion factor: 1 mg/dl x 0.0555 = 1 mmol/l

EXPECTED VALUES

Glucose Fasting:	70 - 110 mg/dl	OR	3.8 – 6.1 mmol/l
Post Prandial:	90 - 140 mg/dl	OR	5.0 – 7.8 mmol/l

It is recommended that each laboratory verifies this range or derives reference interval for the population it serves.

QUALITY CONTROL AND CALIBRATION

It is recommended to perform internal quality control with assayed normal (BioNorm) and assayed abnormal (BioPath), to confirm the validity of the test and assure the accuracy of patient result.

Using the recommended Calibrator (BioCal) or the Standard included, calibrate the assay:

a. When using a new reagent or lot

b. When QC values are out of range

PERFORMANCE CHARACTERISTICS

1. Linearity

As per assay protocol1: The linearity is up to 500 mg/dl (27.5 mmol/l).

As per assay protocol2: The linearity is up to 800 mg/dl (44 mmol/l).

2. Sensitivity/ Limit of detection (LOD)

The limit of detection is 4 mg/dl (0.22 mmol/l).

The limit of quantification is 13 mg/dl (0.72 mmol/l).

3. Interferences

No interference has been observed for the following

Hemoglobin up to 750mg/dl; Bilirubin up to 20 mg/dl

Triglycerides up to 500 mg/dl

4. Precision

Intra-assay precision

	Mean	SD	CV
n = 20	mg/dl	mg/dl	%
sample 1	97.77	0.61	0.63
sample 2	261.55	1.32	0.50

Inter-assay precision

	Mean	SD	CV
n = 20	mg/dl	mg/dl	%
sample 1	92.41	4.47	4.84
sample 2	252.24	2.77	1.16

5. Methods Comparison

Comparison was done between reference Glucose Reagent and CliniQuant - FSR Glucose Reagent (test)

N = 23 $y = 0.961x + 13.74$

$r^2 = 0.987$

LIMITATIONS

Samples with values above 800 mg/dl should be diluted with 0.9% saline, re-run and multiply results by dilution factor.

WASTE DISPOSAL

This product is made to be used in professional laboratories. Please consult local regulations for correct waste disposal.

REFERENCES

1. Thomas L: Clinical Laboratory Diagnostics, 1st ed. Frankfurt: TH-Books Verlagsgesellschaft; 1998, p. 131 - 7.

2. Sacks, D.B.: Carbohydrates. In: Burtis, C.A., Ashwood, E.R., editors. Tietz Textbook of Clinical Chemistry. 3rd ed. Philadelphia, W.B. Saunders Company, 1999, p. 750 – 808.

3. Barham, D., Trinder, P.: An improved color reagent for the determination of blood glucose by the oxidase system. Analyst, 1972, 97; 142 - 5.

4. Data on file: Meril Diagnostics.

IFU/GLUFSR01/00
06-11-2018

Symbols used on Meril Diagnostics labels:

REF	Catalogue No.		Attention See Instruction for Use
LOT	Batch No.	IVD	*In vitro* Diagnostics
	Expiry Date		Consult Instruction for Use
	Manufacturer		Storage Temperature
	Keep Dry		Keep Away from Sunlight
	Manufacturing Date		Do not use if package is damaged
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Appendix 16 **LDH**

Cromatest

LINEAR

LDH BR CE

REF 1141010
2 x 50 mL

CONTENTS
R1. Reagent 2 x 40 mL
R2. Reagent 1 x 20 mL

For *in vitro* diagnostic use only

LDH BR

SFBC
UV enzymatic method
KINETIC

PRINCIPLE

Lactate dehydrogenase (LD/LDH) catalyzes the reduction of pyruvate to lactate (P-L) in the presence of reduced nicotinamide adenine dinucleotide (NADH) at pH 7.5.
The reaction is monitored kinetically at 340 nm by the rate of decrease in absorbance resulting from the oxidation of NADH to NAD^+ proportional to the activity of LD present in the sample.

$$\text{Pyruvate} + \text{NADH} + H^+ \xrightarrow[\text{pH 7.5}]{\text{LD/LDH}} \text{L-Lactate} + NAD^+$$

This test has been formulated according the standarized method described by SFBC.[1]

REAGENT COMPOSITION

R1 **LDH substrate.** TRIS buffer 100 mmol/L pH 7.5, pyruvate 2.75 mmol/L, sodium chloride 222 mmol/L.

R2 **LDH coenzyme.** NADH 1.55 mmol/L.

STORAGE AND STABILITY

Store at 2-8ºC.
All the kit compounds are stable until the expiry date stated on the label. Do not use reagents over the expiration date.
Store the vials tightly closed, protected from light and prevented contaminations during the use.

Discard if appear signs of deterioration:
- Presence of particles and turbidity.
- Blank absorbance (A) at 340 nm < 1.000 in 1cm cuvette.

REAGENT PREPARATION

Working reagent. Mix 4 mL of **R1** + 1 mL of **R2**. Stable for 2 months at 2-8ºC.
Protected from light.

SAMPLES

Serum free of hemolysis separated from the cells as soon as possible after collection. The use of heparin and citrate as anticoagulants have been reported to falsely elevate LD activity.
Freezing results in loss of activity of the enzyme.

INTERFERENCES

- Lipemia (intralipid > 20 g/L) does not interfere.
- Bilirubin (> 40 mg/dL) does not interfere.
- Hemoglobin (> 4 g/L) may affect the results.
- Other drugs and substances may interfere[3].

MATERIALS REQUIRED

- Photometer or spectrophotometer with a thermostatted cell compartment set at 30/37ºC, capable of reading at 340 nm.
- Stopwatch, strip-chart recorder or printer.
- Cuvettes with 1-cm pathlength.
- Pipettes to measure reagent and samples.

PROCEDURE

1. Preincubate working reagent, samples and controls to reaction temperature 30/37ºC.
2. Set the photometer to 0 absorbance with distilled water.
3. Pipette into a cuvette:

Reaction temperature	30/37ºC
Working reagent	1.0 mL
Sample or control	20 µL

4. Mix gently by inversion. Insert cuvette into the cell holder and start stopwatch.
5. Incubate for 30 seconds and record initial absorbance reading.
6. Repeat the absorbance readings exactly after 1, 2 and 3 minutes.
7. Calculate the difference between absorbances.
8. Calculate the mean of the results to obtain the average change in absorbance per minute (ΔA/min).

CALCULATIONS

U/L = ΔA/min x 8095

Samples with ΔA/min exceeding 0.150 at 340 nm should be diluted 1:10 with saline and assayed again. Multiply the results by 10.

If results are to be expressed as SI units apply:
U/L x 0.01667 = µkat/L

QUALITY SYSTEM CERTIFIED
ISO 9001 ISO 13485

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LDH

cromatest

LINEAR

REFERENCE VALUES[4]

Serum

Temperature	37ºC	30ºC
Adults	207 - 414 U/L (3.40 - 6.80 µKat/L)	140 - 280 U/L (2.30 - 4.70 µKat/L)

It is recommended that each laboratory establishes its own reference range.

QUALITY CONTROL

To ensure adequate quality control (QC), each run should include a set of controls (normal and abnormal) with assayed values handled as unknowns.

REF **1980005** HUMAN MULTISERA NORMAL
Borderline level of LDH. Assayed.

REF **1985005** HUMAN MULTISERA ABNORMAL
Elevated level of LDH. Assayed.

If the values are found outside of the defined range, check the instrument, reagents and procedure.
Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

CLINICAL SIGNIFICANCE[2,5]

The enzyme activity found in circulation is a mixture of five isoenzymes. Each organ has a characteristic isoenzyme profile. Leakage of these isoenzymes from a diseased organ results in the elevation of total serum LD.

Increased levels become evident 8-12 hours after myocardial infarction reaching a maximum 4-5 days later. Elevated values in serum are also encountered in cases of pulmonary embolism and in about one third of patients with renal disease, specially those with pyelonephritis or tubular necrosis. In toxic hepatitis with jaundice, Hodking´s disease and abdominal and lung cancers elevations are specially high.

Moderate increases are also observed in cases of liver disease, megaloblastic and pernicious anemia and progressive muscular dystrophy.

Decreases are not important clinically.

ANALYTICAL PERFORMANCE

- ***Detection Limit :*** 15.73 U/L
- ***Linearity :*** Up to 1500 U/L
- ***Precision:***

U/L	Within-run		Between-run	
Mean	495	879	495	879
SD	4.32	7.34	14	13.5
CV%	0.87	0.83	2.83	1.54
N	10	10	10	10

- ***Sensitivity :*** 0.152 mA / U/L LDH
- ***Correlation:*** This assay (y) was compared with a similar commercial method (x). The results were:
  N =52 r = 0.99 y = 1.031x + 3.147

The analytical performances have been generated using on automatic instrument. Results may vary depending on the instrument.

NOTES

1. This method may be used with different instruments. Any application to an instrument should be validated to demonstrate that results meets the performance characteristics of the method. It is recommended to validate periodically the instrument. Contact to the distributor for any question on the application method.
2. Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.

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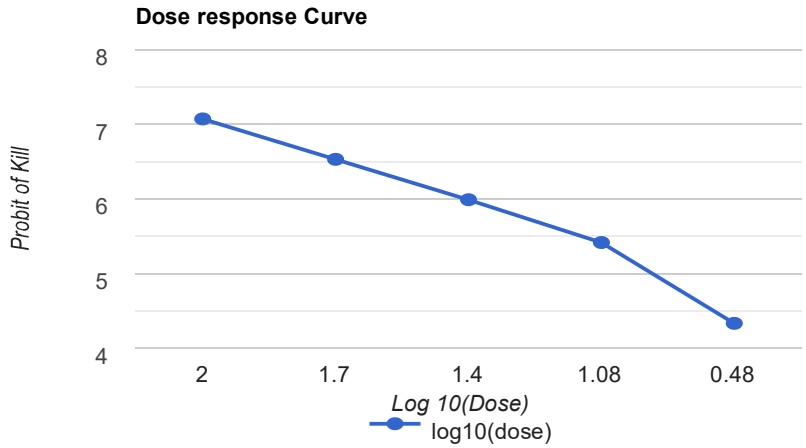
Appendix 17: Statistical data for Effect of CuNPs against Ae. aegypti larvae

Estimation of Probit Line, LC50/LD50 and Fit statistics

Mean X	13.208	**UL**	**13.482**
Mean Y	0.493	Log (LC50)	0.847
Intercept	-1.523	Log LL	0.564
Beta	1.799	Log UL	1.130
LC50	**5.787**	Chi-Square ML	1.573
Sign Chi-Square	0.203	**LL**	**3.662**

Estimation of LC50/LD50 with Confidence interval

Percent	Probit	95 % Confidence Limit for Conc			Variance	95 % Confidence Limit for log (Conc)		
		LC	LL	UL		Log LC	Log LL	Log UL
10.000	-1.282	1.362	0.710	2.614	0.043	0.134	-0.149	0.417
30.000	-0.524	3.591	1.872	6.890	0.019	0.555	0.272	0.838
50.000	0.000	5.787	3.662	13.482	0.011	0.847	0.564	1.130
75.000	0.674	16.662	8.684	31.967	0.008	1.222	0.939	1.505
90.000	1.282	36.581	18.889	69.532	0.015	1.559	1.276	1.842
99.000	2.326	138.045	71.951	264.853	0.045	2.140	1.857	2.423



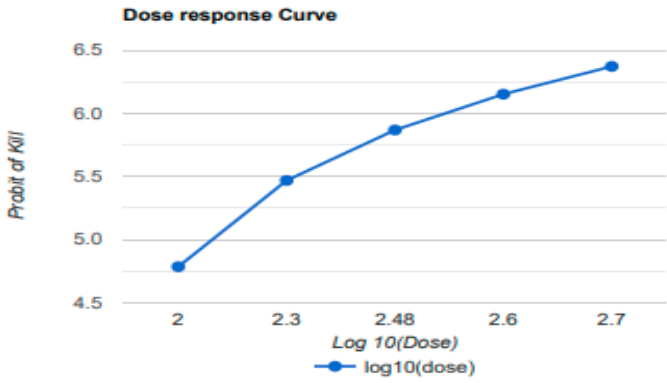
Appendix 18: Statistical data for Effect of Green Coffee against *Ae. aegypti* larvae

Estimation of Probit Line, LC50/LD50 and Fit statistics

Mean X	19.205	LL	95.143
Mean Y	0.383	UL	162.984
Intercept	-1.523	Log (LC50)	2.095
Beta	3.798	Log LL	1.978
LC50	124.526	Log UL	2.212
Sign Chi-Square	0.090	Chi-Square ML	6.482

Eestimation of LC50/LD50 with Confidence interval

Percent	Probit	95 % Confidence Limit for Conc			Variance	95 % Confidence Limit for log (Conc)		
		LC	LL	UL		Log LC	Log LL	Log UL
10.000	-1.282	33.965	25.950	44.454	0.016	1.531	1.414	1.648
30.000	-0.524	73.178	55.911	95.778	0.007	1.864	1.747	1.981
50.000	**0.000**	**124.526**	**95.143**	**162.984**	**0.003**	**2.095**	**1.978**	**2.212**
75.000	0.674	246.729	188.511	322.927	0.001	2.392	2.275	2.509
90.000	**1.282**	**456.555**	**348.826**	**597.554**	**0.003**	**2.659**	**2.543**	**2.776**
99.000	2.326	1,316.705	1,006.015	1,723.347	0.014	3.119	3.003	3.236



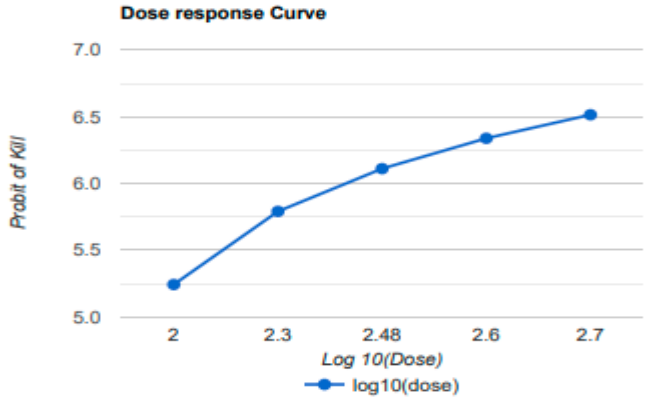
Appendix 19: Statistical data for Effect of Roasted Coffee against *Ae. aegypti* larvae

Estimation of Probit Line, LC50/LD50 and Fit statistics

Mean X	13.208	LL	51.382
Mean Y	0.493	UL	104.995
Intercept	-1.523	Log (LC50)	1.866
Beta	1.799	Log LL	1.711
LC50	73.450	Log UL	2.021
Sign Chi-Square	0.203	Chi-Square ML	4.610

Eestimation of LC50/LD50 with Confidence interval

Percent	Probit	95 % Confidence Limit for Conc			Variance	95 % Confidence Limit for log (Conc)		
		LC	LL	UL		Log LC	Log LL	Log UL
10.000	-1.282	33.965	25.950	44.454	0.016	1.531	1.414	1.648
30.000	-0.524	3.591	1.872	6.890	0.019	0.555	0.272	0.838
50.000	0.000	5.787	3.662	13.482	0.011	0.847	0.564	1.130
75.000	0.674	16.662	8.684	31.967	0.008	1.222	0.939	1.505
90.000	1.282	36.581	18.889	69.532	0.015	1.559	1.276	1.842
99.000	2.326	138.045	71.951	264.853	0.045	2.140	1.857	2.423



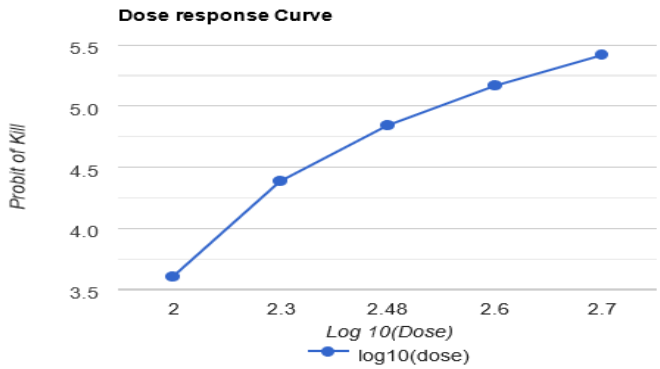
Appendix 20: Statistical data for Effect of CuNPs against *Artemia salina* larvae

Estimation of Probit Line, LC50/LD50 and Fit statistics

Mean X	292.046	LL	275.990
Mean Y	-0.185	UL	429.479
Intercept	-6.584	Log (LC50)	2.537
Beta	2.595	Log LL	2.441
LC50	344.285	Log UL	2.633
Sign Chi-Square	0.715	Chi-Square ML	1.361

Eestimation of LC50/LD50 with Confidence interv

Percent	Probit	95 % Confidence Limit for Conc			Variance	95 % Confidence Limit for log (Conc)		
		LC	LL	UL		Log LC	Log LL	Log UL
10.000	-1.282	110.444	88.536	137.774	0.004	2.043	1.947	2.139
30.000	-0.524	216.207	173.318	269.708	0.001	2.335	2.239	2.431
50.000	**0.000**	**344.285**	**275.990**	**429.479**	**0.001**	**2.537**	**2.441**	**2.633**
75.000	0.674	626.319	502.078	781.303	0.003	2.797	2.701	2.893
90.000	**1.282**	**1,073.231**	**860.338**	**1,338.806**	**0.007**	**3.031**	**2.935**	**3.127**
99.000	2.326	2,711.739	2,173.820	3,382.768	0.019	3.433	3.337	3.529



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E. Papers at national and international scientific seminars and conferences.

1. "Biological and Biochemical Studies of *Biomphalaria arabica* Snails Exposed to Extract *Coffee arabica* Plant, The 5th International Conference on Science, 27 – 28 May 2022, UNHAS, Makassar, Indonesia.
2. "Histopathological and larvicidal study of copper nanoparticles synthesized using *Coffee arabica* extract against the dengue vector *Aedes aegypti* mosquito. 4th ICChSE International Conference on Chemistry and Science Education, 12 - 13 August, 2023 Universitas Negeri Padang, Indonesia.
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