NQLM
Nordic Committee for External
Quality Assurance Programmes
in Laboratory Medicine

NORDIC INTERFERENCE STUDY, March 2000

Effects of Intralipid on some common serum analysis

Enclosed please find the results of the lipid interference study of March 2000. We regret that the report is late, but as this is not a routine survey, some extra statistical work is needed, - however, we hope you will excuse us for the delay. 206 Nordic laboratories participated in this joint survey, - 37 from Denmark; 37 from Finland, 4 from Island, 31 from Norway and 97 from Sweden. The study was initiated by NQLM, Nordic committee for external quality assurance programmes in laboratory medicine, - and run as a collaboration between all the Nordic EQA organisers, according to a protocol worked out by NQLM. The sera were prepared and mailed directly from Sero AS in Oslo. Equalis (the Swedish EQA organisation) has done the data processing and the mailing of the results directly to the participating laboratories.

Material

The material included a series of 6 sera, comprising of 3 sets with varying lipid concentrations, each set with a normal level and a slightly patological concentration level of the 13 analytes selected for this study.

Table 1. Lipid contents

Lipid set	Triglyc. in matrix, mmol/L	Intralipid added mmol/L (g/L)	Triglyc + glycerol theoretical, mmol/L	Triglyc + glycerol analysed, mmol/L
Normal (samples 2 & 3)	0,8	0	0,8	0,8
2.5 g/L (samples 4 & 5)	0,8	3,1 (2,5)	3,9	5,5
5,0 g/L (samples 6 & 1)	0,8	6,2 (5.0)	7,0	10,9

Table 2. Concentration of the components in the serum without intralipid, given as the overall mean value.

Component	Normal	Abnormal
Na, mmol/L	129	126
K, mmol/L	2,8	4,8
Ca, mmol/L	2,15	2,50
Mg, mmol/L	0,9	1,1
Fe, µmol/L	18	22
Bilirubin, µmol/L	17	28
Uric acid, µmol/L	205	508
ASAT, U/L	57	148
ALAT, U/L	49	138
GT, U/L	75	110
Protein g/L	65	79
Albumin, g/L	40	49
Cholesterol, mmol/L	4,5	5,5

Production of the material

Sera from voluntary blood donors from Scandinavian blood centers were spiked the varoius components to give the two final concentration levels above. Intralipid (Mainly triglycerides of palmitin- and stearinacids, Pharmacia Upjohn lot no. 20508-51 and 409905A) was added according to Glick et al. (5), in such a way that the volume and matrix of the added intralipid (0, 2,5 and 5 g intralipid) were exactly equal. There were no other additives than the spiking material.

Mailing and analysis

The sera were mailed as *A-priority mail* directly to the participating laboratories on February 29th, - the following day after production, - packed in polystyrene foam, but with no further temperature control packing. The

samples were mailed and received by the laboratories after 1-3 days and were analysed on March 3rd with a few exceptions.
The analysis were done in triplicates and the mean values transferred to the result form. The labs were asked to omit outliers according to "common sense".

Statistics and result presentation

Grouping. The results are grouped according to the instrument used.

Exclusion of outliers. As a principle all results are processed, also those where the lab have commented that the sample would ordinarily not have been analysed, due to turbidity.
Outliers that clearly do not belong to the result population were excluded, as were also cases of obvious sample mix up.

Numerical summaries. These tables give mean values, SD and CV % in each sample, for each instrument group. For groups of ≤ 3 results SD and CV are not given. Underneath each table the statistics for the total pool of results are given.

Graphs. Graphs are given for each analyte at each of the two concentration levels, as difference plots, using the mean values of the 0 g intralipid/L samples as 100 %. Each instrument group has the same style and colours in all graphs. For groups of ≤ 3 results curves are not given. If your results belong to such a group, we recommend to calculate the points for your group and plot them on the respective graphs for comparison to the others. Especially for analytes where interference is seen,
bilirubin, uric acid and iron this may be informative.

Example for the calculation of one of the 2,5 g/L points:

$$\text{\% on the Y-axis} = \frac{(\text{Xmean 2,5g/L} - \text{Xmean 0 g/L}) \times 100}{\text{Xmean 0 g/L}}$$

Please note that the graph scales are made in 4 sizes:

90 - 110 % (Na, K, Ca, Mg, protein, cholesterol, ASAT, GT)
90 - 120 % (albumin, ALAT and uric acid) and
80 - 140 % (Fe)
80 - 160 % (bilirubin)
For further explanation to the graphs literature reference (5) is recommended.

Comments

The labs were asked to analyse the samples regardless of whether they would have done so if they were routine patient samples. The labs were also asked to give the same comments as they would have done in the routine. The comments were however rather scarce, so we do not know for sure what actions the labs might have taken if these were routine samples. However the main purpose of the interference project is to get information about the robustness of various analysis systems towards various kinds of interference.

We know that abnormal levels of lipids in human sera may cause two kinds of problems on routine analysers, one due to *the water displacement effect* and one due to *the turbidity effect.*

The water displacement effect is generally most apparent on serum Na and Cl determinations, and is clearly seen as the discrepancy between direct and indirect ISE, in the sense that indirect ISE measurements (diluted samples) give "artificially" low molar concentrations on the electrolytes, while direct ISE (undiluted) measurements, which in principle measures molality, reflects the body regulations.

The effects of lipids on the turbidity of the serum samples may be severe, and warnings are usually given with the reagent information on each analyte, saying how high lipid content measured as triglycerides may be tolerated.

To mimic lipemic samples and problems with lipid interference, addition of Intralipid is frequently used, e.g. by Glick and others (1-5). However the lipids of Intralipid have other physical properties than human lipoproteins, and the water displacement effect is not seen at the level that causes turbidity problems. *This study is therefore limited to the optical interference of turbidity.*

Many of the newer instruments apply bi- or polycromatic readings to cope with the blanking problem, as separate serum blank is usually avoided due to increased costs and slow down of the throughput of discrete analysers. Many of these instruments have diode array optics, but it is known that Kone has interference filters. Such instruments (like Hitachi, Integra, Syncron, Axon, and partly Kone instruments) etc, should in principle handle common interference like lipemia better than those which only have monochromatic reading. Also the Vitros instruments, with its special dry chemistry technology is known to handle the blanking problem well.

The results of this lipid interference study show that some of the routine analysers (Hitachi, Vitros, Beckmann) do not seem to be harmed by the turbidity applied to the samples, whereas other analysers seem to be effected for certain analytes. In the cases where interference is seen the variation does not seem to be markely increased, indicating that the interference is rather systematic in nature.

Two general observations concerning optical interference is: A. when interference occur, it is in principle proportional to the amount of intralipid added, and B. the interference, when given as % deviation from the "0 sample", as we have done here, is more pronounced at the lower analyte concentrations than at the higher, - as is expected when the interference is due to a blanking problem.

Bilirubin. The most severe interference observed in this study is the determination of bilirubin on Abbott Aeroset and Cobas Integra. The methods are different, but they are both end point with bichromatic readings. The Integra method sheet gives clear warnings: "avoid lipemia, even slight lipemia interfers with the test". However only 8 out of 21 Integra users had commented that the high lipid samples would not routinely have been analysed until depilidisation (or dilution) was done. The Abbott method sheet for bilirubin claims no interference of triglycerides at 8.5 mmol/l. None of the Abbott users had given any comments about the high lipid samples.

Iron. The iron concentrations were about the same in both series, and so were the interference pattern. Some interference was seen in the high lipid samples on the Kone and Cobas Fara analysers. Konelab offers two alternative methods, one with bichromatic reading and one with sample blank. The Cobas Fara method is run with a sample blank reading at 612nm after addition of all the reagents except the ferrozine dye. The final reading is at 550 nm.

Protein. The Kone instruments show some interference on the low level protein, although the reading is bichromatic.

Uric acid. Some interference is seen in the low uric acid sample on Aeroset and Axon. The Areoset uses a Trinder end point reaction with bichromatic reading. The method sheet informs about ca 9 % positive interference at triglycereide levels of 8.5 mmol/l. No sample comments were given by the Aeroset users.

5-10 % interference was occasionally seen with the remaining analytes, but these observations are not regarded to be of clinical importance.

It is hardly relevant to compare our results here with the results of Glick and coworkers, since most of the instruments (US 10 years ago!) are no longer in use, - new models have taken over. But the interference problem is still with us, as we have seen, both from the hemolysis- and from this study. Next year we will study bilirubin interference, which might be the toughest one to cope with!

Thank you for your participation!

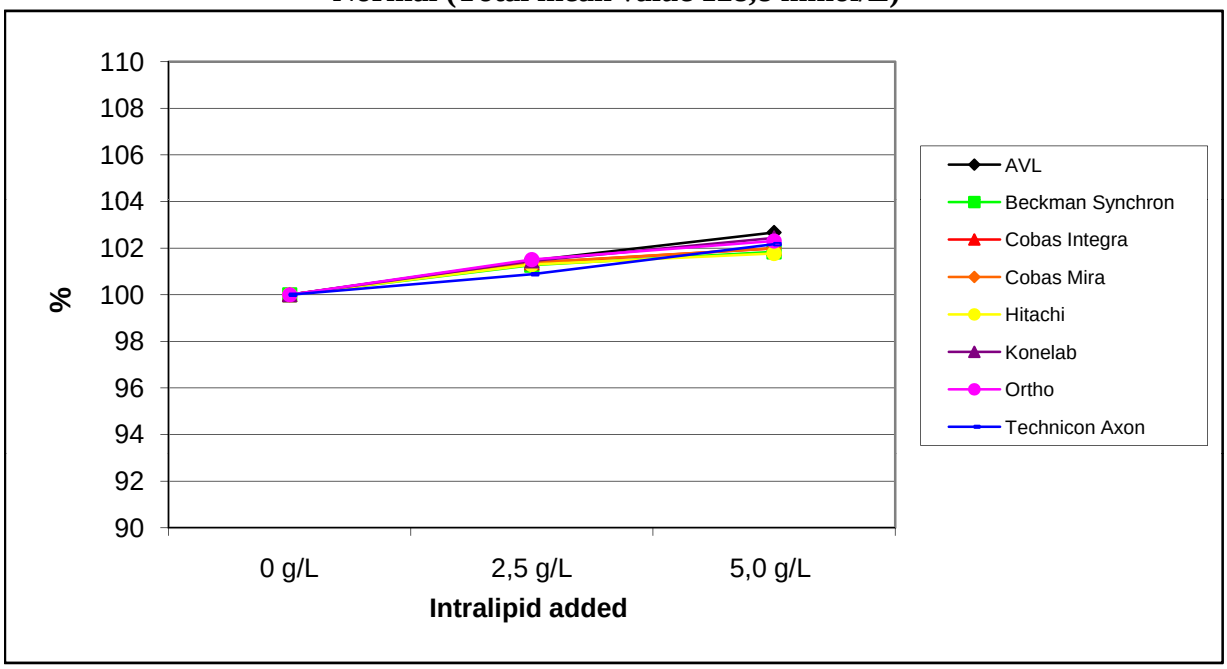
On behalf of NQLM,
Adam Uldall (DEKS), Minna Loikkanen (Labquality), Elin Olafsdottir (Island) ,
Jaak Eintrei Equalis) and Heidi Steensland (NKK)

Literature references:

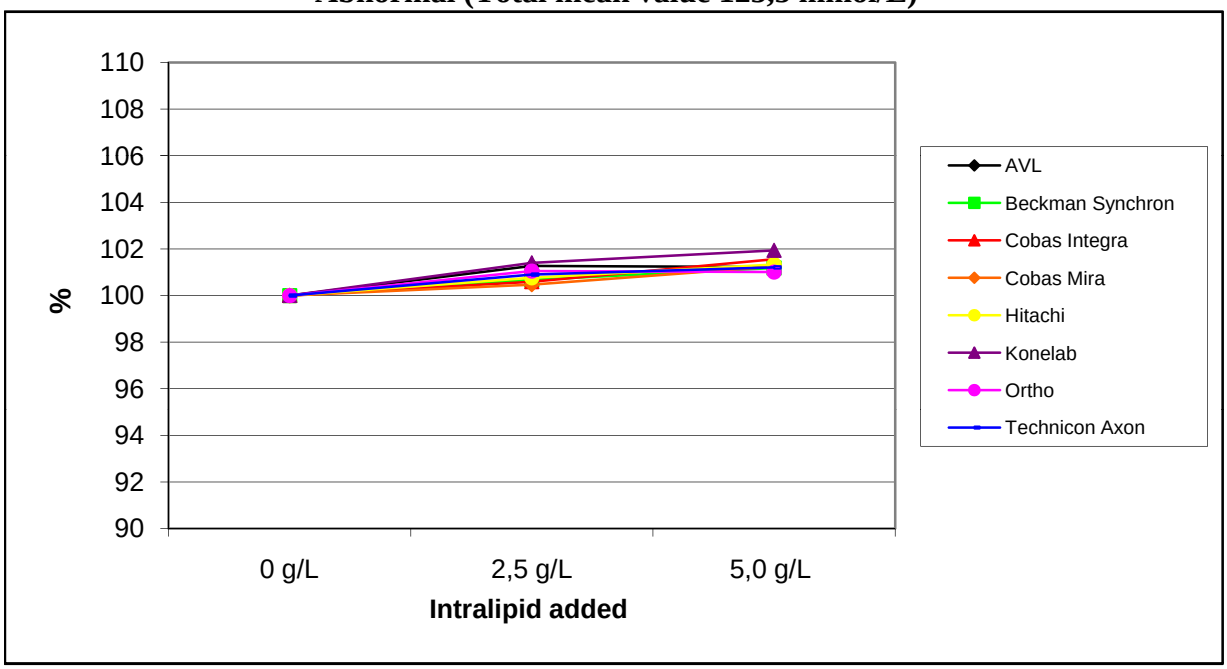
1. Glick MR; Ryder KW. Analytical systems ranked by freedom from interferences. Clin.Chem. 1987;33:1453-8
2. Glick MR; Ryder KW; Jackson S.A Graphical Comparisons of Interferences in Clinical Chemistry Instrumentation. Clin.Chem. 1986;33:470
3. Glick MR; Ryder KW; Glick SJ. Incidence and amount of turbidity, hemolysis and icterus in serum from outpatients. Lab.Med. 1991;22:415-18
4. Glick MR; Ryder KW. Erroneous laboratory results from hemolyzes, icteric and lipemic specimens. Clin.Chem. 1993;39:175-6
5. Glick MR; Ryder KW; Glick SJ. Interferographs, User's guide to interferences in clinical chemistry instruments, 2nd edition, 1991; Science Enterprices, Inc, USA.
ISBN 0-930116-08-9.

Sodium (Na)

Normal (Total mean value 128,8 mmol/L)

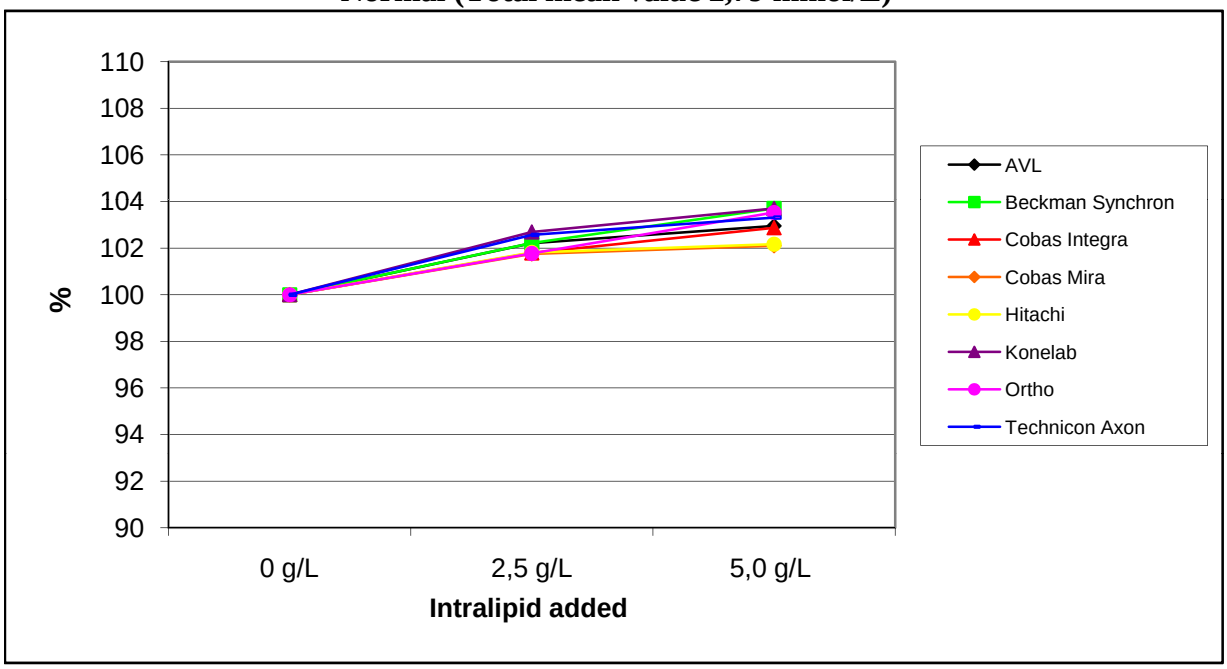


Abnormal (Total mean value 125,5 mmol/L)

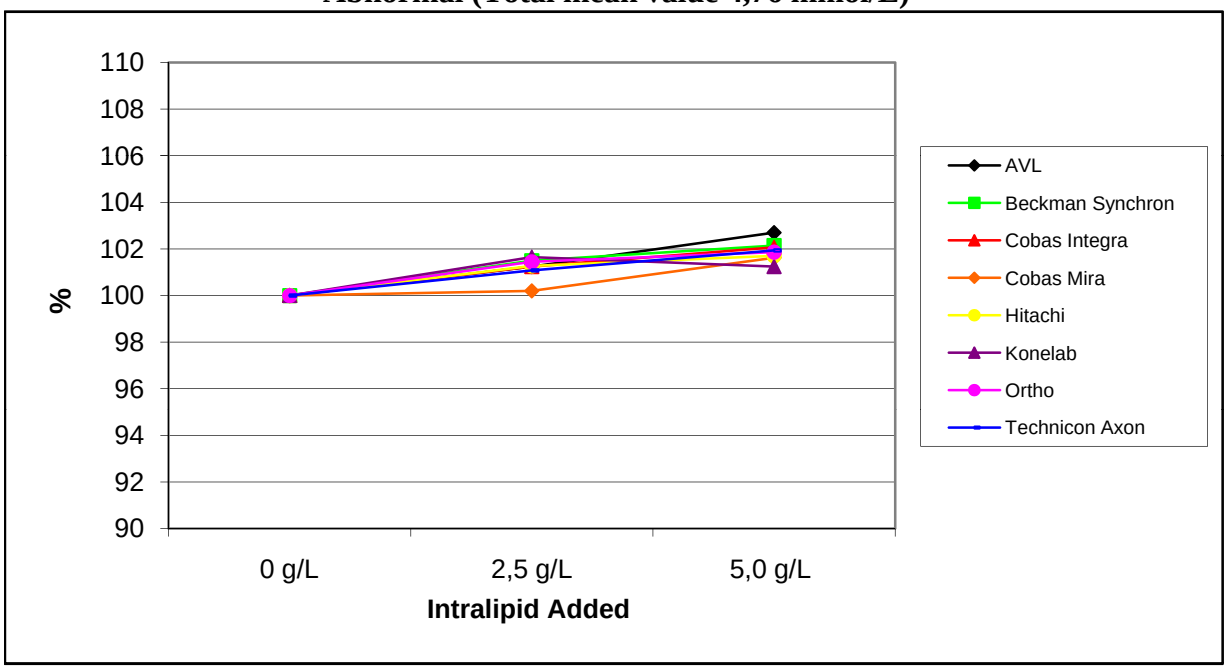


Potassium (K)

Normal (Total mean value 2,79 mmol/L)

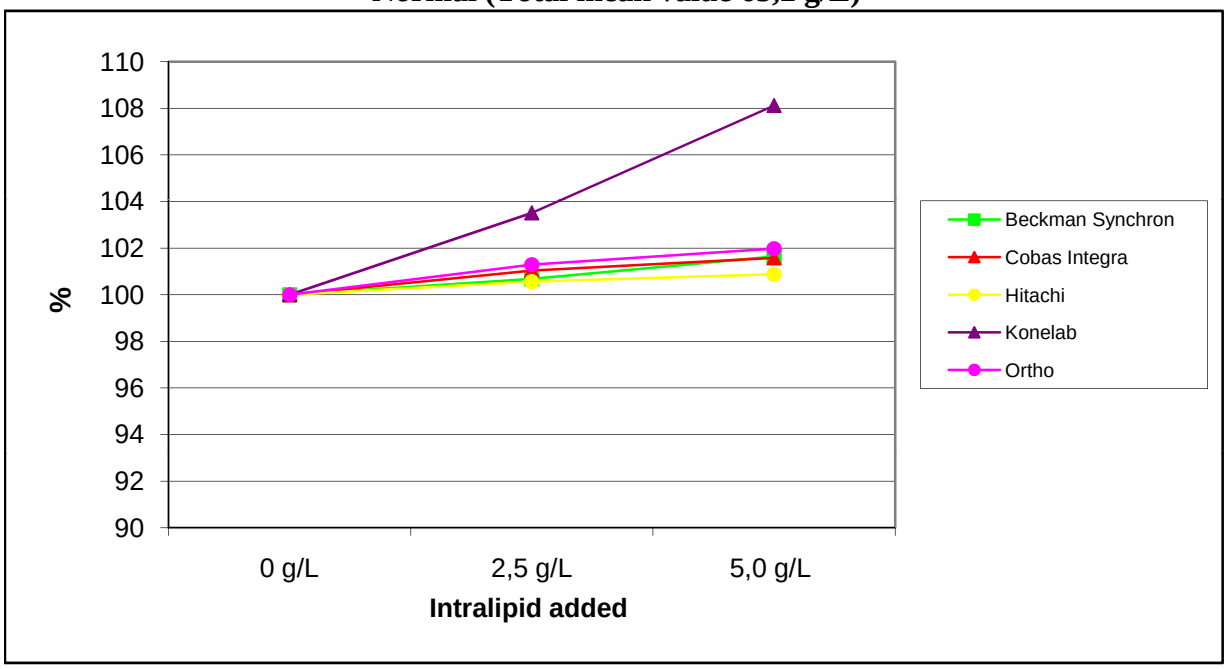


Abnormal (Total mean value 4,76 mmol/L)

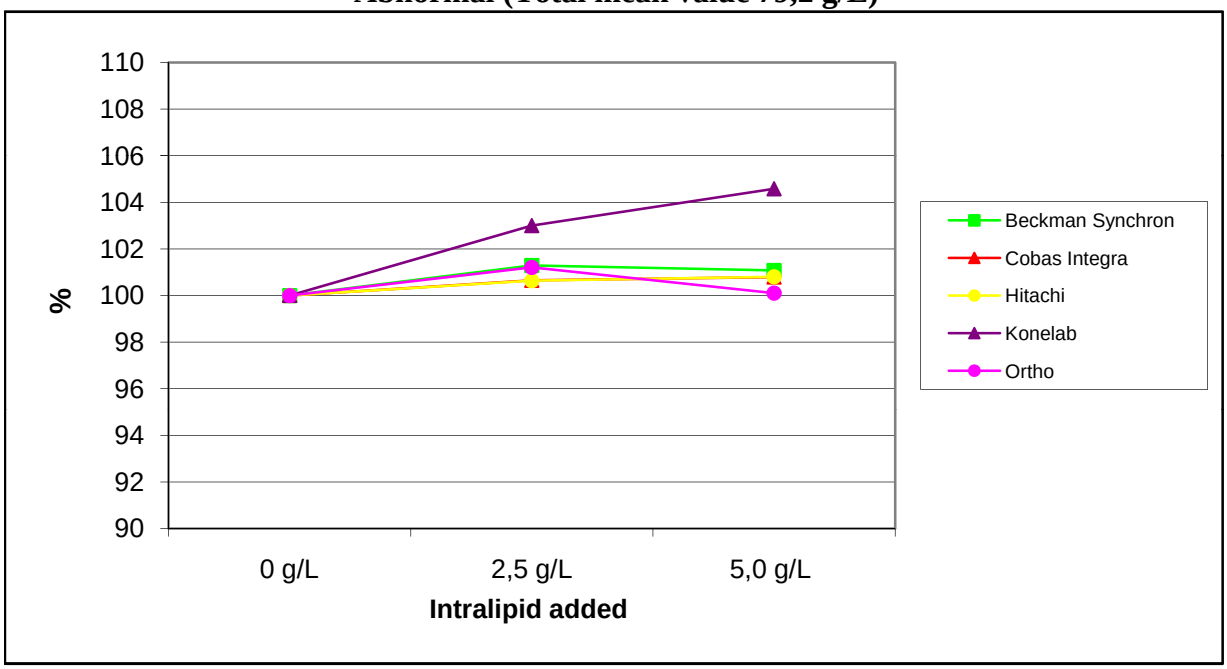


Protein

Normal (Total mean value 65,2 g/L)

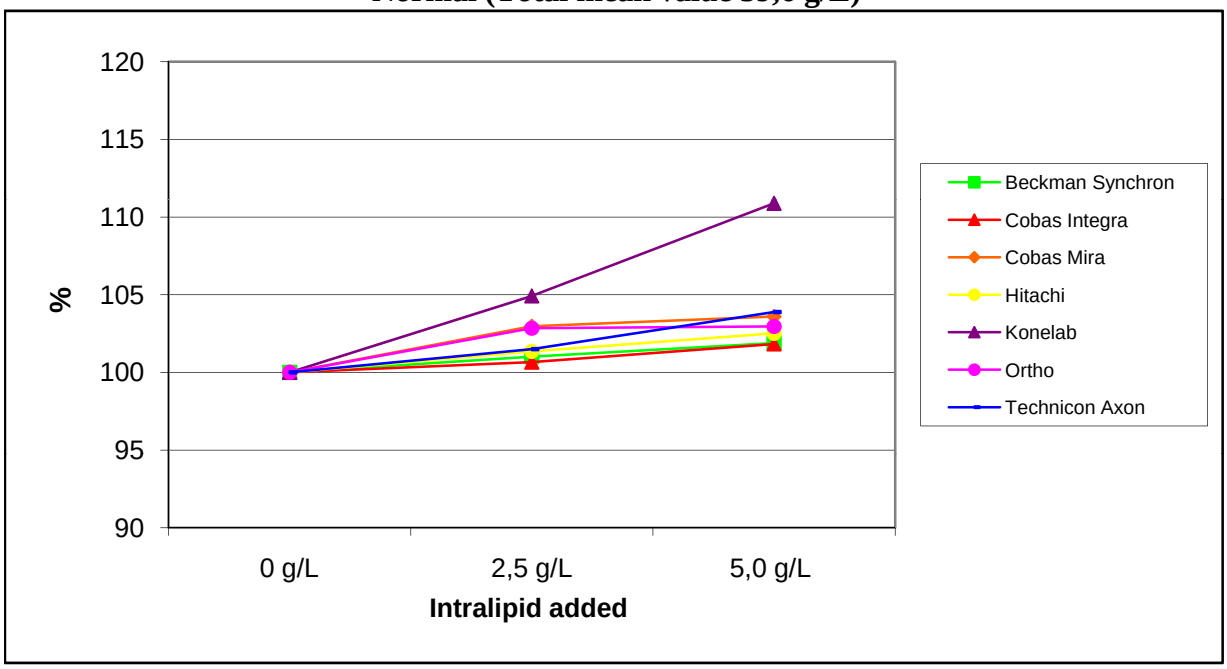


Abnormal (Total mean value 79,2 g/L)

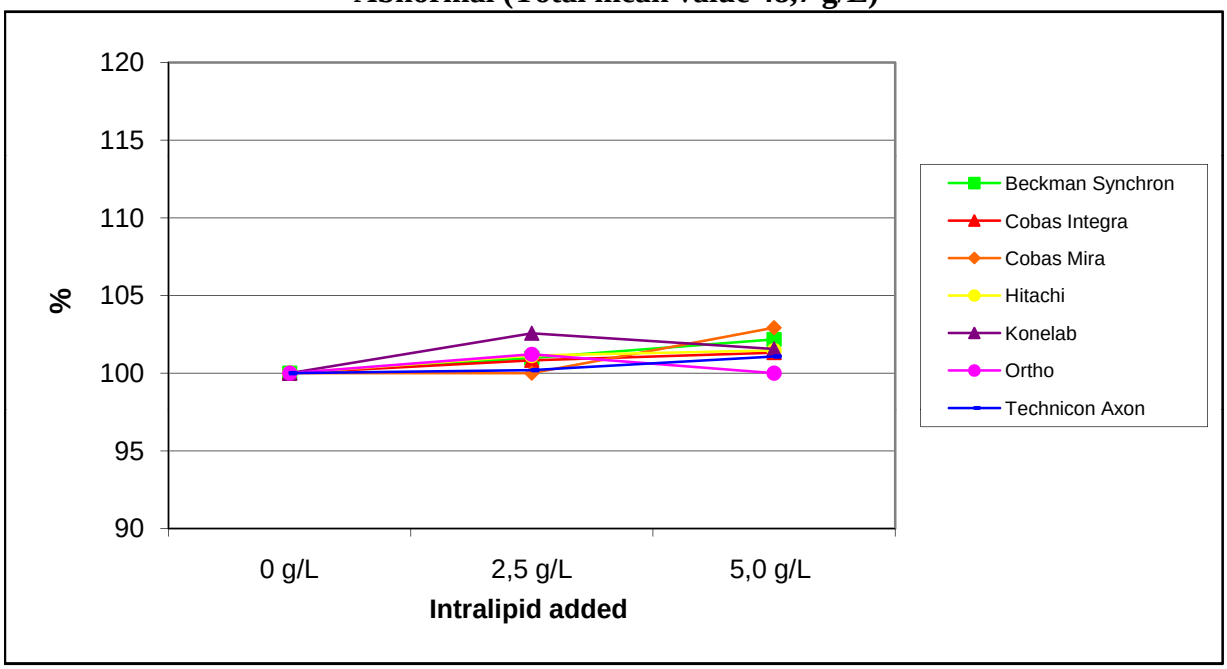


Albumin

Normal (Total mean value 39,6 g/L)

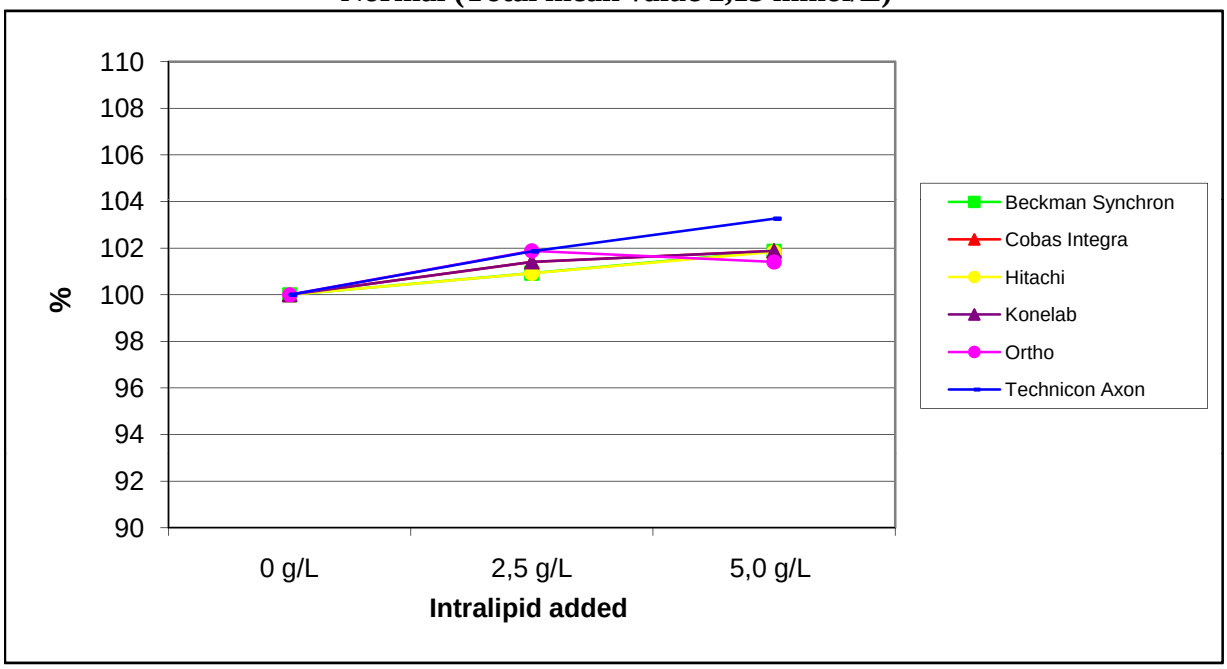


Abnormal (Total mean value 48,7 g/L)

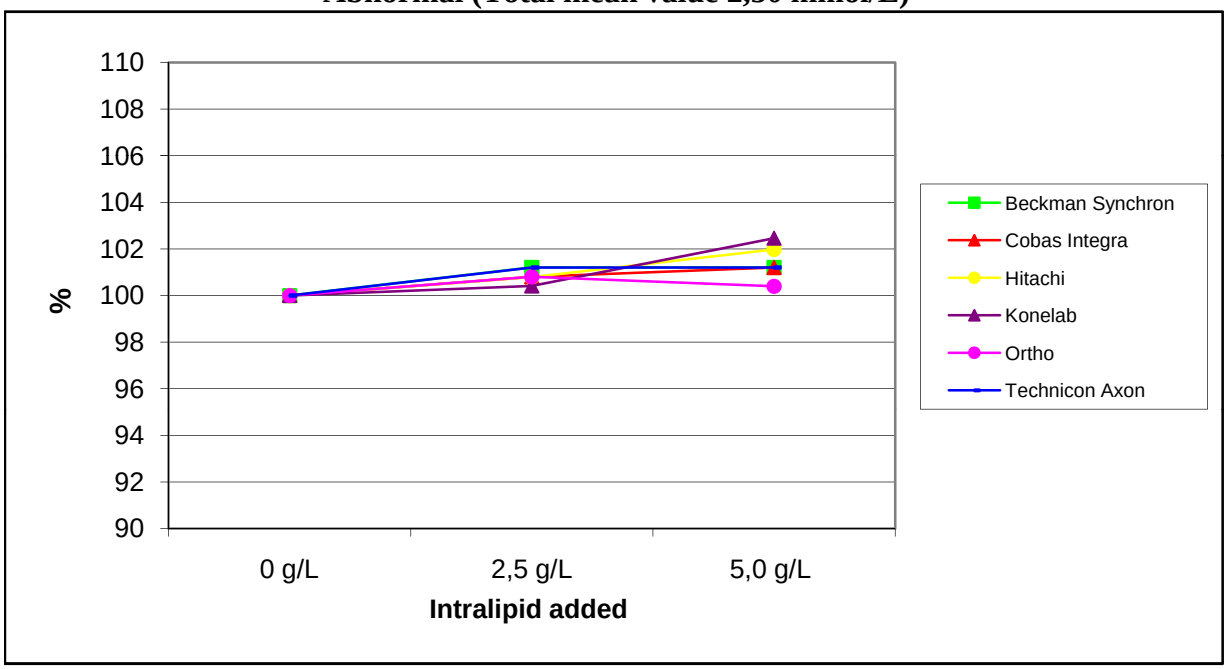


Calcium (Ca)

Normal (Total mean value 2,15 mmol/L)

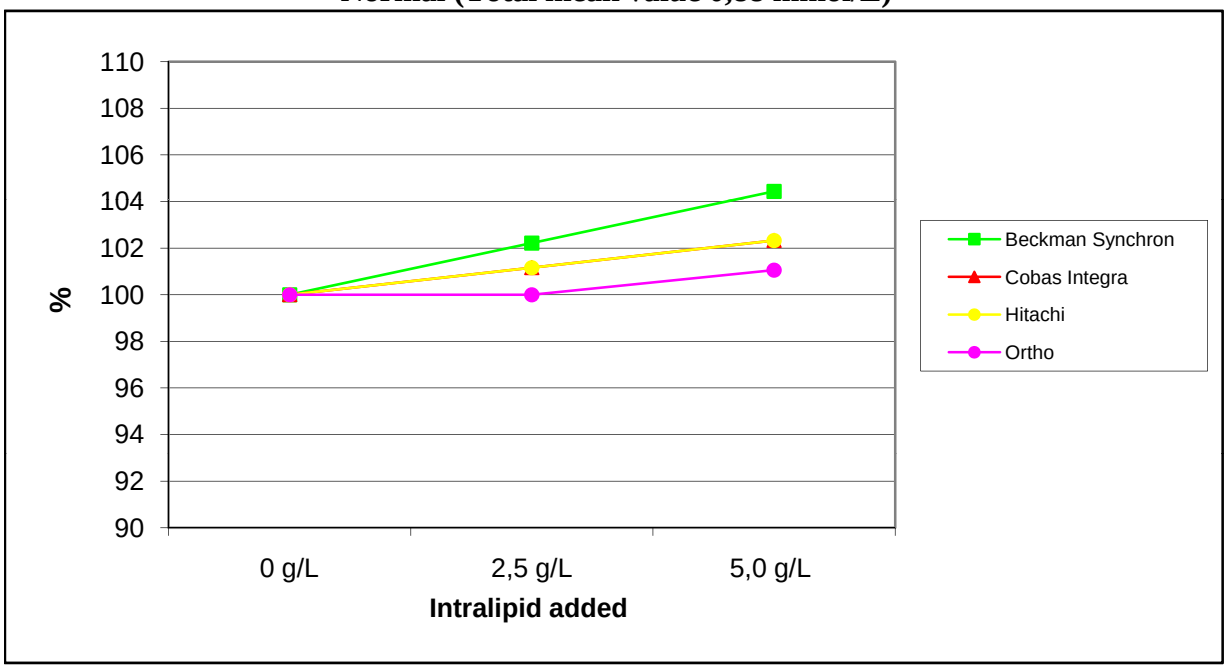


Abnormal (Total mean value 2,50 mmol/L)

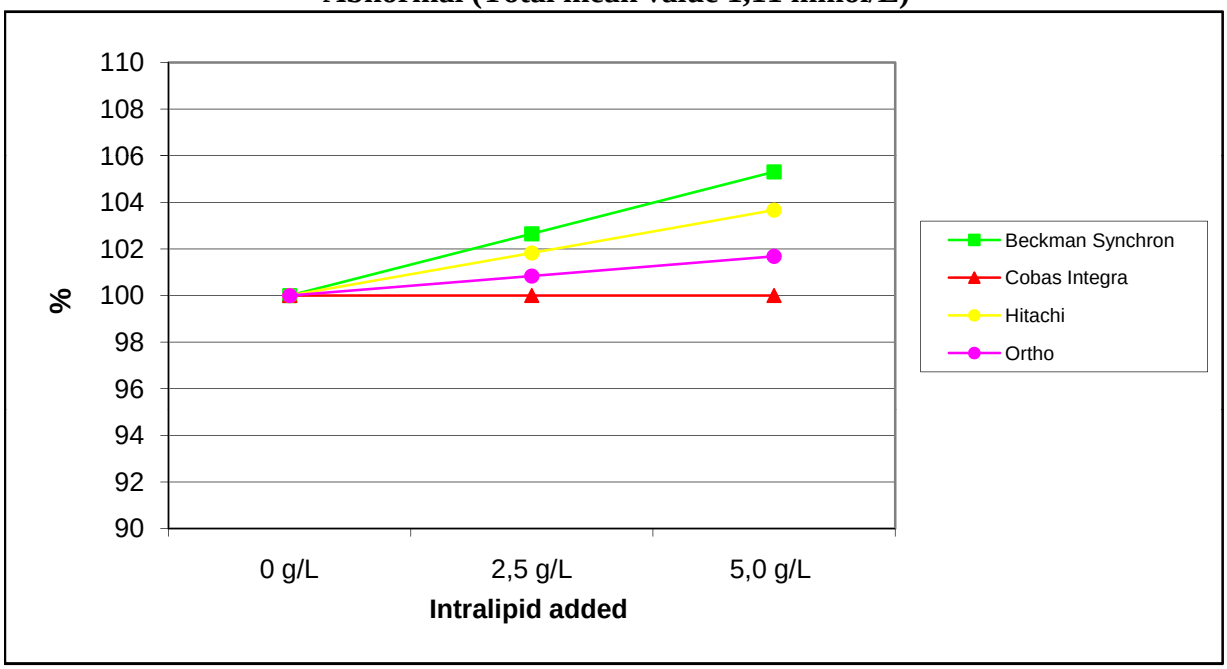


Magnesium (Mg)

Normal (Total mean value 0,88 mmol/L)

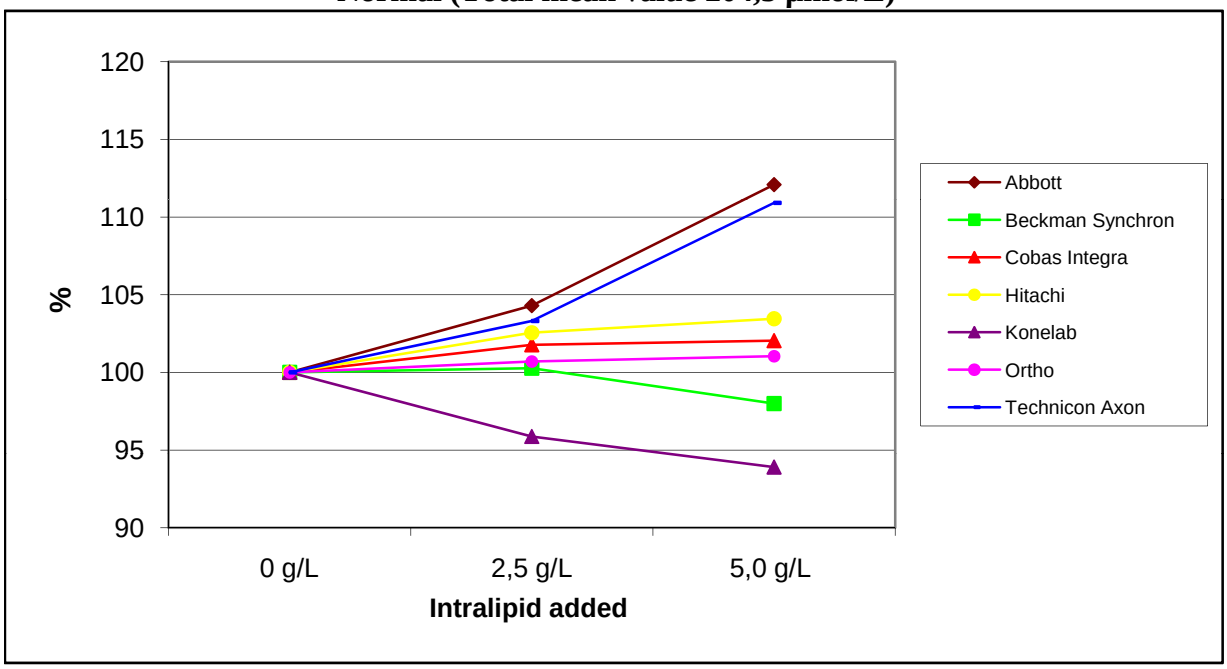


Abnormal (Total mean value 1,11 mmol/L)

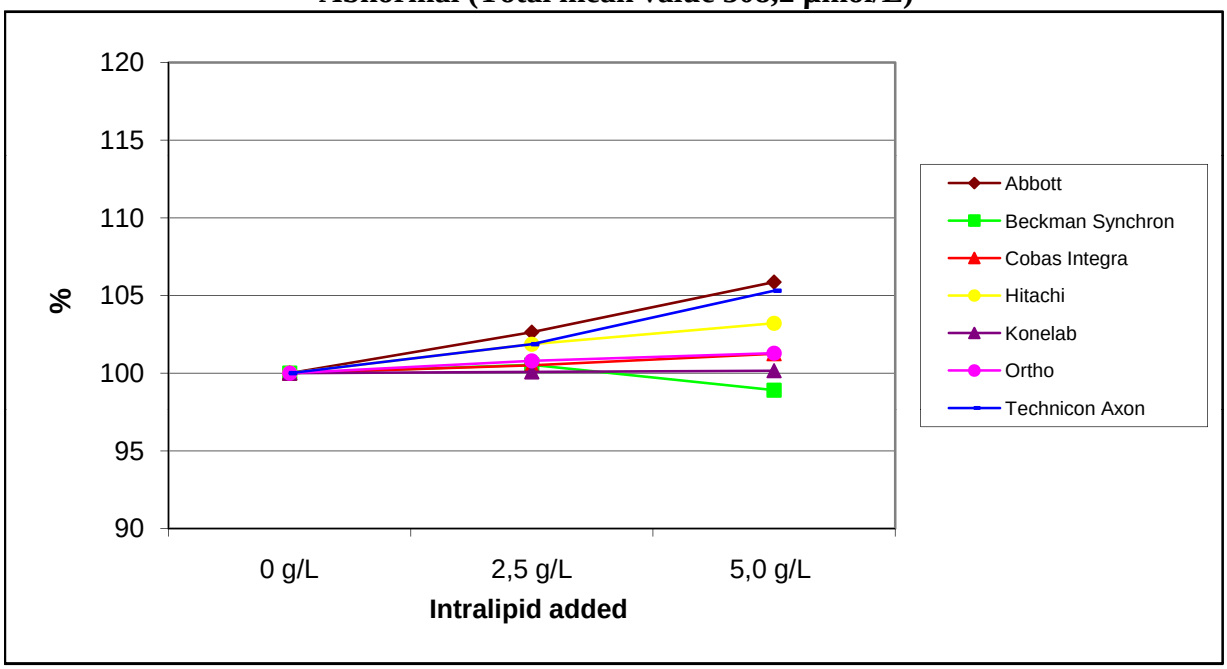


Uric acid

Normal (Total mean value 204,5 µmol/L)

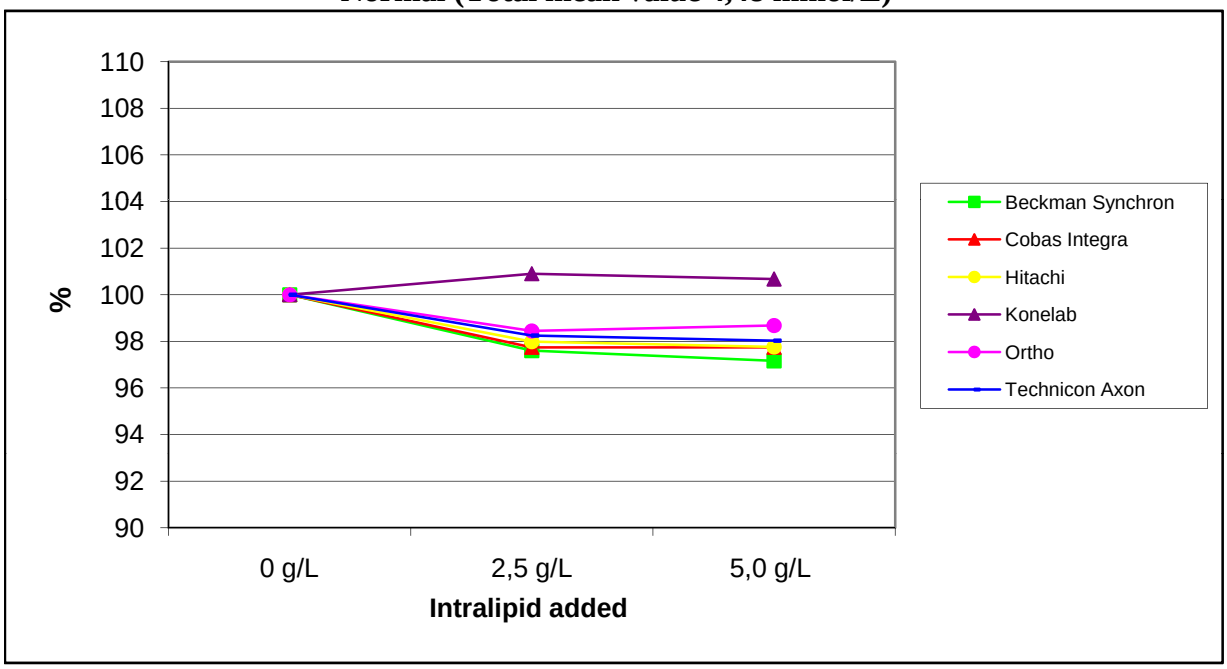


Abnormal (Total mean value 508,2 µmol/L)

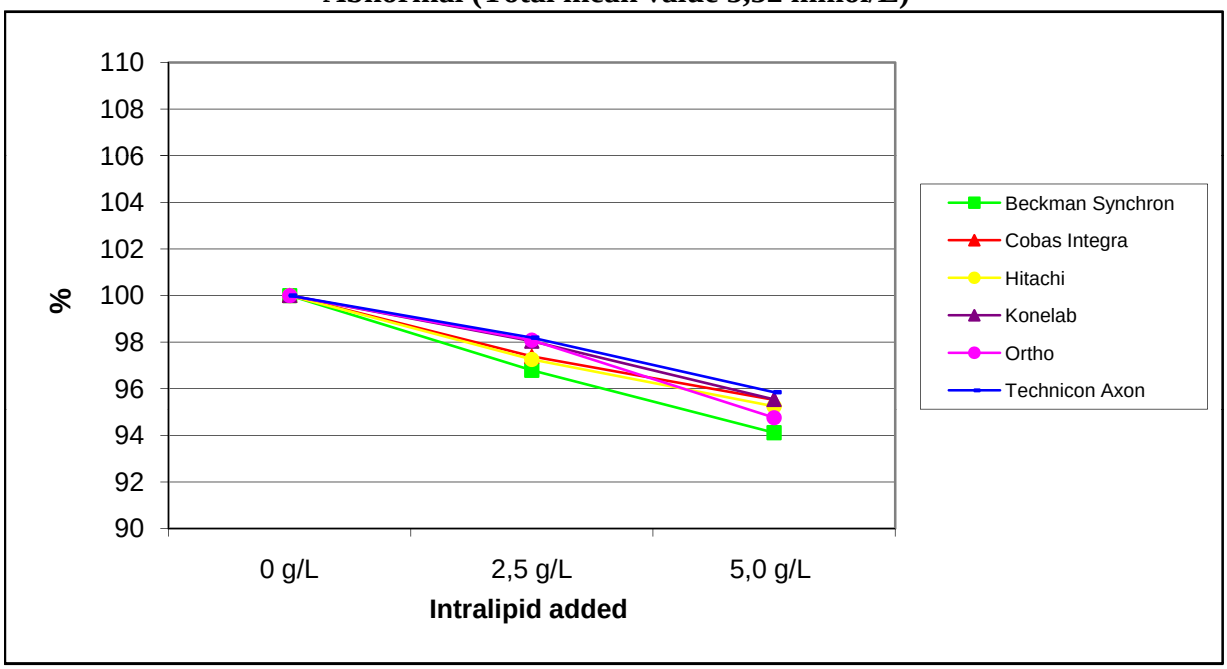


Cholesterol

Normal (Total mean value 4,48 mmol/L)

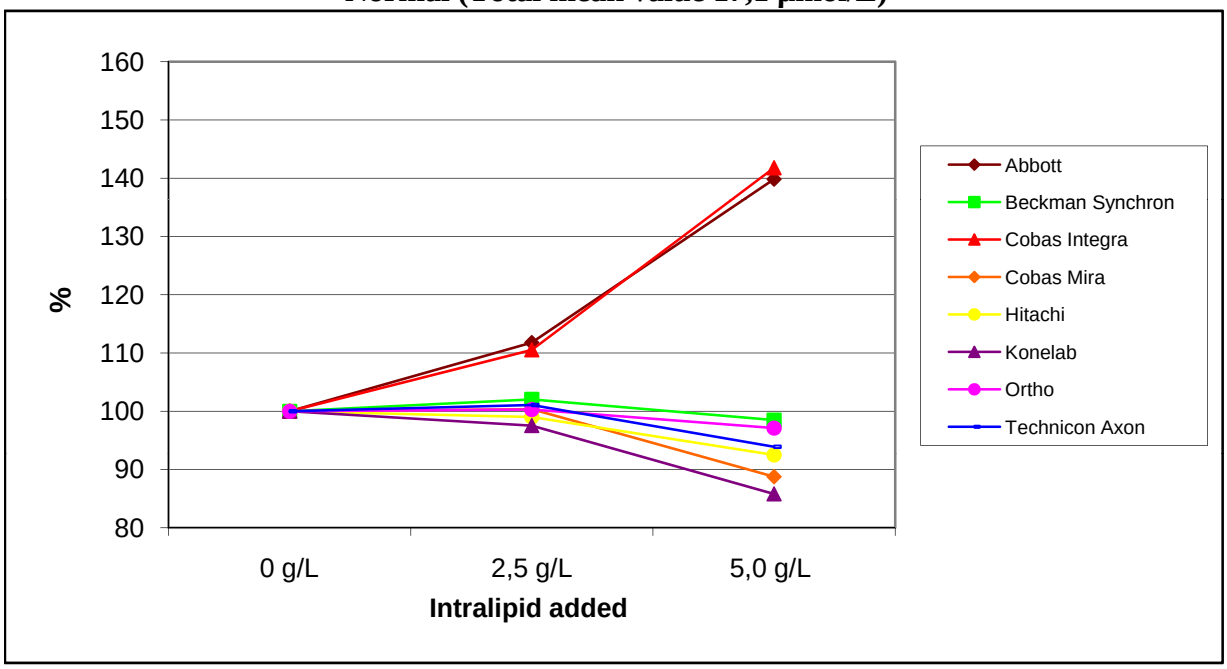


Abnormal (Total mean value 5,52 mmol/L)

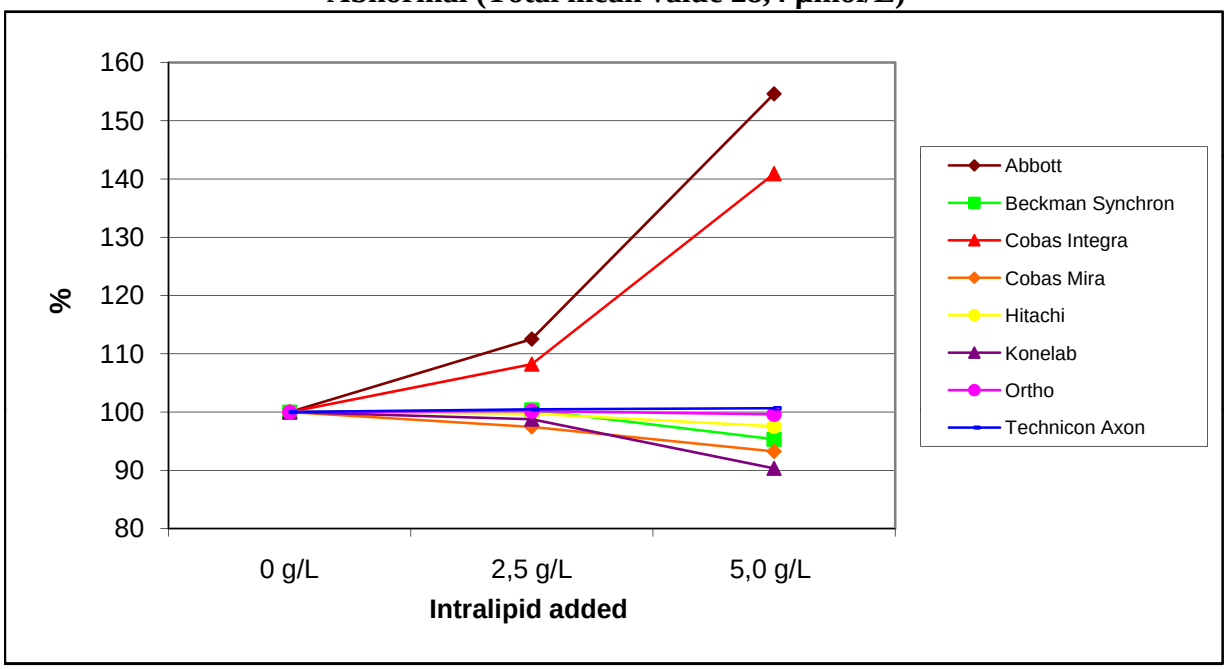


Bilirubin

Normal (Total mean value 17,1 µmol/L)

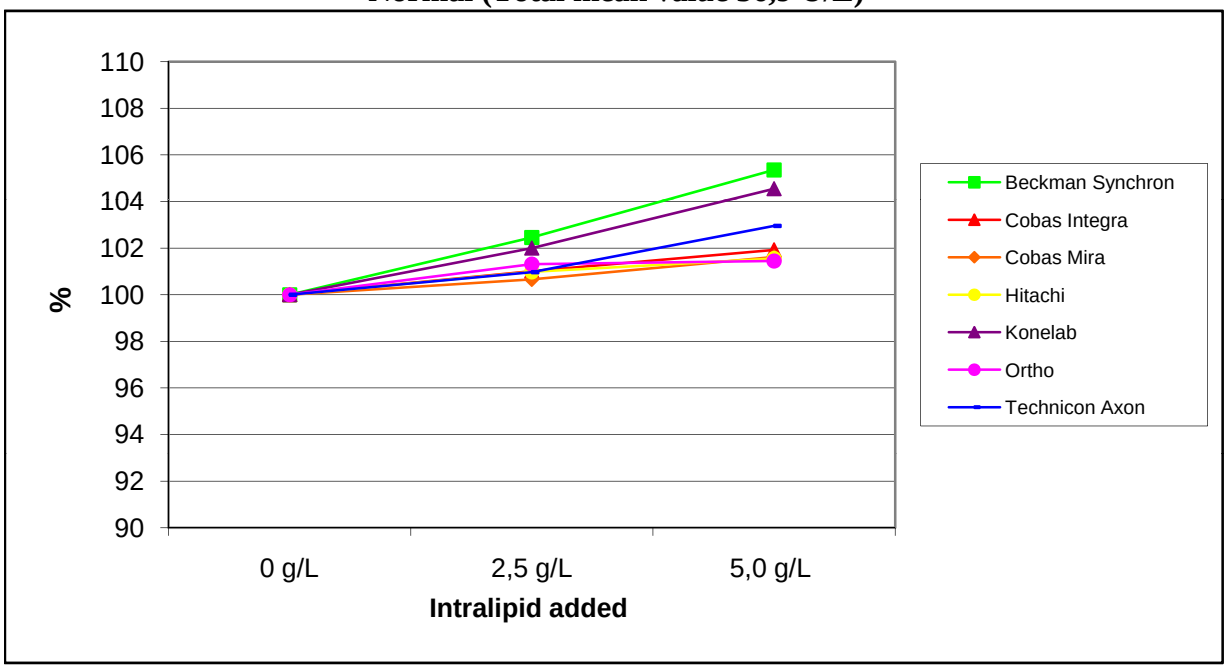


Abnormal (Total mean value 28,4 µmol/L)

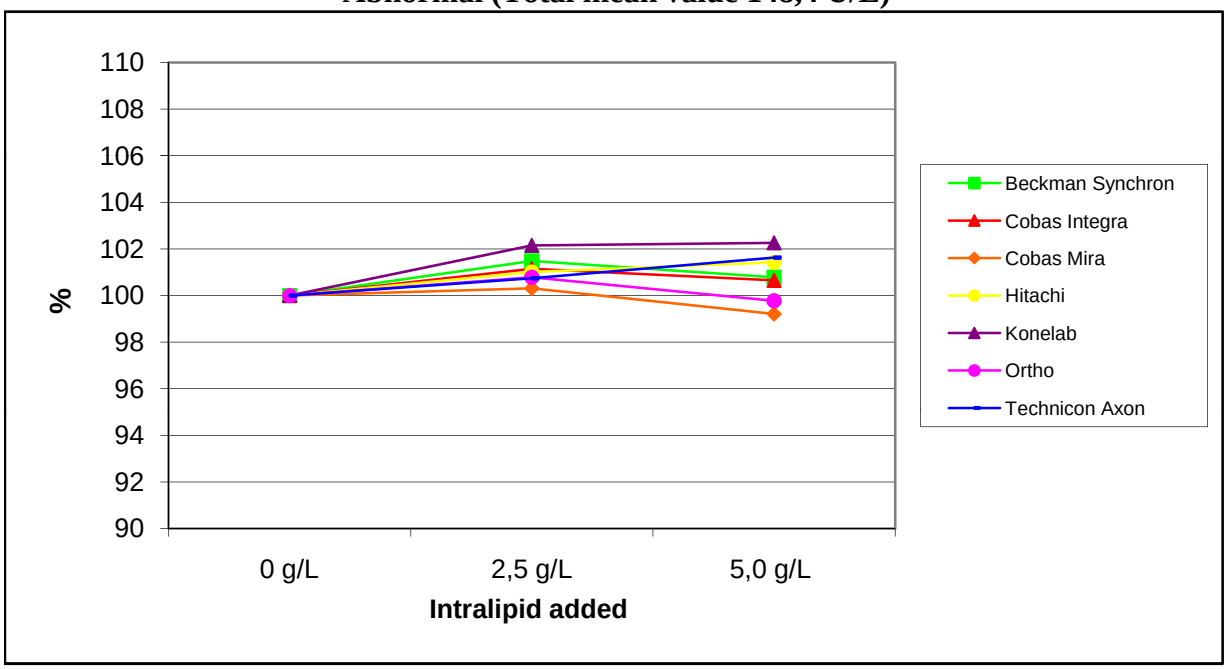


ASAT

Normal (Total mean value 56,9 U/L)

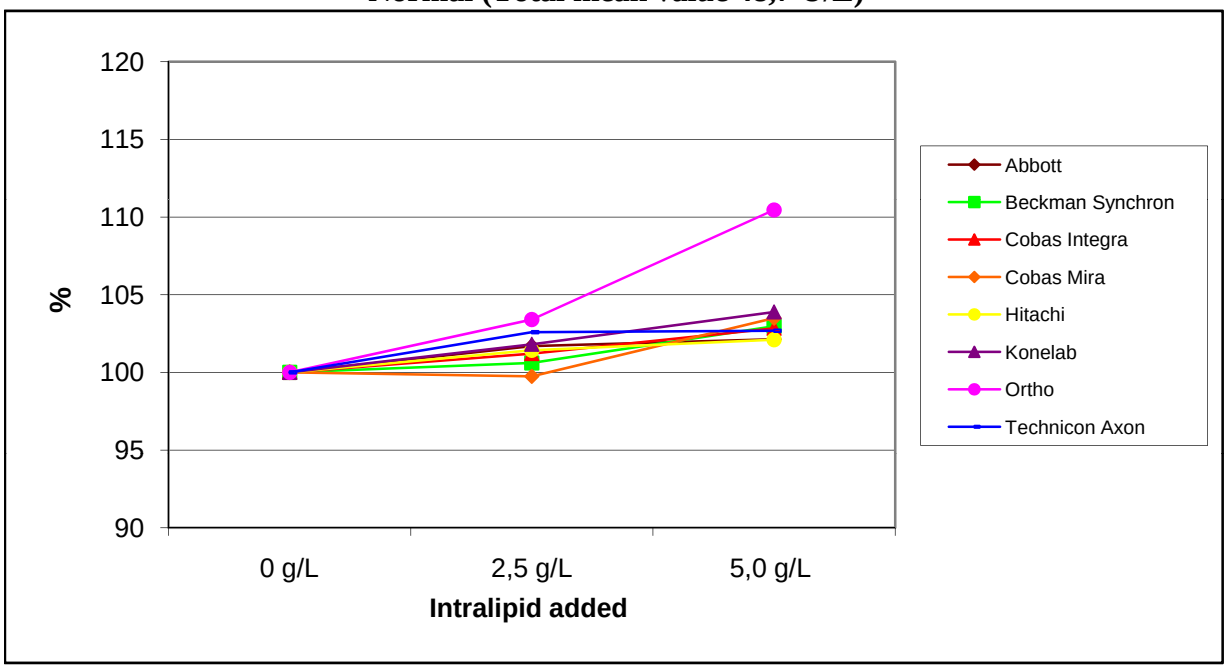


Abnormal (Total mean value 148,4 U/L)

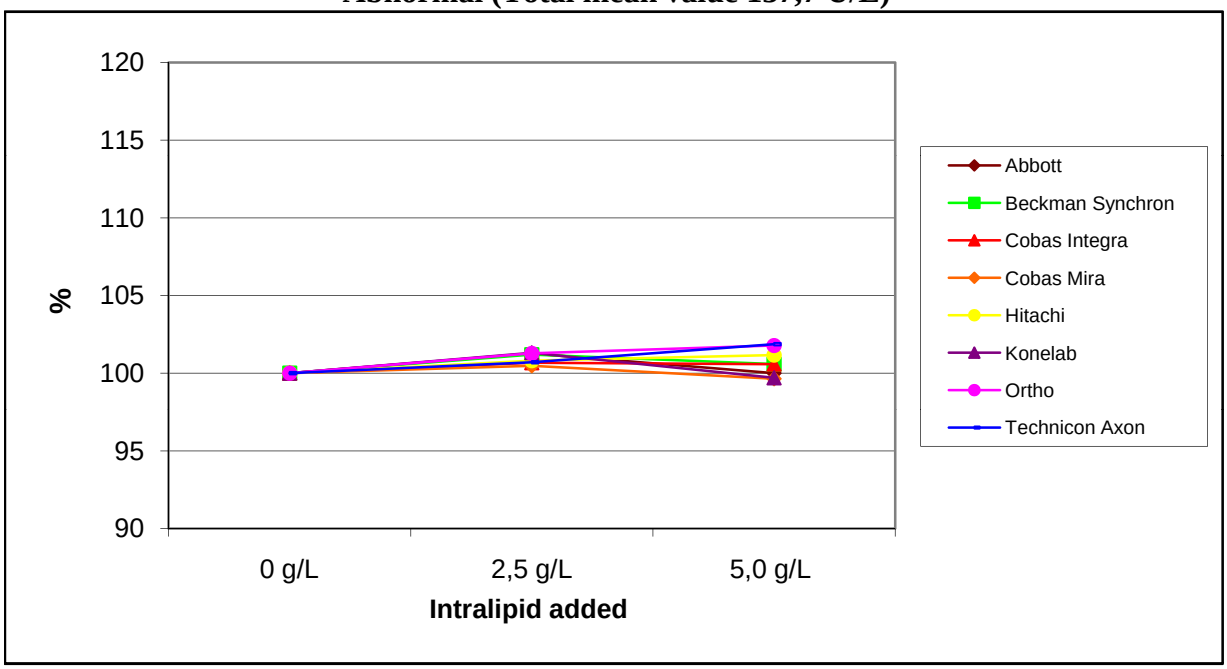


ALAT

Normal (Total mean value 48,7 U/L)

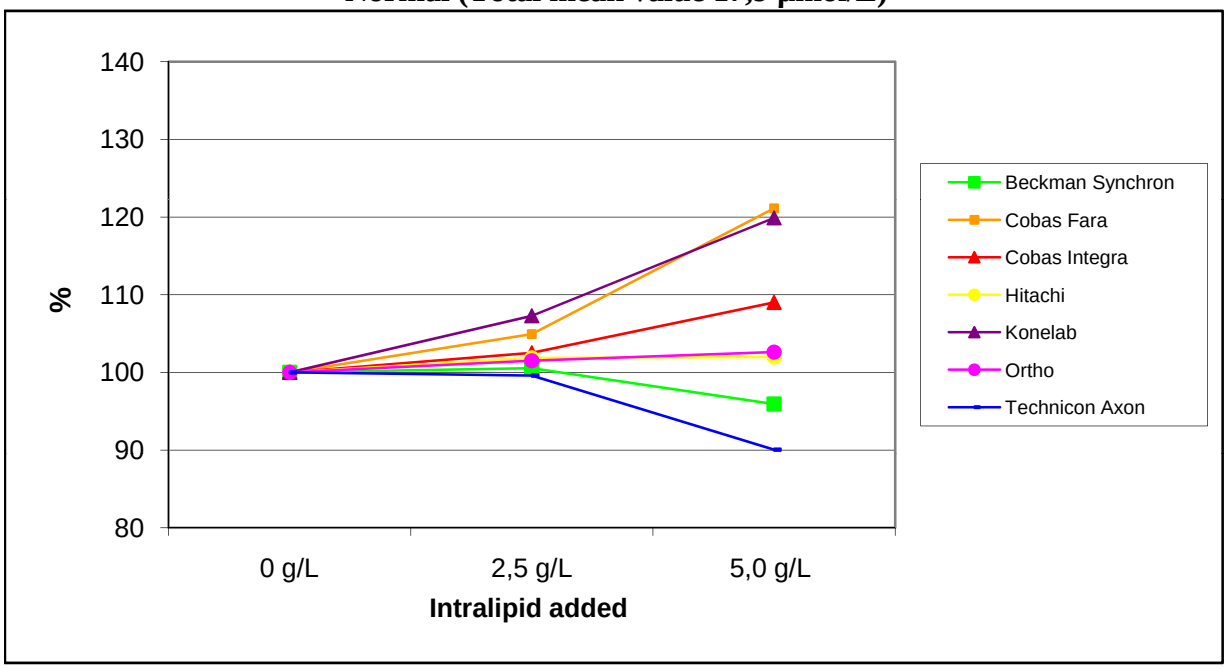


Abnormal (Total mean value 137,7 U/L)

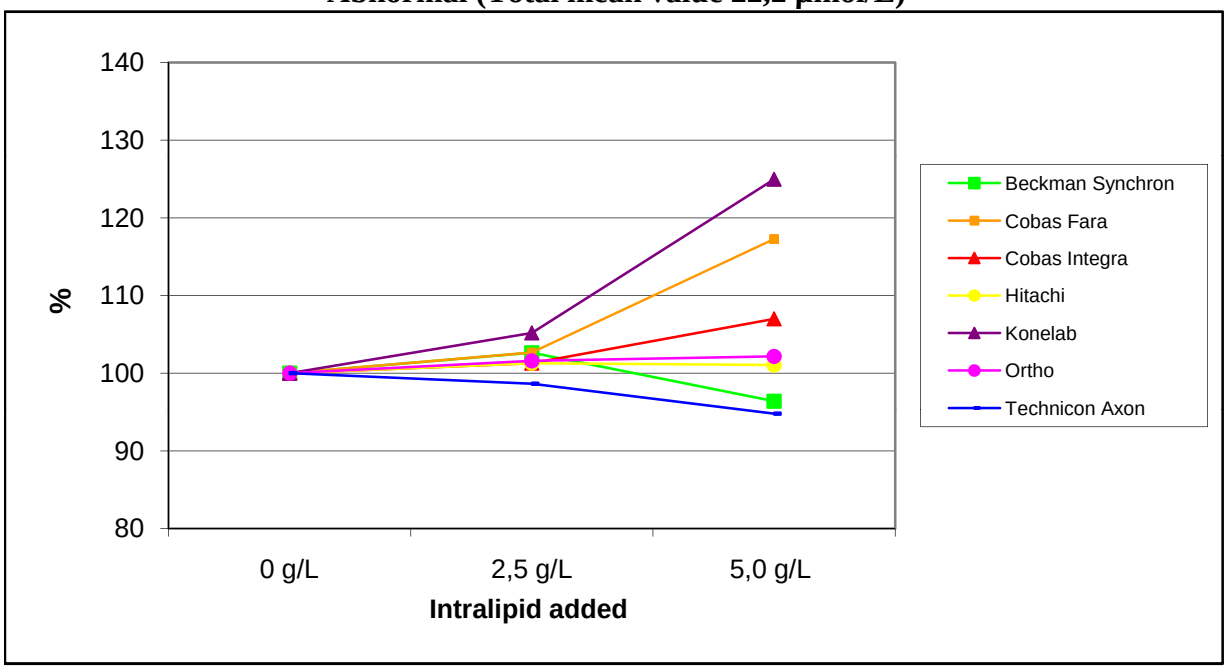


Iron (Fe)

Normal (Total mean value 17,9 µmol/L)

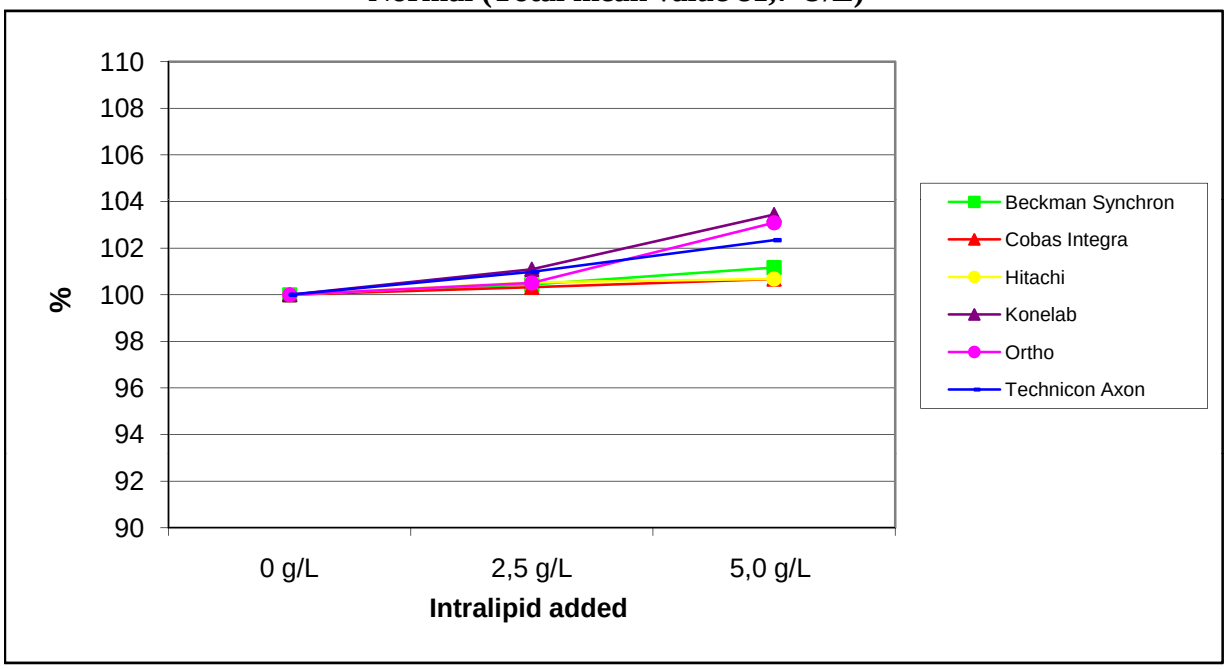


Abnormal (Total mean value 22,2 µmol/L)



GT

Normal (Total mean value 81,7 U/L)



Abnormal (Total mean value 110,8 U/L)