Lipaemia: an overrated interference?

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ABSTRACT

Reagent method sheets for analysis of common serum analytes often highlight the possibility of interference from lipaemia but the information given is often brief and may not be instrument-specific. Thus study assesses the degree of interference from lipaemia in a range of common serum analytes on the Bayer Opera (with a serum blank) using a commercial polymer, LipoClear, as a lipid-clearing agent. Serum samples (mean serum triglyceride 6.89 [range 0.58–28.4] mmol/L) are analysed for 14 common chemistry analytes and the results compared before and after treatment with LipoClear. Results showed no significant critical differences in analyte values before and after treatment, except for an expected fall in total protein, phosphate, cholesterol and triglyceride concentrations. Most of the common analytes in use on the Bayer Opera are not subject to interference from lipaemia; however, we recommend that where method sheets indicate interference from lipaemia then this should be quantified for the analyte in question.

KEY WORDS: Chemistry. Lipids and antilipemic agents. Triglycerides.

Introduction

Lipaemia is reported to interfere in many routine assays. Many reagent suppliers provide information on the effect of lipaemia in their assays, but this is often vague, not quantified and may not be instrument-specific.[1,2]

Lipaemia, like haemolysis and icterus, causes chromophoric interference in photometric analyses due to high background readings, interference at the measured wavelength and light scattering caused by the interfering substance.[2,3] The interference may be dose-dependent for some analytes but not for all.[1]

The interference from lipaemia can be minimised in a number of ways, including the use of a sample blank reading, kinetic analysis, changing the wavelength at which the reaction is read to one at which there is minimal absorbance from the interferant,[4,5] and the use of commercial preparations that clear the lipid content from serum.[6]

In the laboratory setting, staff use different methods – such as visual inspection, lipaemia index, serum indices and triglyceride concentration – to determine the degree of turbidity from lipaemia. These assessments, however, may be inaccurate as the degree of interference from lipaemia is method- and instrument-dependent.[1-3]

The aim of this study, therefore, is to evaluate the effects of lipaemia and LipoClear, a non-toxic polymer for serum lipid clearance, on 14 tests commonly analysed on the Bayer Opera analyser, prior to the introduction of LipoClear into our routine laboratory repertoire.

Materials and methods

A total of 14 analytes were measured in up to 44 serum samples (Table 1) with either no lipaemia or varying degrees of lipaemia (mean serum triglyceride 6.89 [range 0.58–28.4] mmol/L) using methods recommended for use by the instrument manufacturer. Twelve samples had serum triglyceride ≤2 mmol/L; 20 samples had a serum triglyceride >2 and ≤10 mmol/L; and 12 samples had a serum triglyceride >10 mmol/L. Each analyte was determined before and after treatment with LipoClear. (*phi*Tec International, UK) on a Bayer Opera analyser (Bayer AG, Germany).

A 0.5 mL serum sample was added to 0.1 mL LipoClear, mixed and left to stand for 5 min. The mixture was centrifuged and the supernatant analysed. Results were multiplied by 1.2 to correct for the initial dilution.

Significance of differences before and after the use of LipoClear were calculated using the paired *t*-test and Wilcoxon matched pairs for parametric and non-parametric data, respectively. Changes in results were assessed as being significant if the difference between results was greater than 2.8 times the analytical coefficient of variation (CV).[7] This is derived from an application of the *t*-test, and indicates (if the difference between results is greater than 2.8 times the analytical CV) that there is a less than 5% chance of that being due to random variation.

Results

With the exception of alanine transaminase (ALT), amylase and bicarbonate, significant differences in the other analyte values before and after treatment with LipoClear were seen using standard statistical techniques (Table 2).

When analytical CV was taken into account, only phosphate, total protein, cholesterol and triglyceride showed significant analytical change (Table 3).

Discussion

Most methodologies used on the Bayer Opera appeared to be subject to statistically significant interference from lipaemia when evaluated by standard statistical methods,

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Table 1. Method details for analytes in the study

Analyte	Reagent supplier	Method	Package insert details on interference from lipaemia
Urea	Bayer	Kinetic, urease	No significant interference
Creatinine	Bayer	Kinetic, Jaffe	No information given
Albumin	Bayer	BCG succinate	No significant interference
Alkaline phosphatase	Bayer	DEA-optimised, 37˚C	No significant interference
Alanine transaminase	Bayer	IFCC-optimised	Positive interference
Amylase	Bayer	CNPG3, 37˚C	No interference up to triglyceride of 10.5 mmol/L
Phosphate	Bayer	Phosphomolybdate,	UV No information given
Glucose	Bayer	Glucose oxidase	Positive interference
Bicarbonate	Trace	Phosphoenol pyruvate carboxylase	Positive interference
Total protein	Bayer	Biuret	Positive interference
Total bilirubin	Bayer	Diazo	No significant interference
Calcium	Randox	Arsenazo	Positive interference
Triglyceride	Bio-Stat	Enzymic endpoint	Positive interference
Cholesterol	Bio-Stat	Cholesterol oxidase	Positive interference

Table 2. Mean or median analyte results before and after lipid extraction

Analyte	Number tested	Analyte range	Mean or median before lipid extraction	Mean or median after lipid extraction
Urea (mmol/L)	44	1.6–32.9	6.8 (4.8–8.6)	7.1 (5.1–9.0)***
Creatinine (μmol/L)	44	75–854	146 (104–187)	149 (106–191)*
Albumin (g/L)	44	21–60	42 (39–44)	41 (38–43)*
Alkaline phosphatase (iu/L)	37	92–546	211 (174–247)	209 (175–242)*
Alanine transaminase (iu/L)	38	5–150	28 (20–35)	28 (19–35)
Amylase (iu/L)	44	3–96	47 (20)	47 (21)
Phosphate (mmol/L)	39	0.77–2.38	1.37 (0.40)	1.24 (0.22) *
Glucose (mmol/L)	43	3.4–33.4	9.0 (7.1–10.8)	9.3 (7.3–11.2)***
Bicarbonate (mmol/L)	30	9–29	20.7 (5.2)	21.7 (4.5)
Total protein (g/L)	44	59–138	76 (14.0)	65 (6.0)***
Total bilirubin (μmol/L)	43	2–233	23 (8.1–38)	22 (7.4–37)***
Calcium (mmol/L)	44	1.24–2.94	2.35 (2.26–2.44)	2.26 (2.18–2.35)***
Triglyceride (mmol/L)	44	0.58–28	5.28 (4.73–9.07)	1.71 (1.41–2.25)***
Cholesterol (mmol/L)	44	1.7–9.3	5.7 (1.7)	2.6 (1.2)***

Results reported as: mean (standard deviation) for parametric distribution, median (95% confidence intervals) for non-parametric distribution.
* $P<0.05$, ** $P<0.01$, *** $P<0.001$.

but these do not consider the analytical imprecision of assays. When the analytical CV was taken into account, most of the differences failed to achieve critical significance. Only phosphate, protein, cholesterol and triglyceride values remained critically different after the addition of LipoClear.

These findings support a previous study of LipoClear[6] and were expected as LipoClear, a non-ionic polymer, precipitates lipoproteins and phospholipids. Lipaemia did not critically affect measurement of other analytes, probably because the Bayer Opera performs an initial blank reading at the start of the reaction, supporting previous reports recommending the use of serum blanks in minimising lipaemic interference.[1,2]

However, these results are at variance with the manufacturer's method sheets, which indicate lipaemia interference with ALT, amylase, glucose, bicarbonate and calcium methods (Table 1).

It is possible that the manufacturer does not take into account assay imprecision in assessing lipaemic interference. The difference in the results for the tests for significance used is that, for the Mann Whitney and *t*-tests, individual paired points are compared which are considered to have no imprecision. When the calculation of significant change in test results is undertaken, however, the imprecision of the test results is also taken into account. Hence, the apparent significant differences.

Table 3. Comparison of analyte coefficients of variation with % difference in analyte result before and after lipid extraction

Analyte	Analytical CV	2.8x CV	% Difference	Significance
Urea	2.5	7.0	+4.4	Not significant
Creatinine	2.6	7.3	+2.1	Not significant
Albumin	1.3	3.6	–2.4	Not significant
Alkaline phosphatase	4.9	13.7	–1.0	Not significant
Alanine transaminase	3.0	8.4	0	Not significant
Amylase	3.5	9.8	0	Not significant
Phosphate	1.6	4.5	–9.5	Significant
Glucose	2.6	7.3	+3.3	Not significant
Bicarbonate	4.6	12.9	+4.8	Not significant
Total protein	2.3	5.6	–14.5	Significant
Total bilirubin	3.7	10.4	+4.5	Not significant
Calcium	2.1	5.9	–3.9	Not significant
Triglyceride	1.2	3.4	–74	Significant
Cholesterol	2.5	7.0	–54	Significant

In conclusion, LipoClear does reduce lipaemia but most methodologies are often sufficiently robust to avoid interference from lipaemia. Therefore, we recommend that individual laboratories quantify interference from lipaemia for their specific methods and instruments, as the interference could be analyser- and/or reagent-specific. Only if there is significant interference should the use of lipid clearing agents be considered. □

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