the serum groups. The higher total DNA concentration in serum (compared with plasma) was attributable to the release of DNA by hematopoietic cells during the clotting process (11). This concentration decreased by 74-fold in formaldehyde-treated serum, highlighting its cell-stabilizing effect.

We observed no differences in fetal DNA concentrations in the samples treated or not treated with EDTA and formaldehyde. These results support the notion that use of formaldehyde to inhibit enzymes that destroy DNA, such as deoxyribonucleases, can be eliminated. Moreover, Lo et al. (12) previously demonstrated the limited effect of such in vitro DNA degradation. In their study, Dhallan et al. (9) had no control group and compared their values with those of Lo et al. (12), obtained not only with a different methodology but also at different gestational ages, despite the fact that the absolute amount of fetal DNA increases with gestational age. It is most likely that formaldehyde does not affect DNA stability.

Another possible factor is the use of gentle centrifugation. Chiu et al. (10) have shown that centrifugation alone is not enough to obtain maternal cell-free plasma and that the number of cells left in plasma after centrifugation is variable. Therefore, in the study of Dhallan et al. (9), the role of centrifugation is certainly low when compared with the role of formaldehyde. In our study, although we used similar shipping, handling, and processing conditions, we measured a higher absolute concentration of free fetal DNA (169 vs 66.1 kgeq/L), even taking into account the mean gestational age at sampling.

Dhallan et al. (9) also addressed the need for a very short time delay between the venipuncture procedure and the addition of formaldehyde. To circumvent this problem, we added the formaldehyde to the sampling tube before venipuncture. As a result, the formaldehyde enrichment effect was shown for all patients in our study, whereas Dhallan et al. (9) reported a lack of effect in three cases. We observed, however, a high variability for this enrichment (2- to 27-fold for plasma and 21- to 138-fold for serum). This variability seems to be unrelated to gestational age (P >0.05) and to the amount of fetal DNA (P >0.05). We speculate that it reflects a variable effect of the formaldehyde or the natural fluctuation in the amount of cell-free maternal DNA present before processing.

We conclude that formaldehyde increases the percentage of free fetal DNA in maternal plasma or serum by inhibiting maternal cell lysis. This new simple procedure facilitates study of fetal genetic markers such as single nucleotide mutations, but some issues still need to be addressed, especially the reason for the variability in results. Other strategies are actually under evaluation to achieve this final goal (13, 14) before use of this technique for noninvasive prenatal diagnosis.

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References

Automated Processing of Serum Indices Used for Interference Detection by the Laboratory Information System, Henricus J. Vermeer, Evert Thomassen, and Niels de Jonge (Department of Clinical Chemistry and Hematology, Leyenburg Hospital, PO Box 40551, 2504 LN The Hague, The Netherlands; * author for correspondence: fax 31-70-3592191, e-mail n.dejonge@leyenburg-ziekenhuis.nl)

In this report, we focus on improvements in patient results with respect to interferences in analytical results obtained from a routine chemistry analyzer (Synchron LX20PRO; Beckman Coulter Inc.). Although many authors have reported on improvements in process performance to reduce analytical errors, assay interference by endogenous or exogenous substances is an underrated problem with potential detrimental effects for the patient (1–5). Four major endogenous compounds that often interfere with most laboratory results are hemoglobin, bilirubin, lipids, and paraproteins (3–5). In our laboratory, the vast majority of hemolyzed samples (>95%) are attributable to in vitro processes resulting from incorrect sampling procedures or transport (6). Icteric or lipemic
samples are indicative of a physiologically, and possibly clinically, important process. Although differentiating between in vivo and in vitro hemolysis is clinically important, extensively hemolyzed samples may be difficult to evaluate (6, 7).

The frequency of interferences in clinical laboratory analyses is very difficult to determine (3). In principle, interferences can be reduced by use of adequately blanked analytical methods (8). Graphical displays of instrument- and analyte-specific interferences have been produced previously (6, 9). The LX20 analyzer has the capability to detect hemolysis, icterus, and lipemia in samples and produces semiquantitative unitless index values for hemoglobin, bilirubin, or Intralipid (so-called serum indices), but these serum index functions are not intended for diagnostic purposes. With increasing amounts of the specific interfering compounds, increasing index values are generated, which are linearly correlated with the amount of interferent.

We assessed the effect of interference by hemolysis, icterus, or lipemia on various analytes, using interference data provided by the manufacturer (Beckman Coulter, Inc.), experimental data from three Dutch hospitals, and our own research. We performed interference studies with serum pools to which interferent solutions were added (up to a maximum of 10% by volume), according to the CERMAB protocol (10). These combined data were used to generate tolerance tables indicating the extent of bias at specific index values. Arriving at a consensus in the field of LX20 serum index decision thresholds was difficult, however. In fact, individual index decision thresholds are in most cases not fully concordant at present. In this context, we have recently begun a harmonization study in The Netherlands of serum index decision thresholds for different analytes assayed on Synchron LX20 systems, and the results of this project will be published elsewhere.

We next developed an algorithm for the detection and processing of clinically or analytically relevant amounts of hemolysis, icterus, and lipemia in assays for 28 analytes, including electrolytes, enzymes, metabolites, and proteins (see Fig. 1 and Table 1 in the Data Supplement available with the online version of this Technical Brief at http://www.clinchem.org/content/vol51/issue1/). Before we developed this algorithm, we defined several initial postulates. The first postulate was that comments should be added to the result only when the amounts of analytically relevant or clinically interferents were significant. We decided to tolerate a deviation of ± 10% from the initial value for most analytes, in concordance with published recommendations (7). However, for some analytes this cutoff point is too high in view of their clinical significance. For this reason, we set the maximum tolerable deviation attributable to interference in assays for sodium and chloride at 5% to avoid wrong interpretation resulting from pseudohyponatremia or pseudohypochloremia (11). The second postulate was that the source of hemolysis initially be considered the result of inappropriate preanalytical conditions (in vitro hemolysis). The third postulate was that the presence of increased lipid concentrations in any patient sample should be detected and that this finding should be communicated to the clinician, followed by lipid removal by centrifugation or treatment with LipoClear® (12). The final postulate was that the workload for laboratorians should be as low as possible.

The developed algorithm has a fully open character and covers different processing layers: detection, automated notification of technicians and physicians, comment reporting, validation, and possible sample rejection. The transparency of the procedure is a great advantage because ongoing research results for interference effects can easily be implemented in the algorithm (e.g., results of the harmonization study). Processing of interference is done automatically for selection of chemistries for which interference testing is necessary, for interference detection, for application of decision rules, and for addition of comments to the report (see below). Further (manual) actions depend, of course, on the strategy chosen by the clinical laboratory in agreement with the clinicians.

The algorithm was implemented in two steps: The decision rules and actions to be taken were introduced first, and the algorithm was subsequently implemented in the laboratory information system (LIS), allowing complete automation of both detection and processing. The effectiveness of the algorithm was studied retrospectively.

In our laboratory, the algorithm was written in a specific LIS-dependent language, Mips Internal Site Programming Language (MISPL®). The LIS in use is General Laboratory Information Management System (GLIMS), Ver. 5.5H01 (MIPS). The underlying database is Progress, Ver. 9.1c/d, and the operating system is IBM Unix, Ver. 5.2.0. A graphical representation of the architecture of the algorithm is shown in Fig. 1. Processing occurs if serum indices indicate significant interference and depends on the extent of analytical error (see Table 2 in the online Data Supplement). If interference is present below a

![Fig. 1. Algorithm for the automated processing of serum indices.](http://www.clinchem.org/content/vol51/issue1/)

H-index, hemolysis index; I-index, icterus index; L-index, lipemia index.)
specific critical concentration, the result will be automatically accompanied by a warning comment to alert the clinician. If the index is above this critical concentration, the result is flagged, and the technician is informed by the LIS that the result cannot be reported to the clinician (see Tables 3 and 4 in the online Data Supplement). Principally, rejection of a sample always includes an extra validation step by the technician. An audit trail is generated automatically for every result. To test the performance of the algorithm, we collected laboratory data retrospectively from the LIS for relevant time periods of 12 months before, during, and after introduction of automated detection of serum indices (Table 1).

If a patient suffers from intravascular hemolysis, a specific attribute in the LIS can be linked temporarily or permanently to that patient, giving, if desired, specific comments and thereby bypassing the detection and unnecessary flagging for in vitro hemolysis. The occurrence of intravascular hemolysis in a patient is likely if a second ordered sample from that patient shows the same extent of hemolysis.

To determine whether the unacceptable result rate attributable to endogenous interference improved over time, we performed a retrospective analysis to study the effect of the stepwise introduction of systematic detection and the subsequent automatic rule-based algorithm for the actions to be taken on behalf of the clinician (correction of result by adding a comment to the report) or patient (redraw of specimen).

In the year 2000, interference was still detected by visual inspection, and the processing of the finding (e.g., sample rejection, correction, addition of a comment to the report) was a manual action taken by the technician handling the specimen. If the correct action was taken, it was recorded in the LIS. Automated spectrophotometric detection of interference by the chemistry analyzer, as well as decision rules for the systematic, although manual, handling of specimens based on tolerance tables for clinically relevant interference, were introduced in the first half of 2001. In July 2002, the algorithm for completely automated decision-making by the LIS for the processing of serum interference was introduced, and all reported actions were automatically archived in the LIS.

We retrospectively examined the (relative) numbers of samples with clinically relevant serum interferences over three 1-year periods (2000, 2001–2002, and 2002–2003). We traced back the reporting of analytically or clinically relevant serum interference in the LIS for all analytes for each 1-year period. The total number of analyses for each 1-year period for which the algorithm would have been applicable (first two periods) or was applied (last period) and the number of reported incidents of relevant serum interference as recorded in the LIS are shown in Table 1.

<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>Method for detection of interference</td>
<td>Visual inspection</td>
<td>Automated (spectrophotometry)*</td>
<td>Automated (spectrophotometry)</td>
</tr>
<tr>
<td>Processing of interference</td>
<td>Manual</td>
<td>Manual</td>
<td>Automated (LIS)</td>
</tr>
<tr>
<td>Total analyses</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>123 819</td>
<td>121 826</td>
<td>124 567</td>
</tr>
<tr>
<td>Fraction</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Hemolysis (&gt;0.5 g/L)</td>
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<td></td>
</tr>
<tr>
<td>Number</td>
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<td>113</td>
<td>1.326</td>
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<tr>
<td>Fraction</td>
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<td>9.28 $\times 10^{-4}$</td>
<td>1.06 $\times 10^{-2}$</td>
</tr>
<tr>
<td>95% CI*</td>
<td>0.80–2.20 $\times 10^{-4}$</td>
<td>0.76–1.10 $\times 10^{-4}$</td>
<td>1.06–1.07 $\times 10^{-2}$</td>
</tr>
<tr>
<td>Ratio compared with manual detection and processing</td>
<td>1</td>
<td>6.0</td>
<td>69.4</td>
</tr>
<tr>
<td>Icterus (&gt;103 μmol/L)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>0</td>
<td>66</td>
<td>674</td>
</tr>
<tr>
<td>Fraction</td>
<td>0</td>
<td>5.42 $\times 10^{-4}$</td>
<td>5.41 $\times 10^{-3}$</td>
</tr>
<tr>
<td>95% CI*</td>
<td>0</td>
<td>4.10–6.70 $\times 10^{-4}$</td>
<td>5.37–5.45 $\times 10^{-3}$</td>
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<tr>
<td>Ratio compared with manual detection and processing</td>
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<td>NA</td>
<td>NA</td>
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<tr>
<td>Lipemia (&gt;400 mg/L Intralipid)</td>
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<td></td>
<td></td>
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<td>Number</td>
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<td>1018</td>
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<td>Fraction</td>
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<td>8.17 $\times 10^{-3}$</td>
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<tr>
<td>95% CI*</td>
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<td>8.13–8.22 $\times 10^{-3}$</td>
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<tr>
<td>Ratio compared with manual detection and processing</td>
<td>1</td>
<td>0.0</td>
<td>1011.9</td>
</tr>
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</table>

* In this 1-year period, the automated method for detection was activated in 2002 (6-month period). In 2001 (6-month period), detection was done as in 2000 (visual inspection).

* 95% CI; 95% confidence interval; NA, not applicable.
Introduction of spectrophotometric detection of serum indices and decision rules to be carried out manually increased the detection rate of relevant hemolysis 6.04-fold. After introduction of the algorithm in the LIS, the detection rate for relevant serum interference was 69.37-fold higher than in the first 1-year period. For icteric samples, a large increase in reported interference was observed: whereas the occurrence of icterus in the sample was not reported at all in the first period, during the second 1-year period, 0.05% of specimens were reported as significantly icteric, causing deviations in the assay results. This number was increased 10-fold (0.54%) in the third period.

Interestingly, only one case of lipemia was reported in the first period and none in the second period. Apparently, automated spectrophotometric detection together with written instructions in the second 1-year period did not lead to adequate reporting to the clinician. In the third year, 1012-fold more cases of clinically relevant lipemia were reported than in the first year. It should be pointed out here that the evaluation performed and the observed quality improvement depend on the validity of the automated procedure.

Summarizing, we believe that this algorithm can be used as a blueprint for processing of test results in general. Every test result is now evaluated automatically in real time against predetermined tolerance limits for the extent of interfering substances, and the algorithm is designed as an almost technician-independent method. We encourage cooperation in this project, and copies of the MISPL algorithm and tolerance tables can be obtained free of charge from the corresponding author.

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References


We report a case of γ-heavy chain disease (γ-HCD) detected in the course of routine serum assay by CE, characterized by immunosubtraction, and confirmed by further techniques.

The patient was a 91-year-old man who had a complete medical examination, supplemented with numerous laboratory tests, including serum protein electrophoresis, ~2 years before the admission, without any significant finding but mild anemia. At admission he presented with bilateral peripheral edema, no dyspnea, and no palpable lymph node enlargement. Abdominal echography showed an enlarged steatotic liver and enlarged spleen. Laboratory investigation showed normocytic anemia (blood hemoglobin, 77.0–99.0 g/L; mean corpuscular volume, 99.3–102.6 fl), increased serum lactate dehydrogenase, low serum total protein (41.0 g/L) and albumin (28.0

Heavy Chain Disease Can Be Detected by Capillary Zone Electrophoresis, Paola Luraschi,1 Ilenia Infusino,1 Irene Zorzoli,2 Giampaolo Merlini,2 Camilla Fundaro,1 and Carlo Franzini1* (1 Dipartimento di Scienze Cliniche Luigi Sacco, Università degli Studi di Milano, Milan, Italy; 2 Laboratorio di Biotecnologie e Tecnologie Biomediche, IRCCS Policlinico San Matteo, Dipartimento di Biochimica, Università degli Studi di Pavia, Pavia, Italy; * address correspondence to this author at: Dipartimento di Scienze Cliniche Luigi Sacco, Via G.B. Grassi 74, 20157 Milan, Italy; fax 39-02-3564-018, e-mail carlo.franzini@unimi.it)

Capillary electrophoresis (CE) is an analytical technique for the separation of molecules on the basis of molecular size, electric charge, and hydrophobicity. Since automated clinical instruments have become available, the major clinical application of this technique has been the rapid and effective separation of serum proteins.

The detection of monoclonal protein components (MCs) is the main purpose of serum protein separation assays. Several reports have dealt with the feasibility of CE for the detection of MCs, and some pitfalls of this technique have also been described (1–13). Immunosubtraction is used in conjunction with CE for typing any detected MC, for confirming the diagnosis of monoclonal gammopathies, and for identifying the heavy and light chains constituting the monoclonal immunoglobulin molecule. Different types of MCs (IgG, IgA, or IgM, with associated κ or λ light chains) and free light chains (either κ or λ) have been reported to be detectable by CE, but there have been no observations concerning the behavior of free heavy chains in CE.


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