nmol/L (r = 0.90). P was <0.001 for all correlation coefficients.

Among the five samples distributed in January 2004 were two (samples 4 and 5) containing endogenous $25OHD_2$. A summary of the results is presented in Table 1.

The wide range of results submitted by users of the same method illustrates the degree to which 25OHD assays are operator-dependent. The one automated procedure (Nichols) produced more consistent values, but for the samples (1, 2, and 3) containing only $25OHD_3$, the results were again higher than those of other methods.

For samples 4 and 5, in which $25OHD_2$ is the predominant metabolite, method means for the IDS RIA and the Nichols automated assay were lower than those of other methods. The inference is that the IDS RIA and the Nichols automated procedure underestimate $25OHD_2$, the latter by a considerable margin. Indeed, the presence in these samples of $25OHD_3$, albeit in smaller amounts than $25OHD_2$, disguises the true extent of the problem. IDS acknowledge that their RIA has only a 75% cross-reactivity with $25OHD_2$ *(11)*, but Nichols claims a 100% cross-reactivity and has presented data to support this *(12)*. The reason for the disparity between the manufacturer's claims and the DEQAS findings is unclear. Low recovery of $25OHD_2$ by the Nichols automated assay has also been observed in clinical samples (Dr. Carol Wagner, Department of Pediatrics, Medical University of South Carolina, Charleston, SC, personal communication). Vitamin-D-deficient neonates failed to show an increase in 25OHD, despite receiving large doses of ergocalciferol. When the samples were reanalyzed by the DiaSorin assay, the expected increase in 25OHD was observed. This suggests that the problem is not confined to DEQAS samples, an explanation originally proposed by the manufacturer to explain the overrecovery of $25OHD_3$. In the IDS RIA, the underrecovery of $25OHD_2$ can be attributed to differences in antibody specificity for the two forms of the metabolite. Interestingly, the same antibody is used in the nonisotopic version of the IDS assay, which despite the published cross-reactivity data of 75%, did not appear to underestimate $25OHD_2$ in the DEQAS samples.

In summary, an international quality assessment scheme has demonstrated that, for samples containing only $25OHD_3$, most commercial 25OHD methods are capable of giving results close to the target value, but the results are highly operator dependent. The Nichols automated assay gives more consistent results but generally produces higher values than other methods. For samples containing predominantly $25OHD_2$, the Nichols procedure and, to a lesser extent, the IDS RIA gave considerably lower results than other methods. The underrecovery of $25OHD_2$ by the Nichols assay, which has also been observed in clinical samples, occurred despite the manufacturer's claim that the method is equally specific for both forms of 25OHD. The treatment of vitamin-D-deficient patients could be severely compromised by the use of assays that underestimate $25OHD_2$.

The validity of 25OHD results will, justifiably, continue to be questioned. The only way for laboratories to demonstrate the accuracy of their results is to participate in an external quality assessment scheme and to make details of their performance available to clinical colleagues.

Since this article was submitted, Nichols Institute Diagnostics has issued a technical note in which they acknowledge that some samples containing substantial quantities of $25OHD_2$ give low results in the Advantage automated assay.

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Assay-Specific Differences in Lipemic Interference in Native and Intralipid-Supplemented Samples, *Joshua A. Bornhorst,[1] Richard F. Roberts,[2] and William L. Roberts[1]** ([1] Department of Pathology, University of Utah Health Sciences Center, Salt Lake City, UT; [2] ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT; * address correspondence to this author at: ARUP Laboratories, 500 Chipeta Way, Salt Lake City, UT 84108; fax 801-584-5207, e-mail William.Roberts@aruplab.com)

Lipemia is a potential cause of analytical interference *(1, 2)*. Determinations of the lipemic index (L-index) or triglyceride concentrations are used to quantify lipemia *(3, 4)*. The soy-based lipid emulsion, Intralipid, has been

used to simulate lipemia in interference studies *(2, 5)*, but without evidence of how well it simulates naturally occurring lipemia.

Many serum proteins can be quantified by immunoturbidimetric assays *(6)*. Lipemia interferes by altering light scattering *(1)*. Manufacturers often provide guidelines for the maximum acceptable lipemia that have been established by interference experiments using Intralipid-supplemented samples. For the Modular Analytics P analyzer (Roche Diagnostics), the maximum allowable triglyceride values range from 4000 mg/L for the prealbumin assay to 20 000 mg/L for the haptoglobin assay. Sample turbidity is only weakly correlated with triglyceride concentration in patient samples *(4)*. Thus, to test the validity of the lipemic thresholds, we directly compared interference from lipemic patient samples with interference induced by supplementation with Intralipid.

We prepared pooled samples by mixing excess serum from two to five individual patient samples. Samples were assayed immediately or were stored at 4 °C for up to 2 weeks before testing. Samples were mixed by multiple manual inversions before analysis. The 16 pooled samples were simultaneously assayed for L-index, triglycerides, α_1-antitrypsin, ceruloplasmin, haptoglobin, prealbumin, and transferrin on a Modular Analytics P 800 analyzer. All results are the means of duplicate measurements.

The protein assays are non-particle-enhanced immunoturbidimetric assays. The primary assay wavelength is 700 nm and the secondary wavelength 340 nm, except for transferrin (secondary wavelength, 505 nm). All absorbances measured at these wavelengths were within the linear range of the photometer. Triglycerides were measured by a colorimetric enzymatic assay with a primary wavelength of 700 nm and a secondary wavelength of 505 nm. The L-index was determined by the difference between the absorbances of the sample, diluted in isotonic saline, at 660 nm and 700 nm.

The protein assays used similar reagent and sample volumes. For the ceruloplasmin and prealbumin reactions, 20 μL of sample was mixed with the first reagent (250 μL), and then the second reagent (83 μL) was added. The α_1-antitrypsin and haptoglobin reactions were similar except that the sample volumes were 10 μL. In the transferrin assay, the initial sample volume is 35 μL, the first reagent volume is 270 μL, and the second reagent volume is 100 μL.

After analysis, the samples were subjected to ultracentrifugation in an Airfuge (Beckman Coulter) at 134 000*g* for 5 min. This markedly reduced the visual turbidity of the sample infranate. The infranate was then reassayed in duplicate for the same set of analytes. Intralipid (200 mL/L; Baxter Healthcare Corp.) was then added to each infranate to produce L-index values comparable to or exceeding those of the original serum pool. The addition of Intralipid had a very small impact on the total volumes of the samples. In general, the addition of 10 μL of 200 mL/L Intralipid to a 1-mL nonlipemic serum sample generated a L-index increase of ~225. For each pool, the concentration of triglycerides in the Intralipid-supplemented infranate exceeded that found in the original pool. The Intralipid-supplemented samples were then assayed in duplicate. The ratio of the measured analyte concentrations in each sample after ultracentrifugation to that in the original patient sample was calculated. Sample dilution was performed with sterile normal saline. All studies using samples obtained from humans were approved by the Institutional Review Board of the University of Utah.

The relationship between L-indices and triglycerides in patient samples and Intralipid-supplemented patient samples were examined (Fig. 1, A and B). The correlation coefficients for triglyceride concentrations and L-indices in Intralipid-supplemented serum pools (r = 0.84) and lipemic patient samples (r = 0.88) were comparable.

The effects of lipemia on test results were examined (Fig. 1C). Increasing triglyceride concentrations in the original pool were associated with substantial depressions of reported results for both ceruloplasmin and prealbumin. The degree of interference varied linearly with the triglyceride concentrations and correlated less well with L-index values (data not shown). Linear best fits of the data indicated that at a triglyceride concentration of 10 000 mg/L, the measured ceruloplasmin and prealbumin concentrations were depressed 52% and 38% compared with the observed values in infranates (Fig. 1C). Measured transferrin concentrations in the pooled sera were slightly depressed in lipemic samples (14% depression at a triglyceride concentration of 10 000 mg/L). These triglyceride concentrations (10 000 mg/L) roughly correspond to a L-index of 200 in patient samples (Fig. 1A). Lipemia in patient samples had little effect on measured concentrations of α_1-antitrypsin and haptoglobin. In contrast to native lipemic samples, supplementation of pooled sera with Intralipid to a triglyceride concentration of 16 000 mg/L (L-index of ~400) had minimal effect on all analytes tested (Fig. 1D), although the measured ceruloplasmin concentration was observed increased slightly. As indicated by a linear fit of the data, at a triglyceride concentration of 10 000 mg/L, the measured ceruloplasmin concentration increased 7%.

All mean analyte concentrations after ultracentrifugation were near the middle of their respective reference intervals. The mean (SD) for the analytes after ultracentrifugation were 300 (100) mg/L for ceruloplasmin (reference interval, 200–600 mg/L), 1600 (700) mg/L for haptoglobin (reference interval, 300-2000 mg/L), 280 (60) mg/L for prealbumin (reference interval, 200–400 mg/L), 1400 (300) mg/L for α_1-antitrypsin (reference interval, 1000–2000 mg/L), and 2800 (700) mg/L for transferrin (reference interval, 2000–4000 mg/L). The L-index in the samples after ultracentrifugation was dramatically reduced relative to the original samples and had a mean (SD) L-index of 17 (9). The residual triglyceride concentrations in the samples after ultracentrifugation were reduced less uniformly, yielding a mean (SD) concentration of 3900 (2900) mg/L. Finally, ultracentrifugation of five Intralipid-supplemented samples was, in some cases, less effective in reducing L-index [mean (SD) L-index, 37 (28)] than ultracentrifugation of native patient samples.

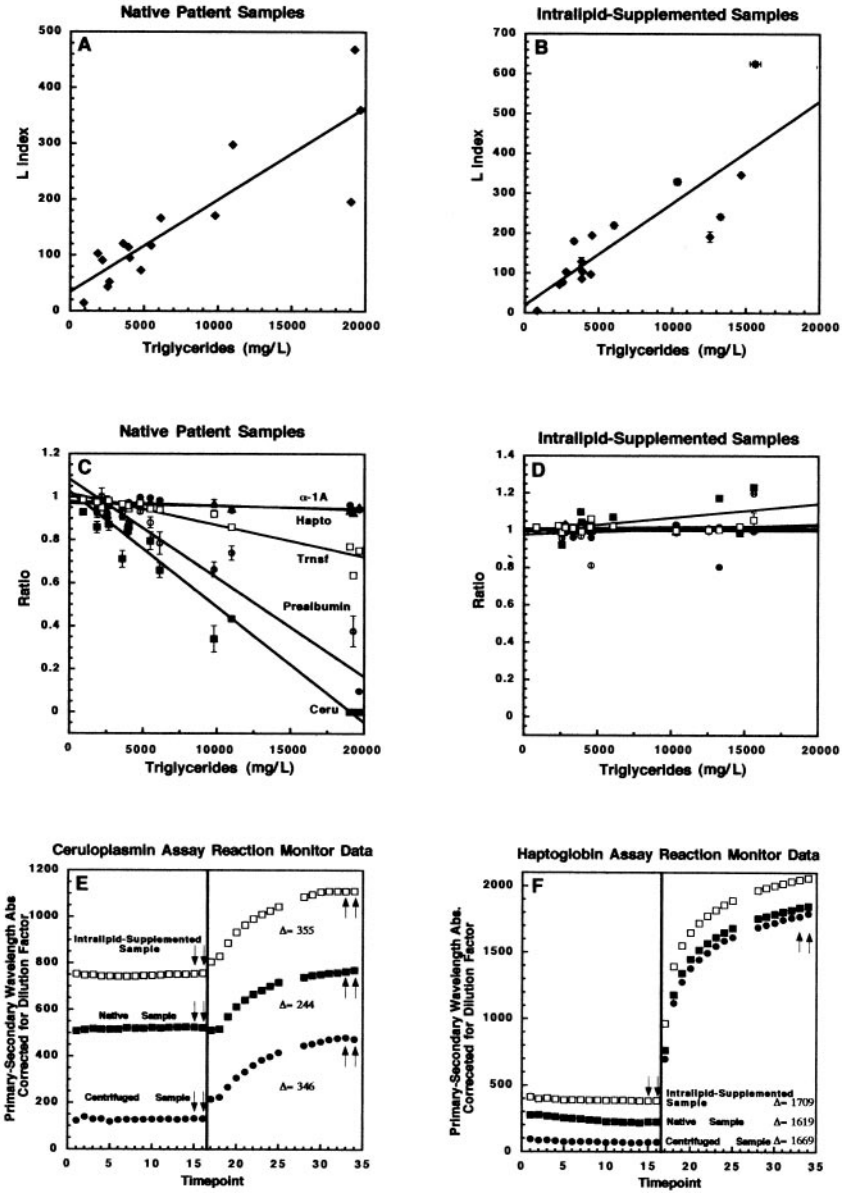

Fig. 1. Results for the various analytes (*A–D*) and reaction monitor data (*E* and *F*).

(*A–D*), individual results (*error bars*, 1 SD) and linear regression lines are shown. For points with no apparent *error bars*, the SD is less than the size of the data point. (*A*), relationship between L-index values and triglyceride concentrations for 16 patient samples. The slope is 0.165; $r = 0.876$. (*B*), L-index values vs triglyceride concentrations for the same 16 samples after ultracentrifugation and supplementation with Intralipid. The slope is 0.256; $r = 0.842$). (*C* and *D*), results for α_1-antitrypsin (●), ceruloplasmin (■), haptoglobin (▲), transferrin (□), and prealbumin (○). (*C*), the ratio of the analyte concentration measured in patient samples to the analyte concentration measured after removal of lipemia by ultracentrifugation is plotted on the *y axis*. (*D*), the ratio of analyte measured in Intralipid-supplemented samples to analyte measured after removal of lipemia by ultracentrifugation is plotted on the *y axis*. (*E* and *F*), reaction monitor data for ceruloplasmin and haptoglobin. Data were obtained from a single patient sample (■; L-index, 121; triglycerides, 3570 mg/L), after ultracentrifugation (●; L-index, 12; triglycerides, 1920 mg/L), and after Intralipid supplementation (□; L-Index, 195; triglycerides, 4560 mg/L). The mean net absorbances of time points 15 and 16 are subtracted from the net absorbance readings at time points 33 and 34 to obtain the absorbance change for each sample. *Arrows* indicate these time points. (*E*), volume-corrected data for the net absorbance ($A_{700} - A_{340}$) for the ceruloplasmin assay. The sample (20 μL) is mixed with the first reagent (250 μL) at time point 0. At time point 16, the second reagent (83 μL) is added (represented by the *thick vertical line*). The net absorbance after time point 16 is corrected for sample dilution by dividing by 0.765. The ceruloplasmin concentrations were 149 mg/L for the original sample, 216 mg/L for the sample after ultracentrifugation, and 225 mg/L for the Intralipid-supplemented sample. (*F*), volume-corrected data for the haptoglobin assay. All conditions are as described for the ceruloplasmin assay except for an initial sample volume of 10 μL and a dilution correction factor of 0.758. The measured haptoglobin concentrations were 1246 mg/L for the original sample, 1336 mg/L for the sample after ultracentrifugation, and 1308 mg/L for the Intralipid-supplemented sample.

Refrigerated storage of serum pools did not appear to significantly affect the lipemic interference. A freshly pooled sample with a triglyceride concentration of 6450 mg/L and a L-index of 160 exhibited lipemic interference comparable to the lipemic interference effects observed in stored serum pools as described above. For example, the original patient sample had a measured ceruloplasmin concentration of 174 mg/L and a prealbumin concentra-

tion of 220 mg/L, which increased after ultracentrifugation to 319 and 319 mg/L, respectively. After storage at 4 °C for 6, 9, and 15 days, the measured L-index and all analyte concentrations were identical or increased only slightly. After 15 days of storage, the measured L-index was 177, the measured triglyceride concentration was 6900 mg/L, and the ceruloplasmin and prealbumin concentrations were 208 and 230 mg/L, respectively.

Dilution was examined as an additional method to reduce assay interference. After 15 days of storage, the same pooled sample was diluted 1:3 with normal saline, and the concentrations of the analytes were measured. The L-index was 168, the triglyceride concentration was 6690 mg/L, and the ceruloplasmin and prealbumin concentrations were 318 and 345 mg/L, respectively, comparable to concentrations observed after ultracentrifugation. Thus, dilution appears to markedly reduce lipemic interference in these assays.

Examination of the P 800 analyzer reaction monitors for the ceruloplasmin and haptoglobin assays revealed a possible explanation for the negative interference by native lipemia seen for ceruloplasmin. The original sample examined was moderately lipemic and had a L-index of 121 as is frequently observed in patient samples. When reagent was added after timepoint 16, the absorbance decreased slightly at timepoints 17 and 18 for the native sample but increased noticeably for both the centrifuged and Intralipid-supplemented samples (Fig. 1E). This decrease in absorbance after reagent addition to a lipemic sample likely plays a role in the 30% depression of the measured ceruloplasmin concentration because it decreases the difference between the initial sample absorbance measurements before second reagent addition and the final absorbance measurements. This effect was not observed for the measurement of haptoglobin, although a larger change in absorbance during the haptoglobin assay may render the effect relatively inconsequential (Fig. 1F). Finally, a slight slowing of the rate of the reaction in the lipemic sample relative to that seen after ultracentrifugation cannot be entirely excluded.

The observed correlation between the triglyceride concentration and turbidity was relatively poor ($r < 0.90$) in both the Intralipid-supplemented and patient lipemic samples. The poor correlation between measured turbidity and triglycerides in patient samples has been noted previously, although supplementation with Intralipid led to a good correlation between triglycerides and patient sample turbidity when lipid emulsion was titrated into a single pool of patient samples *(4, 7)*. The nonlinear relationship between triglyceride concentration and sample turbidity in lipemic patient samples likely results from nonhomogeneity in the organization of lipids in patient samples *(4, 8–10)*. Triglycerides are likely to be present in various macromolecular forms, including both VLDL particles and chylomicrons. Chylomicrons can contribute substantially to sample turbidity, whereas VLDL particles make more minor contributions. The distribution of triglycerides in these macromolecular complexes is patient-specific.

Lipemia found in patient samples, patient samples after ultracentrifugation, and in samples supplemented with Intralipid elicited markedly different degrees of interference in some clinical assays. Turbidimetric assays may be particularly sensitive to selective perturbation by lipemic samples. Differences in particle size in lipemic samples can dramatically affect light scattering and alter turbidity *(11)*. Ultracentrifugation preferentially removes larger buoyant macromolecular lipid complexes such as chylomicrons from patient samples and thus alters the lipid profile. The lipid composition in the soy-based emulsion Intralipid consists predominantly of small, relatively dense, phospholipid-rich liposomes and triglyceride-rich artificial chylomicrons *(12)*, whereas patient samples contain a complex mixture of macromolecular lipid-and-protein structures *(8)*.

The assay-specific nature of the interference in lipemic patient samples appears to be predominantly attributable to disruptions of complex macromolecular structures on the introduction of assay-specific reagents. This may be caused by differences in the composition of these reagents or by the presence of trace amounts of detergents. Polyethylene glycol, which is a component of all of the reaction reagents, may contain trace amounts of contaminating detergents. Patient samples, after either ultracentrifugation or Intralipid supplementation, exhibit only minor assay interference, presumably because of the lack of higher-order lipid-lipoprotein complexes. However, assay-specific interference in lipemic samples arising through phase-partitioning of the analyte and increases in the volume of the nonpolar phase that slow the reaction rate cannot be completely ruled out *(13)*.

These results have important implications for the evaluation of lipemic interference in the clinical laboratory. In some assays, the lipemic interference in patient samples was not observed in Intralipid-supplemented samples with comparable or higher L-index and triglycerides values. This may not be limited to turbidimetric assays, as it has been noted that for a rate-blanked compensation assay for creatinine, lipemic interference can occur in patient samples but not in samples containing synthetic lipid emulsions *(7)*. Thus, the use of samples supplemented with Intralipid does not provide a universally applicable method to estimate endogenous assay interference in lipemic patient samples. This suggests that interference studies should be performed using lipemic patient samples rather than Intralipid-supplemented samples.

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Locked Nucleic Acid-Enhanced Detection of *1100delC*CHEK2* Germ-Line Mutation in Spanish Patients with Hematologic Malignancies, *María Collado,[1] Olfert Landt,[2] Eva Barragán,[1] Ulrich Lass,[2] José Cervera,[3] Miguel A. Sanz,[3] and Pascual Bolufer[1]** ([1] Laboratory of Molecular Biology, Department of Medical Biopathology, and [3] Clinical Hematology, Service of Hematology, Hospital Universitario La Fe, Valencia, Spain; [2] TIB MOLBIOL, Berlin, Germany; * address correspondence to this author at: Laboratory of Molecular Biology, Escuela de Enfermería 7°, Hospital Universitario La Fe. Avda. Campanar 21, 46007 Valencia, Spain; fax 34-91-806-1206, e-mail bolufer_pas@gva.es)

The prediction that there might be common DNA sequence variants that confer a small but appreciably enhanced risk of cancer has been validated with the discovery of the germ-line mutation *1100delC* in the cell cycle checkpoint kinase gene (*CHEK2*; OMIM 604373) *(1, 2)*. *CHEK2* is located on chromosome 22q and encodes the human ortholog of yeast Cds1 and Rad53, which are G_2-checkpoint kinases *(3)*. CHEK2 is a protein kinase activated in response to DNA damage involved in cell-cycle arrest. It serves as a link in the ATM-CHEK2-CDC25A-CDK2 pathway that checks genomic integrity in response to DNA damage *(4)*. The *1100delC* mutation in exon 10 abolishes the kinase function of *CHEK2 (5)* and has been reported in patients with Li–Fraumeni syndrome in the United States and in Finnish families with a cancer phenotype suggestive of Li–Fraumeni syndrome, including breast cancer *(5)*.

There have been recent reports of a higher incidence of the *1100delC* mutated allele in patients with a family history of breast cancer who are not carriers of *BRCA1* (OMIM 113705) or *BRCA2* (OMIM 600185) mutations, compared with healthy controls (4.2% and 5.5% in breast cancer cases vs 1.4% and 1.1% in controls, respectively) *(1, 6)*. The presence of the mutated allele approximately doubles the breast cancer risk in women and increases it 10-fold in men *(1)*. It has also been reported that 4.8% of individuals with familial prostate cancer are carriers of distinct *CHEK2* germ-line mutations *(7)*. These mutations may contribute to prostate cancer risk, highlighting the importance of the integrity of DNA damage-signaling pathways in the development of prostate cancer.

The few reports for patients with hematologic malignancies have been concerned mainly with *CHEK2* somatic mutations *(8–10)*. Thus, in a series of 109 patients with leukemia and myelodysplastic syndrome (MDS), two somatic mutations were reported: one among 55 patients with acute myeloid leukemia and another among seven with non-Hodgkin lymphomas *(8)*. Similarly, two somatic mutations were found in a study carried out on 10 patients with MDS and 3 with acute myeloid leukemia *(9)*, and in a study carried out on 60 patients with non-Hodgkin lymphoma, there was a germ-line mutation in 1 with a mantle cell lymphoma *(10)*. Thus, in general, there is a low incidence and low relevance of *CHEK2* somatic mutations in the etiology of leukemia.

However, little is known about the relevance of the germ-line mutations of *CHEK2* in the risk for developing leukemia and whether the germ line *1100delC CHEK2* sequence variant confers higher risk of leukemia, especially for treatment-related leukemia (TRL) and MDS. To address this point, we screened for the *1100delC CHEK2* germ-line mutations in patients with leukemia or TRL/MDS and in a control group.

We studied 107 patients with acute leukemia (AL). Two patients had AL (one type B, common; and one biphenotypic), and 105 had acute myeloid leukemia: 1 with French-American-British subtype Mo, 11 with subtype M1, 13 with subtype M2, 65 with subtype M3, 4 with subtype M4, 2 with subtype M4Eo, 5 with subtype M5, 2 with subtype M6, 1 who was not classified, and 1 with subtype M1 at relapse. There were 52 males and 55 females, and the median age was 46 years (range, 1–78 years). We also studied a group of 26 patients with TRL/MDS (15 males and 11 females) with a median age of 64 years (range, 7–87 years) at the time of diagnosis of the primary tumor (Table 1). The control group consisted of 176 healthy volunteers (69 males and 107 females) who had a median age of 36.5 years (range, 16–75 years).

DNA was extracted from 500 μL of whole blood anticoagulated with EDTA by use of MagNA Pure LC DNA Isolation Kits-Large Volume (Roche Diagnostics) with the MagNA Pure LC System (Roche).