for dibucaine-inhibited activity; and \( y = 1.4x - 27 \) (\( r = 0.995 \)) for dibucaine number.

By the criteria established for the KDA methodology (1), both methods classified five of the seven as normal homozygotes (dibucaine numbers 72–80) and the other two as heterozygotes (dibucaine numbers 50–65).

Thus the method we originally developed for the KDA analyzer has been successfully applied to the RA-1000 and yields similar classifications for pseudocholinesterase variants.

Reference


How Accurate Are Your IgE Determinations? Joyce G. Schwartz,1 Richard W. Brown,1 and Carole L. Gage2

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We received a request for IgE quantification in a serum sample from a 13-year-old Latin-American boy who had had severe atopic dermatitis since birth. The patient also complained of a chronic cough and congestion exacerbated by exposure to dust. He had no personal history of asthma; however, his family history was positive for maternal asthma and paternal atopic dermatitis.

Pertinent laboratory values included an increased leukocyte count of 13 100 mm\(^{-3}\) (13.1 \( \times \) 10\(^{9}\)/L) with 20% eosinophils; hemoglobin 163 g/L; and hematocrit 48%. Values for IgA, IgM, IgG, and IgG subclasses were within normal limits. Radioallergosorbent (SAA) testing included markedly increased values for all antigens (e.g., foods, pollens, dust) tested.

At the request of the patient’s physician, we divided the patient’s serum sample for IgE analysis, sending half the sample to a local immunology laboratory and assaying half in our laboratory.

We used the Kallestad Quanticlone IRMA (two-site) kit (Kallestad Laboratory, Inc., Austin, TX 78701) for the IgE assay. Our initial analysis had revealed a concentration of >50 000 kilo-units/L; the sample eventually had to be diluted 100-fold before an accurate quantitative result of 59 000 kilo-units/L was obtained. [The upper linear limit of the Quanticlone IgE kit is 1000 kilo-units/L.]

The immunology laboratory used the Phadebas IgE "Prist" kit (Pharmacia Diagnostics, Piscataway, NJ 08854), a radioimmunosorbent test. Using only one dilution of 1:20, they reported a result of 1400 kilo-units of IgE per liter. [The upper linear limit of the IgE Prist Kit is 100 kilo-units/L.]

When our laboratory noted the discrepancy between the two values, we contacted the immunology laboratory and told them that they might have been experiencing a high-dose "hook effect." When they repeated the assay with multiple dilutions (a final dilution of 9000-fold), the immunology laboratory obtained an IgE result of 54 000 kilo-units/L.

A known, but often ignored, disadvantage of the two-site IRMA kits and radioimmunosorbent tests is the high-dose "hook effect," a paradoxical decrease in radioactive counts at high concentration of antigen, such that the curve of radioactivity concentration is not strictly linear but includes a peak. Because the hook effect takes place at very high concentrations in serum, this problem does not affect samples with mild to moderate increases in IgE. However, because some patients can have extremely high IgE concentrations, it is a good practice to assay two dilutions of each patient’s sample and compare results. If the calculated IgE concentration of the more dilute sample is significantly greater than that of the less-dilute sample, it could be an indicator of the hook effect (1).

To decrease possible interference by the hook effect and for optimum patient care, laboratories utilizing radioimmunosorbent test kits or two-site commercial IRMA kits, not only for IgE but also for ferritin, thyrotropin, and (especially) chorionic gonadotropin, should always assay two dilutions of each patient’s sample and seek those kits with high upper limits of linearity. We question how many patients are given falsely decreased values for these assays.

Reference


GC/MS Confirmation of Urinary Opiates by Use of Trimethylsilyl Derivatives, Amitava Dasgupta1 and Virginia Hauer2

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Gas–liquid chromatographic (GLC) analysis for codeine can be done directly, but the phenolic hydroxy group in morphine must be derivatized for optimal separation and detection. Most commonly used is the acetyl derivative (1), but the potential use of trimethylsilyl (TMS) derivatives has been poorly investigated (2). We adapted the procedure of Saady et al. (3) and Paul et al. (4), using TMS to derivatize.

To 5–10 mL of urine in a 25-mL round-bottom flask, add 1 mL of concentrated HCl, reflux at 100 °C (we used an oil bath) for 30 min, cool to room temperature, and adjust the pH to 9.7 ± 0.2 with concentrated NaOH. Extract the opiates using 10 mL of a mixture of toluene/heptane/isoamyl alcohol (70/20/10 by vol). This mixture gives a cleaner preparation than chloroform/isopropanol (70/30).

Evaporate the organic layer to dryness under nitrogen. Add 50 mL of bis(trimethylsilyl) trifluoroacetamide. Cap and heat in a water bath at 55 °C for 30 min. Cool to room temperature and inject 1–2 μL into the GC/MS. We used a Model 5995 GC (Hewlett Packard) with a direct capillary system to the ion source, a splitless injection system, and a 5% phenylmethyl capillary column (0.25 mm × 0.3 mm i.d.). The flow rate of carrier gas (helium) was 1 mL/min. Injector temperature was 250 °C, oven temperature 250 °C. The column temperature was increased at 10 °C/min to a final temperature of 270 °C and held there for 16 min. The detector voltage was set at the "autotune" level.

We found the TMS derivative of opiates to be more volatile (as evidenced by shorter retention times) and produced better quality of mass spectra with more characteristic peaks than the corresponding acetyl derivatives (Figure 1). The retention times for the TMS derivatives of codeine and morphine were 8.4 and 9.2 min, respectively. Retention times of the acetyl derivatives were about 2 min longer. We observed no abnormal silylation in the double bond of the

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allylic alcohol moiety of codeine. The TMS derivatives can be injected directly into the GC/MS, and do not require a drying step—an advantage compared with the acetyl derivatization procedure.

We found acid hydrolysis better than enzymatic hydrolysis for liberation of the conjugated opiates. Analytical recoveries (larger peaks) of morphine were better and the acid hydrolysis is shorter than the enzymatic procedure, which takes several hours or an overnight incubation. Opiates decompose, however, if urine is refluxed for >30 min. Similar findings were noted by Fish and Hayes (5).

Operating in the "scan" mode, the on-column sensitivity for morphine is about 40 ng (with a signal/noise ratio >10), indicating the method has sufficient sensitivity for use in drug testing and confirmation.

References

Misidentification of Urine Lipid Bodies Owing to Use of Starch-Powdered Gloves, Pierre-Etienne Senécal and Jacques Rochette (Département de biochimie médicale, Hôpital du St-Sacrement, 1050 Chemin Ste-Foy, Québec, QC, Canada G1S 4L8)

We have found that the powder from at least one brand of powdered gloves ("Tru-Touch"; Becton Dickinson, Mississauga, Canada) can give rise to false urine lipid bodies (ULB) under microscopy. This is noteworthy in light of the increasing trend to wear gloves during manipulations of body fluids (1).

This is of clinical importance because the presence of positively birefringent ULB may lead to the diagnosis of the nephrotic syndrome (2, 3). ULB are sometimes called "oval fat bodies" (4, 5), and they can form fatty casts. If contamination has already occurred or is suspected, three criteria can be used to distinguish true from false ULB. First, as shown in Figure 1, false ULB are not as regular and round as true ULB. Secondly, under crossed polarized light, the conical texture of the false ULB is more isometric. This is the "French cross pâtée" (6), tend to be off center. Thirdly, true ULB tend to accompany proteinuria, and in the nephrotic syndrome (2); usually the degree of proteinuria has already been determined by dipstick analysis. We found no advantage in using a stain ("Sedi-Stain"; Clay-Adams, Parsippany, NJ) to differentiate true ULB from false. Sudan III stain at 70% alcohol has been advocated for this use. Martin and Small (7) used the temperature of the transition from anisotropism to isotropism (~42 °C) to characterize oval fat bodies (7), but we find this impractical in the usual clinical laboratory.

The contaminant was shown to be corn starch by the color it developed with an iodine solution; this finding was confirmed by the manufacturer. A commercial talcum powder did not resemble ULB at all when examined under the same conditions.

Thus we suggest that all laboratories performing microscopic urinalyses verify whether this artifact could occur in their own laboratory: scrape a glove's interior with a slide, cover the residue so obtained with normal urine, and search for contamination, using the aforementioned criteria.

References
4. Derman H. Identification and purification of formed elements in the urine. In: Sunderman FW, Sunderman FW, eds. Laboratory