
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 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/mm3 (13.1 × 10^3/L) with 20% eosinophils; hemoglobin 163 g/L; and hematocrit 48%. Values for IgA, IgM, IgG, and IgG subclasses were within normal limits. Radioallergosorbent (RAST) 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 Quanti clone IgE kit is 1000 kilo-units/L.]

The immunology laboratory used the Pharmacia 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) choriogonadotropin, 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 Haver1,2 (1 Dept. of Lab. Med., Univ. of Washington, Seattle, WA 98195; and 2 VA Med. Center, Seattle, WA 98108; use second author and second address for correspondence)

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 μL 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 (33 m × 0.32 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