



Fig. 1'. Box-plot presentation of the ratios of three different mAb-mAb immunofluorometric LH assays to an oligo mAb IFMA (7 capture mAbs + 10 detector mAbs).

Serum samples tested were from control subjects ($n = 10$) and subjects with PCOD ($n = 50$), HA ($n = 8$), and CRF ($n = 15$). ** $P < 0.01$, *** $P < 0.001$.

compared with the oligo IFMA. The restricted reactivity of the genetic variant of LH with intact specific mAbs should therefore be distinguished from the restricted reactivity of CRF samples, which is apparent also when using β - β or β - α mAb combinations.

The authors seem to put strong faith in the accuracy of the DELFIA LH kit as it "interacts with all LH subtypes." Although responsible for the development of this kit, I can neither refute nor confirm this statement despite their reference to our previous work. Such an assertion would demand a more thorough effort than we or the Marseille group has undertaken so far. What we have shown (2) is that DELFIA LH_{spec}, in comparison with four assays involving intact LH-specific mAbs (two of which were commercial), does not show the same restricted reactivity of LH in certain individuals as do these, and that the correctness of the LH_{spec} results is supported by determination of LH bioactivity.

Another important aspect of the present report, echoing previously made claims by Carayon et al. (7, 8), relates to the designation of the correct specificity of the LH mAbs as reported by us. According to this argument, we incorrectly determined the specificity of one of the two antibodies used by DELFIA LH_{spec}. Whereas we state that this kit uses two LH β antibodies, Carayon and co-workers claim, as a result of their collaborative epitope mapping study, that one of the antibodies is an α - β mAb. This assertion totally dismisses the documentation presented by us earlier (2, 9), where we documented the high cross-reactivity of this assay with free LH- β preparations. Furthermore, this assay was recently

(10) used to measure the free β -subunit concentrations after an adsorption step to remove the intact LH dimers in sera from patients with pituitary adenomas.

I can think of two possible explanations for this disagreement. Free LH β with, e.g., a 10-fold lower affinity for a mAb compared with the intact LH dimer, may still be determined with equal potency with excess reagent in an immunometric assay. In our previous studies, the designation of an LH antibody to be specific for intact LH means $<5\%$ binding of LH β or LH α subunits to that antibody when tested in an excess reagent system. Well-defined and clearly stated quantitative criteria are needed when assigning a certain specificity to a mAb.

A more serious shortcoming of the previous studies by Carayon et al. (7) is the way the specificities were determined, i.e., by estimating the binding of each mAb to antigen preparations directly adsorbed onto a plastic surface. As has been reported (11), the immobilization of antigens to a plastic surface may result in deformation or preferential blocking of some of the epitopes compared with their appearance in solution. This phenomenon is widely recognized in the development of monoclonal antibodies. Screening of antibodies by using coated antigens will frequently detect only the coated antigen and not the native conformation. The results from an epitope study with such a basic methodological flaw are inevitably a target for justifiable doubt.

A detailed dissection of the results from our and Carayon's group according to these lines may possibly resolve some of the apparent contradictions that Carayon et al. have referred to previously (6, 7) and in the present communication. The question relating to what information is misleading may hopefully also be seen in a new, more constructive light.

References

- Pettersson K, Söderholm J. Individual differences in lutropin immunoreactivity revealed by monoclonal antibodies. *Clin Chem* 1991;37:333-40.
- Pettersson K, Ding Y-Q, Huhtaniemi I. Monoclonal antibody-based discrepancies between two-site immunometric tests for lutropin. *Clin Chem* 1991;37:1745-8.
- Pettersson K, Ding Y-Q, Huhtaniemi I. An immunologically anomalous luteinizing hormone variant in a healthy woman. *J Clin Endocrinol Metab* 1992;74:164-71.
- Pettersson K, Mäkelä M-M, Dahlén P, Lamminen T, Huoponen K, Huhtaniemi I. Gene polymorphism found in the LH beta gene of an immunologically anomalous

- variant of human luteinizing hormone [Abstract]. *Eur J Endocrinol* 1994;130:65.
- Furui K, Suganuma N, Tsukahara S-I, Asada Y, Kikkawa F, Tanaka M, et al. Identification of two point mutations in the gene coding luteinizing hormone (LH) β -subunit, associated with immunologically anomalous LH variants. *J Clin Endocrinol Metab* 1994;78:107-13.
 - Okuda K, Yamada T, Imoto H, Komatsubara H, Sugimoto O. Antigenic alteration of an anomalous human luteinizing hormone caused by two chorionic gonadotropin-type amino-acid substitutions. *Biochem Biophys Res Commun* 1994;200:584-90.
 - Costagliola S, Niccoli P, Florentino M, Carayon P. European collaborative study of luteinizing hormone assay: 1. Epitope specificity of luteinizing hormone monoclonal antibodies and surface mapping of pituitary and urinary luteinizing hormone. *J Endocrinol Invest* 1994;17:397-406.
 - Costagliola S, Niccoli P, Florentino M, Carayon P. European collaborative study on luteinizing hormone assay: 2. Discrepancy among assay kits is related to variation both in standard curve calibration and epitope specificity of kit monoclonal antibodies. *J Endocrinol Invest* 1994;17:407-16.
 - Pettersson K, Söderholm J. Ultrasensitive two-site immunometric assay of human lutropin by time-resolved fluorometry. *Clin Chem* 1990;36:1928-33.
 - Gil-del-Alamo P, Pettersson K, Saccomanno K, Spada A, Faglia G, Beck-Peccoz P. Abnormal response of luteinizing hormone beta subunit to thyrotropin-releasing hormone in patients with non-functioning pituitary adenoma. *Clin Endocrinol* 1994;41:661-6.
 - Schwab C, Bosshard H. Caveats for the use of surface-adsorbed protein antigen to test the specificity of antibodies. *J Immunol Methods* 1992;147:125-34.

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Bupropion Metabolites Produce False-Positive Urine Amphetamine Results

To the Editor:

The phenethylamine derivative bupropion (Wellbutrin®; Burroughs Wellcome Co., Research Triangle Park, NC) was introduced in the US in 1989. Oral doses of ≤ 450 mg per day are prescribed in treatment of depression (1). The drug is metabolized to *threo* and *erythro* amino alcohol metabolites, a morpholinol metabolite, and to a lesser extent other compounds, including diol and acidic

derivatives. The stereoselective reduction of the carbonyl group adjacent to the benzene ring of bupropion is reported to favor the *threo* amino alcohol metabolite in humans (1, 2). We believe that bupropion use can cause false-positive results in an assay designed to detect amphetamine abuse, as evidenced by the following data.

A patient in no acute distress was admitted to this hospital's inpatient psychiatry unit for treatment of depression. The patient had a history of polysubstance abuse and had been prescribed bupropion (300 mg per day) three weeks before admission. On admission a drug screen (3) detected bupropion metabolites in the patient's blood. The patient's urine was positive for "amphetamines" in the Emit II monoclonal immunoassay (Syva Co., San Jose, CA) performed on a Hitachi 717 analyzer (Boehringer Mannheim, Indianapolis, IN), but was negative for methamphetamine and amphetamine by liquid chromatography (3). The patient denied use of drugs other than bupropion and ethanol within the past month. We then quantitatively analyzed the urine by liquid chromatography as stated (3), except that we used a 150 × 4.6 mm ABZ column (Supelco, Bellefonte, PA) and a mobile phase of 350 mL of acetonitrile mixed with 550 mL of a pH 6.4, 30 mmol/L phosphate buffer. Retention times for the analytes were: morpholinol metabolite, 2.7 min; *threo* amino metabolite, 3.0 min; *erythro* amino metabolite, 3.4 min; bupropion, 4.3 min; and the internal standard (proprietyline), 6.3 min. Urine concentrations of bupropion-related substances were: bupropion, 12 mg/L; *erythro* amino metabolite, 45 mg/L; *threo* amino metabolite, 335 mg/L; and morpholinol metabolite, 19 mg/L.

We further investigated the role of bupropion in the immunoassay by preparing saline solutions of bupropion and three of its major metabolites and analyzing these solutions with the monoclonal Emit II immunoassay. The results (Table 1) indicate that all four compounds cross-react to some extent. The *erythro* amino alcohol metabolite appeared to cross-react the most; the *threo* and morpholinol metabolites and bupropion itself were less reactive. On a molar basis, the assay is quite selective: even the *erythro* metabolite at 417 μmol/L gave only about the same response as the 6.7 μmol/L methamphetamine calibrator.

The patient's urine exhibited reac-

Table 1. Reactivities of bupropion and metabolites in Emit II assay of amphetamines.

Conc tested, mg/L	Reactivity, mA/min at 340 nm			
	Bupropion	Morpholinol metab.	Erythro metab.	Threo metab.
0	-1	2	-1	3
10	0	6	6	1
20	3	8	18	9
50	12	16	33	15
100	19	28	47	27
200			63	48
300	52	58		57
400			85	

The reactivity of the negative calibrator was set at 0 on the Hitachi 717. The calibrator containing 1 mg/L *d*-methamphetamine gave a reading of 48 mA/min; urine samples with values greater than that of this calibrator are considered to be "positive" according to the manufacturer.

tivities (1 unit of reactivity being defined as 1 mA/min at 340 nm) in the 60–65 range (see Table 1), significantly greater than the 48 mA/min for the 1 mg/L methamphetamine calibrator. The combination of the high concentration of the *threo* metabolite and its modest cross-reactivity (Table 1) explains a large portion of the assay reactivity observed in the patient's urine. Despite its much lower concentration, the *erythro* metabolite may also have been a significant contributor; the morpholinol metabolite and the parent drug probably were not.

This patient's bupropion dosage regimen was not high. Welch et al. (4) reported urinary bupropion metabolite concentrations after a single 200-mg oral dose given to seven healthy male volunteers; the concentrations of unconjugated urinary metabolites were lower than those reported here. It is possible, however, that at steady-state the urinary bupropion metabolite concentrations will be higher than Welch et al. reported (4). We recently encountered a random urine sample from a patient receiving 450 mg per day bupropion and found: *erythro* metabolite, 20 mg/L; *threo* metabolite, 90 mg/L; morpholinol metabolite, 29 mg/L; and bupropion, 7 mg/L. This patient's urine was also positive for amphetamines by the Emit II assay, suggesting that typical doses may generate sufficient metabolites to cause "false positives."

From these findings, we believe bupropion should be added to the list of psychotropic drugs/metabolites that may cross-react in certain amphetamine drug-of-abuse immunoassays (5, 6).

References

1. Physician's desk reference, 47th ed. Oradell, NJ: Medical Economics, 1992: 842–4.
2. Rohrig TP, Ray NG. Tissue distribution of bupropion in a fatal overdose. *J Anal Toxicol* 1992;16:343–5.
3. Puopolo PR, Volpicelli SA, Johnson DM, Flood JG. Emergency toxicology testing (detection, confirmation, and quantification) of basic drugs in serum by liquid chromatography with photodiode array detection. *Clin Chem* 1991;37:2124–30.
4. Welch RM, Lai AA, Schroeder DH. Pharmacological significance of the species difference in bupropion metabolism. *Xenobiotica* 1987;17:287–98.
5. Olsen KM, Gulliksen M, Christophersen AS. Metabolites of chlorpromazine and brompheniramine may cause false-positive urine amphetamine results with monoclonal Emit® d.a.u.® immunoassay [Letter]. *Clin Chem* 1992;38:611–2.
6. Crane T, Dawson CM, Tickner TR. False-positive results from the Syva Emit d.a.u. monoclonal amphetamine assay as a result of antipsychotic drug therapy [Letter]. *Clin Chem* 1993;39:549.

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Demonstration of Macroamylasemia by Polyethylene Glycol (PEG) Precipitation Requires Correct PEG Concentration

To the Editor:

We recently encountered an anomaly while following a method published in this journal for confirming macroamylasemia by polyethylene glycol (PEG) precipitation (1).

A 43-year-old man (patient A) complaining of abdominal pain was found to have persistently increased amylase activity. The surgeon could find no cause for this and suspected macroamylasemia. Analysis of a serum and a urine sample gave: serum amylase 388 IU/L (reference range 10–90), serum creatinine 110 μmol/L (50–120), urine amylase 156 U/L (25–550), and urine creatinine 12 780 μmol/L (9000–12 000). The fractional excretion of amylase (amylase clearance/creatinine clearance) was calculated as 0.3% (reference range 2.3–6.3%), which was regarded as consistent with reduced renal clear-