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Serum Total Prolactin and Monomeric Prolactin Reference Intervals Determined by Precipitation with Polyethylene Glycol: Evaluation and Validation on Common ImmunoAssay Platforms

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BACKGROUND: Macroprolactin is an important source of immunoassay interference that commonly leads to misdiagnosis and mismanagement of hyperprolactinemic patients. We used the predominant immunoassay platforms for total prolactin and bioactive monomeric prolactin to assay serum samples treated with polyethylene glycol (PEG) and establish and validate reference intervals for macroprolactin.

METHODS: We used the Architect (Abbott), ADVIA Centaur and Immulite (Siemens Diagnostics), Access (Beckman Coulter), Elecsys (Roche Diagnostics), and AIA (Tosoh) analyzers with samples from healthy males (n = 53) and females (n = 93) to derive parametric reference intervals for total and post-PEG monomeric prolactin. Concentrations of immunoreactive prolactin isoforms in serum samples from healthy individuals were established by gel filtration chromatography (GFC). We then used samples from 22 individuals whose hyperprolactinemia was entirely attributable to macroprolactin and 32 patients with true hyperprolactinemia to compare patient classifications and prolactin concentrations measured by GFC with the newly derived post-PEG reference intervals.

RESULTS: Parametric reference intervals for post-PEG prolactin in male and female serum samples, respectively, were (in mIU/L): 61–196, 66–278 (Centaur); 63–245, 75–381 (Elecsys); 70–301, 92–469 (Access); 72–229, 79–347 (Architect); 73–247, 83–383 (AIA); and 78–263, 85–394 (Immulite). Concordance between GFC and immunoassay-specific post-PEG reference intervals was observed in 311 of 324 cases and for 31 of 32 patients with true hyperprolactinemia and 17 of 22 patients with macroprolactinemia. Results leading to misclassification occurred in a few analyzers for 5 macroprolactinemia patient samples with relatively minor increases in post-PEG prolactin (mean 61 mIU/L).

CONCLUSIONS: Our validated normative reference data for sera pretreated with PEG and analyzed on the most commonly used immunoassay platforms should facilitate the more widespread introduction of macroprolactin screening by clinical laboratories.

Prolactin-secreting pituitary adenomas and prolactinoma are the most common endocrine disorders of the hypothalamic-pituitary axis. Clinical manifestations include symptoms of hypogonadism and galactorrhoea, which are a direct result of prolactin excess *(1, 2)*. Laboratory measurements demonstrating hyperprolactinemia are essential for the diagnosis, but a major challenge facing laboratories is the correct differentiation of patients with true hyperprolactinemia, who have supraphysiological concentrations of bioactive monomeric prolactin, from those with macroprolactinemia, who have increased concentrations of macroprolactin together with normal concentrations of monomeric prolactin. Although rare in the general population, macroprolactinemia is frequently found in individuals receiving medical attention *(3–7)*. Unlike monomeric prolactin, macroprolactin is biologically inactive and its presence is clinically irrelevant *(3, 6, 8–10)*. Unfortunately, macroprolactin is detected to varying degrees by all prolactin immunoassays *(11–14)* and its presence commonly leads to diagnostic confusion and misdiagnosis *(15–19)*.

Monomeric prolactin (M_r 23 000) is the most common form of circulating prolactin in healthy in-

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dividuals and most patients with true hyperprolactinemia, but forms with higher molecular mass are also present, such as big prolactin (M_r 60 000) and big-big prolactin or macroprolactin [M_r 150 000) *(4, 8, 20, 21)*]. Macroprolactin is reported to be the cause of hyperprolactinemia in up to 26% of cases, with percentages varying depending on the assay used *(3, 22)*. Because some laboratories fail to screen hyperprolactinemic sera for macroprolactin, hyperprolactinemia attributable to macroprolactin has in some cases led to misdiagnosis and unnecessary medical and surgical intervention *(15–19)*. In the UK approximately 80% of laboratories now screen hyperprolactinemic sera for the presence of macroprolactin *(23)*. There are no data available on the screening protocols undertaken by US laboratories, although anecdotally it appears that such screening is performed infrequently. This lack of screening is surprising given the publicity the topic has received during the past decade, which includes the listing of macroprolactin by the Pituitary Society as a diagnostic pitfall *(24)* and authoritative recommendations to screen all patients for macroprolactin in the routine investigation of hyperprolactinemia *(25, 26)*.

Although gel filtration chromatography (GFC)[3] is the gold standard method for measurement of monomeric prolactin in sera, this method is slow, labor-intensive, and costly and hence is not suited to routine use. In a recent report of a comprehensive examination of alternative approaches we recommended polyethylene glycol (PEG) precipitation *(27)*, a method that is simple and inexpensive to perform and has been extensively compared with GFC *(4, 5, 28)*. PEG precipitation does have drawbacks, however. Recovery of monomeric prolactin is not quantitative, and up to 25% of the monomer present may be coprecipitated *(27)*. Furthermore, PEG has been reported to cause immunoassay interference on some analyzers, the AxSym (Abbott), Access 2 (Beckman Coulter), and the Immulite 2000 (Siemens) *(29–31)*. As a consequence, our group and others have recommended comparing post-PEG monomeric prolactin concentrations to a reference interval derived by use of identically PEG-treated sera from healthy individuals *(15, 27, 32)*. When an increased total serum prolactin concentration decreases to within an appropriate reference interval after PEG treatment, macroprolactinemia, as we have previously defined it *(15)*, is confirmed. In this setting, the increased total prolactin is of no clinical significance and may be reported as such, and the hyperprolactinemia initially observed is accounted for by the combined presence of excess macroprolactin together with big prolactin in the presence of concentrations of monomeric prolactin that are within the reference interval.

The current convention is to report results as a percentage of total prolactin recovered after PEG treatment, generally employing a cutoff of 40% to distinguish macroprolactinemia from true hyperprolactinemia. This latter approach lacks specificity, so the reported result may be misinterpreted in cases in which excess macroprolactin occurs simultaneously with supraphysiological concentrations of monomeric prolactin *(15)*. From a clinical point of view, the presence of excess monomeric prolactin is of overriding concern, and a diagnosis of macroprolactinemia in this setting is misleading and inappropriate. Moreover, the priority for the laboratory should be to determine whether the bioactive monomeric prolactin concentration is increased rather than simply to measure the percentage of macroprolactin present *(22, 33)*.

To overcome such problems we and others have advocated the necessity of establishing absolute method-specific reference intervals for prolactin in PEG-treated sera *(15, 27, 32)*. This approach would enable more rigorous definition of macroprolactinemia than previously employed by laboratories using PEG precipitation, and would prevent confusion when excess biologically active prolactin is present along with macroprolactin. In this study, we sought to derive reference intervals for serum prolactin measured before and after PEG treatment with assays on 6 widely used immunoassay platforms. We evaluated the diagnostic accuracy of these reference intervals relative to GFC.

Patients and Methods

STUDY PARTICIPANTS

For the determination of reference intervals, blood samples from healthy healthcare professionals (53 males and 97 females) were collected into plain Becton Dickinson Vacutainers. After collection, samples were left to clot at room temperature for 30 min, then centrifuged. The serum was then divided into aliquots and stored at −40 °C before analysis.

To compare classification of patient samples performed with GFC and the new analyzer-derived post-PEG reference intervals, we used samples from 19 females and 3 males whose hyperprolactinemia could be explained entirely by macroprolactin (macroprolactinemic group), and 28 females and 4 males with true hyperprolactinemia.

Approval for this study was obtained from the Research Ethics Committee, St. Vincent's University Hospital.

[3] Nonstandard abbreviations: GFC, gel filtration chromatography; PEG, polyethylene glycol.

ASSAY METHODS

We measured prolactin with the Access 2 (Beckman Coulter), ADVIA Centaur and Immulite 2000 (Siemens Diagnostics), AIA 1800 (Tosoh), and Architect (Abbott) and Elecsys 2010 (Roche Diagnostics, prolactin II assay). Immunoassays were calibrated against the WHO third international standard for prolactin, IS 84/500, except for the AIA, which used the second international standard, IS 83/562. Prolactin units (in mIU/L) can be converted (to ng/mL) by dividing by 21.2 (Access, Architect, Centaur, Elecsys and Immulite) or 27.0 (AIA). All sera were also treated with PEG *(15)*. Briefly, 250 μL of sera, mixed with an equal volume of PEG 6000 (VWR International UK, product 29577), 250 g/L in PBS (Sigma, 137 mmol/L sodium chloride, 10 mmol/L sodium phosphate), pH 7.4, was incubated for 10 min at room temperature. The suspension was clarified by centrifugation at 14 000*g* for 5 min, before prolactin measurement. The 25% (wt/vol) stock PEG solution was stored at 4 °C for no more than 14 days and allowed equilibrate to room temperature before use. To assess the stability of prolactin in 12.5% (wt/vol) PEG, 50 normal sera were treated with PEG, and the supernatants analyzed immediately and again after 24 h at 4 °C. All measurements of post-PEG prolactin for a given assay were completed within 24 h. In the case of Access and Immulite assays, prolactin was also measured in the PEG supernatant after a dilution of 1 in 5 with PBS.

GEL FILTRATION CHROMATOGRAPHY

We analyzed sera by using GFC as described previously *(4)*, but we used a 60-cm × 1.6-cm column of Superdex SD-75 with an AKTA protein purification system (Pharmacia Biotech) optimized to separate all 3 components of serum immunoreactive prolactin. Up to 1 mL of serum was applied to the column and eluted with Tris-buffered saline, pH 7.4. Fractions (1.4 mL) were collected and prolactin was measured with the AIA. Macroprolactin, big prolactin, and monomeric prolactin were determined from the area under the peaks. Using a frozen pool of serum with monomeric prolactin of 455mIU/L, we observed a CV of 4.5% during a period of 15 months, n = 8.

RECOVERY OF MONOMERIC PROLACTIN ADDED TO SERUM

We used a monomeric prolactin standard [IS 84/500, National Institute for Biological Standards and Controls, Hertfordshire, UK] to assess the effect of PEG on immunoassay performance. The standard was reconstituted in prolactin-naive sera (<9 mIU/L) and diluted to a final concentration of approximately 700 mIU/L before assay (n = 6) pre- and post-PEG treatment. To investigate precipitation of monomer by PEG, we washed the PEG precipitate with 12.5% (wt/vol) PEG in Tris-buffered saline, then dissolved the precipitate in Tris-buffered saline containing 40 g/L BSA before subjecting the resulting solution to GFC.

STATISTICAL ANALYSIS

We calculated reference intervals, defined as 95% confidence limits, with an IFCC-approved parametric method by using RefVal *(34)*. For data analysis we used the Student unpaired *t*-test, $P < 0.05$, or one-way ANOVA followed by a Newman-Keuls test, performed by using GB-STAT version 9 (Dynamic Microsystems).

Results

REFERENCE PANELS

The male reference panel consisted of samples from 53 individuals (age 20–64 years, median 28.0 years). For this panel of samples visual inspection of total prolactin and post-PEG distribution histograms for the 6 methods revealed data with a relatively gaussian distribution. Mean and median values for each of the analytical methods were very close, and Anderson-Darling coefficients were <0.752 ($P = 1.000$), indicating that the data were not overtly negatively or positively skewed (Table 1).

Prolactin in 4 sera in the female panel deviated by more than 3 SDs from the group mean and were excluded from analyses (see Discussion). The final female reference panel consisted of samples from 93 individuals (age 19–59 years, median 29.0 years). In contrast to the male data, female total and post-PEG prolactin distribution histograms for all methods appeared to be nongaussian and positively skewed on visual inspection, as confirmed by Anderson-Darling coefficients of 1.407–2.619 ($P = 0.000$) and a noticeable difference between mean and median values (Table 1). ANOVA and pair-wise comparisons (Newman-Keuls) indicated method-related differences in results for both male and female panels (Table 1).

Because both the total and post-PEG prolactin distributions for females were rendered normal by log transformation, as reflected in the considerably reduced Anderson-Darling coefficients ($P = 1.000$) (Table 1), we used transformed data for all subsequent analyses of female results. For consistency male data were also log transformed.

TOTAL AND POST-PEG PROLACTIN REFERENCE INTERVALS

The 95% reference intervals for total prolactin in male and female sera for each method are illustrated in Table 2, in addition to reference intervals provided by the manufacturers. Upper reference limits ranged from 262 mIU/L (Centaur) to 365 mIU/L (AIA) in males and from 348 mIU/L (Centaur) to 548 mIU/L (AIA) in females.

Table 1. Descriptive statistics for total prolactin and post-PEG prolactin (mIU/L) in samples from healthy males (n = 53) and females (n = 93) for each immunoassay platform.

Method	Minimum	Maximum	Mean	Median	SD	Differences[a]	A^{2b}
Total prolactin							
Males							
Centaur	51	298	146	139	50	a,b,d,e,f	0.452 (0.172)
Access	51	303	158	157	57	a,b,c,d,f	0.252 (0.258)
Immulite	55	305	167	159	58	a,b,c,d,e	0.364 (0.227)
Elecsys	62	391	180	171	66	a,b,c,e,f	0.450 (0.281)
Architect	70	322	188	185	61	a,c,d,e,f	0.375 (0.229)
AIA	75	393	211	201	72	b,c,d,e,f	0.271 (0.174)
Females							
Centaur	61	404	168	147	75	a,b,d	1.992 (0.285)
Access	66	527	192	162	91	a	2.073 (0.280)
Immulite	68	479	196	174	90	a	1.407 (0.456)
Elecsys	72	577	222	195	108	a,c	2.426 (0.279)
Architect	86	527	225	196	97	a,c	1.632 (0.197)
AIA	89	604	257	229	120	b,c,d,e,f	2.152 (0.226)
Post-PEG prolactin							
Males							
Centaur	56	214	123	122	37	a,e,f	0.264 (0.168)
Architect	60	228	141	142	42	e	0.764 (0.568)
Elecsys	54	279	144	142	48	e	0.298 (0.175)
AIA	60	246	151	148	47	c	0.474 (0.497)
Immulite	65	262	163	154	53	c	0.536 (0.441)
Access	61	347	172	176	61	b,c,d	0.405 (0.337)
Females							
Centaur	58	314	143	128	59	a,b,d,e,f	1.726 (0.266)
Architect	66	422	175	154	72	c,e	1.756 (0.261)
Elecsys	64	437	177	151	81	c,e	2.375 (0.251)
AIA	74	448	190	166	83	c	1.885 (0.317)
Immulite	73	462	198	187	85	c	1.546 (0.324)
Access	79	533	213	184	100	b,c,d	2.619 (0.218)

[a] One-way ANOVA followed by Newman-Keuls test, $P < 0.05$; a, vs AIA; b, vs Architect; c, vs Centaur; d, vs Elecsys; e, vs Access; f, vs Immulite.
[b] A^2 represents the Anderson-Darling coefficient with the coefficient generated using transformed data in parentheses.

Post-PEG reference intervals derived for male and female sera for the 6 analytical methods are listed in Table 3. The upper reference limits ranged from 196 mIU/L (Centaur) to 301 mIU/L (Access) in males and from 278 mIU/L (Centaur) to 469 mIU/L (Access) in females.

DISTRIBUTION OF PROLACTIN ISOFORMS IN NORMAL SERA AND UPPER REFERENCE INTERVAL FOR MONOMERIC PROLACTIN AFTER GFC

To establish an upper reference interval for monomeric prolactin measured with GFC (Fig. 1A), we studied normal female sera in the upper quintile of results after PEG precipitation, n = 16, and male sera with prolactin concentrations above a sensitivity threshold of 300 mIU/L, n = 5. GFC revealed that monomeric prolactin accounted for on average 72% of the total prolactin present in these normal sera (Fig. 2), although this percentage ranged from 30% to 85% (females, 226–444 mIU/L, median 354 mIU/L; males 104–288 mIU/L, median 238 mIU/L). Big prolactin was a fairly constant, although minor, component in all sera (mean 15%, range 9%–23%). In contrast, the contribution of macroprolactin was more variable and

Table 2. Reference intervals for total prolactin (mIU/L) in serum samples from males and females for each immunoassay platform.

Method	Parametric lower	Estimate upper	Manufacturer's range
Samples from males			
Access	58	277	56–278
Centaur	63	262	45–375
Immulite	70	281	53–360
Elecsys	72	331	86–324
Architect	85	310	54–381
AIA			
Samples from females	89	365	97–440
Centaur	71	348	59–619
Immulite	75	396	40–530
Access	77	408	71–566
Elecsys	88	492	102–496
Architect	98	447	25–629
AIA	105	548	111–780

ranged from 2% to 55%, with a mean of 13%, but in the majority of cases (17 of 21) it was <10%. Two samples exhibited macroprolactin percentages that were >50% of the total prolactin.

RECOVERY OF MONOMERIC PROLACTIN AND METHOD IMPRECISION, INTERFERENCE, AND STABILITY OF PROLACTIN IN THE PEG SUPERNATANT

Recovery of IS 84/500 monomeric prolactin after treatment with PEG varied. For results obtained with the AIA, Centaur, Architect, and Elecsys, mean recoveries were lower than expected but relatively constant at 74%, 88%, 80%, and 82%, respectively. GFC of the PEG precipitate demonstrated the presence of monomeric prolactin (Fig. 1B) confirming that the low recoveries were due to precipitation rather than interference. In contrast, over-recovery of the standard was observed with the Access (122%) and to a lesser extent the Immulite (107%), indicating positive interference by PEG. This interference was decreased by a 1-in-5 dilution of the PEG supernatant with PBS before assay, to yield a 100% recovery of standard with Access and 74% with Immulite.

Method imprecision (CV) was satisfactory and ranged from 2.0% (Elecsys) to 3.1% (Centaur) in untreated sera and from 1.1% (Elecsys) to 3.2% (Architect) in PEG-treated sera at prolactin concentrations of approximately 700 mIU/L, n = 6. Prolactin in sera

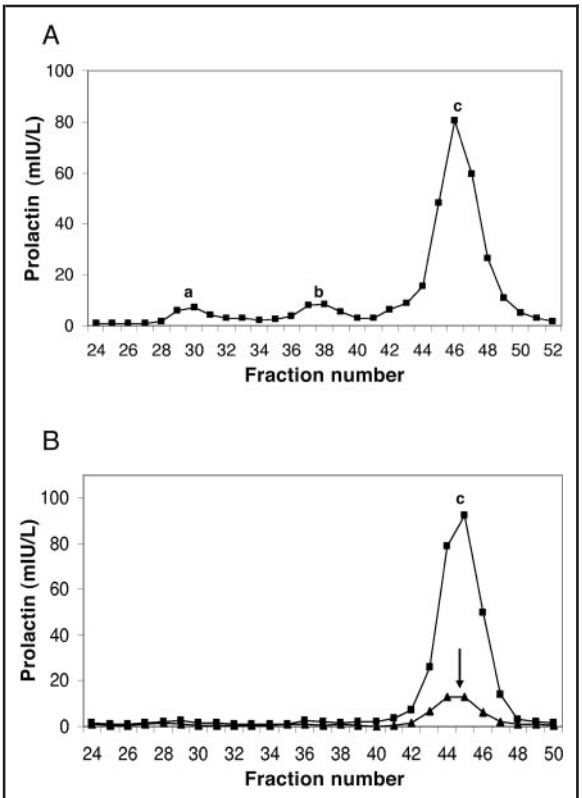

Fig. 1. Representative chromatograms illustrating the resolution and size distribution of immunoreactive prolactin species in normal sera (A) and IS 84/500 (B) when subjected to high-resolution GFC over Superdex SD-75.

After PEG precipitation of IS 84/500, the resulting pellet was resuspended and chromatographed over Superdex SD-75 (▲), confirming the presence of monomeric prolactin (arrow). Chromatography of untreated IS 84/500 (■) is shown for comparison. a, macroprolactin; b, big prolactin; c, monomeric prolactin.

Table 3. Parametric reference intervals for post-PEG prolactin (mIU/L) in male and female sera for each immunoassay platform.

	Male range		Female range	
Analyzer	Lower	Upper	Lower	Upper
Centaur	61	196	66	278
Elecsys	63	245	75	381
Access	70	301	92	469
Architect	72	229	79	347
AIA	73	247	83	383
Immulite	78	263	85	394

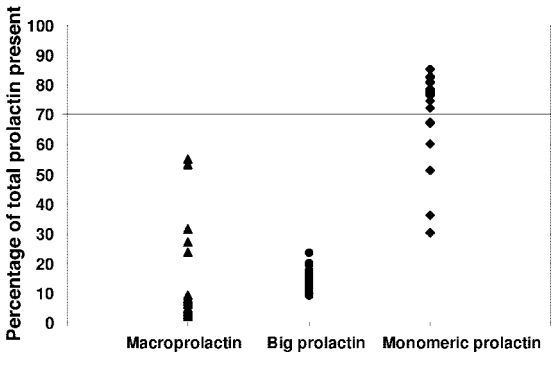

Fig. 2. Distribution of prolactin isoforms in normal sera.

Sera from healthy individuals, 16 females and 5 males, containing the highest total prolactin concentrations in the normal reference panels were subjected to GFC to ascertain the relative contributions of macroprolactin, big prolactin and monomeric prolactin.

treated with PEG exhibited no significant change ($P < 0.05$) when reanalyzed after storage for 24 h at 4 °C.

VALIDATION OF THE NEWLY ESTABLISHED POST-PEG REFERENCE INTERVALS

To validate our approach to post-PEG reference intervals, we compared the classifications of hyperprolactinemic patients obtained by using the appropriate analyzer-derived post-PEG reference interval to the classification according to GFC. Female patients with increased total prolactin concentrations and GFC monomer concentrations >444 mIU/L were categorized as true hyperprolactinemic and those with GFC monomer concentrations ≤444 mIU/L as macroprolactinemic. The comparable cutoff for male patients was 288 mIU/L.

The samples from 32 patients (28 female, 4 male) with true hyperprolactinemia had total prolactin concentrations by AIA that ranged from 873 mIU/L to 7265 mIU/L, with corresponding GFC monomer concentrations that ranged from 452 mIU/L to 4428 mIU/L. Concordance in categorization was obtained between GFC and each of the 6 immunoassay methods examined in 31 of 32 patients. A discrepancy was observed in a sample from 1 patient, which had with a total serum prolactin by AIA of 9082 mIU/L. Fractionation by GFC revealed 8052 mIU/L of macroprolactin, 182 mIU/L of big prolactin, and 848 mIU/L of a monomer, findings indicative of true hyperprolactinemia. After this sample was treated with PEG, the Architect and the AIA results led to correct categorization of this patient as having true hyperprolactinemia. The post-PEG prolactin in this sample, however, was within the newly established reference intervals of the Access, Immulite, Centaur, and Elecsys, indicating macroprolactinemia (Table 4, patient 1).

Total prolactin concentrations in samples from 22 patients (19 female, 3 male) with macroprolactinemia ranged from 544 mIU/L to 3045 mIU/L, with corresponding GFC monomer concentrations ranging from 114 mIU/L to 413 mIU/L. Using the GFC monomer concentration as a reference, we determined that in 17 of the 22 cases, results for each of the 6 immunoassay methods examined led to correct patient categorization.

EFFECT OF PEG DILUTION ON THE IMMULITE AND ACCESS ASSAYS

In an effort to minimize the positive interference PEG exerts on the Immulite and Access immunoassays, we measured prolactin in the PEG supernatant of samples from both healthy individuals and patients after a 1-in-5 dilution with PBS. For samples from both males and females, post-PEG reference intervals obtained

Table 4. Discordant classification of 6 patients.[a]

Patient	GFC monomeric prolactin, mIU/L (classification)	Immunoassay post-PEG prolactin, mIU/L (classification)					
		Access	Immulite	Architect	AIA	Centaur	Elecsys
1	848 (H)	385 (M)	298 (M)	414 (H)	596 (H)	214 (M)	275 (M)
2	413 (M)	376 (M)	284 (M)	302 (M)	494 (H)	244 (M)	294 (M)
3	405 (M)	380 (M)	270 (M)	284 (M)	450 (H)	228 (M)	305 (M)
4	296 (M)	517 (H)	302 (M)	346 (M)	378 (M)	300 (H)	444 (H)
5	295 (M)	495 (H)	340 (M)	328 (M)	380 (M)	298 (H)	425 (H)
6	262 (M)	228 (M)	174 (M)	212 (M)	390 (H)	150 (M)	184 (M)

[a] Classification of 6 patients, 5 females and 1 male, with discordant results as either hyperprolactinemic (H) or macroprolactinemic (M) based on monomeric prolactin concentrations after GFC compared to post-PEG concentrations from the 6 immunoassay platforms examined. Discordance in patient classification is illustrated by shading

with diluted normal sera were considerably lower than those in undiluted sera. The reference interval for the Immulite was 99–211 mIU/L for males and 103–291 mIU/L for females. Corresponding Access ranges were 73–233 mIU/L and 83–347 mIU/L. Applying the adjusted ranges to the evaluation panels of hyperprolactinemic and macroprolactinemic sera diluted with PBS post-PEG failed to correct the discrepancies with respect to GFC that were previously identified with patient 1 (Immulite and Access) and patients 4 and 5 (Access) (Table 4). Moreover, results from both Immulite and Access led to miscategorization of patient 2 samples as hyperprolactinemic.

Discussion

Manufacturer-published reference intervals for total prolactin in male sera are very similar to our newly derived ranges for only the Access and the Elecsys (Table 2). The upper limits of the reference interval given by the manufacturers for the AIA, Architect, Centaur, and Immulite are 21%, 23%, 43%, and 28% higher, respectively, than those established in this study (Table 2). Reference intervals for females were similar for the Elecsys but considerably higher for Centaur (78%), AIA (42%), Architect (41%), Access (39%), and Immulite (34%) (Table 2). Although some variability may be explained by ethnicity and age differences in the reference populations, the magnitude of the variability noted in this study clearly raises questions about the applicability of universal reference intervals. As far as we are aware this is the first study to use the same reference populations to determine reference intervals for several widely used prolactin immunoassays. Although such an approach might be expected to harmonize ranges provided by differing manufacturers, we found significant variations between methods. For the 5 methods (Access, Centaur, Immulite, Elecsys, and Architect) calibrated against IS 84/500, the spread of the upper limit of the reference interval in females (144 mIU/L) at a mean concentration of 418 mIU/L was considerable at 35%. Moreover, comparison of the reactivity of IS 84/500 vs sera revealed differing patterns of reactivity. Such findings suggest that the variability in reference intervals may reflect subtle differences in immunoassay antibody specificity for the isoforms of prolactin present in sera and emphasize the difficulties in establishing common reference intervals for immunoassays, particularly when mixtures of isoforms are present.

With the Architect, Centaur, Elecsys, and AIA assays the upper reference limits for post-PEG prolactin in healthy males and females were 20%–32% lower than for total serum prolactin, reflecting the precipitation of macroprolactin, big prolactin, and a portion of the monomeric prolactin present in sera. Smaller differences were seen with the Immulite (6% lower in females, 1% lower in males), and for both male and female samples post-PEG results with the Access assay were 9%–15% higher than for total serum prolactin, reflecting the positive interference of PEG in these assays. Although further dilution of the PEG supernatant decreased PEG interference with the Immulite and Access assays, it did not eradicate it entirely, and with the Access assay, results for post-PEG prolactin after dilution were frequently higher than for total prolactin. We do not recommend dilution in routine use because the dilution step complicates the PEG precipitation technique and invariably leads to poorer precision because of the lower prolactin concentrations. Moreover, we found that sample dilution was of no added benefit in decreasing patient misclassification. To avoid confusion, users of the Access assay may need to explain to clinicians the reason why the reference interval for monomeric prolactin is higher than for total prolactin.

Upper reference interval endpoints for prolactin in male (279 mIU/L) and female (424 mIU/L) sera treated with PEG have been derived recently with a less rigorous approach for the Elecsys assay *(35)* and compare reasonably well with those determined here. Post-PEG ranges have also been established for the Access, and although these ranges are similar to the ranges we derived, they are not directly comparable because Triton X-100 was added to the PEG solution in an attempt to decrease the positive immunoassay interference caused by PEG *(32)*.

We observed prolactin concentrations in 4 female sera that deviated by more than 3 SDs from the group mean, and we excluded these from the normal panel. Samples from 2 of these patients had total prolactin concentrations of 1598 mIU/L and 794 mIU/L, and these patients were referred for clinical follow-up. The remaining 2 samples had total prolactin concentrations that normalized from 1211 mIU/L and 644 mIU/L to 138 mIU/L and 298 mIU/L, respectively, after PEG treatment, results indicative of macroprolactinemia. GFC revealed monomer concentrations of 207 mIU/L and 350 mIU/L, respectively, confirming macroprolactinemia.

Comparison of the patient classifications we determined by using post-PEG prolactin to those obtained with GFC generated 324 results, with the classifications in agreement in 311 instances (96%). Discrepancies occurred with samples from 6 individuals (Table 4). Misclassification by PEG precipitation in 5 of 6 cases involved macroprolactinemic patients (patients 2–6) who were deemed to have true hyperprolactinemia according to the results of 4 of the immunoassays, (Access, AIA, Centaur and Elecsys), and these results were identified as false positives (Table 4). The 9 dis-

crepancies observed with these patients were relatively minor and involved incursions of prolactin concentrations of 20 to 143 mIU/L into the true hyperprolactinemia range. In 7 of the 9 cases, the discrepancies involved a post-PEG prolactin incursion of <67 mIU/L into the true hyperprolactinemia range. In the case of patient 1 (Table 4), the discrepancies observed between GFC monomeric prolactin and immunoassay post-PEG prolactin concentrations were considerably greater. This pattern of reactivity was confirmed by analysis of a further sample obtained from this patient. Our reference intervals are based on 2.5% and 97.5% reference limits, and we anticipate that a small number of values will fall outside these ranges. Allowance must also be made for the combined imprecision contributions of the PEG and GFC methods. In the setting of hyperprolactinemia, the discrepancies identified were relatively minor and generally unlikely to cause diagnostic confusion.

Measurement of prolactin in the UK and the US is dominated by a small number of instruments; 95% of participants of in the UK National External Quality Assessment Program and 83% of participants in the College of American Pathologists prolactin proficiency-testing program use 1 of the 6 platforms we examined *(23, 36)*. We and others have previously demonstrated that such immunoassays are susceptible to clinically significant interference from macroprolactin *(11)*. Reactivity of macroprolactin has in the past commonly resulted in misdiagnosis and mismanagement of patients with hyperprolactinemia *(15)*. This problem is further compounded by the relatively common association of the nonspecific symptoms of hyperprolactinemia with macroprolactinemia, which may lead the clinician to presume that a cause-and-effect relationship has been identified for a patient presenting with, for example, infertility *(15)*. From both a clinical and biochemical perspective, the overriding concern should be to correctly identify patients with true hyperprolactinemia in the first instance of laboratory testing to avoid subsequent unnecessary investigation, imaging, or treatment. Investigations for macroprolactinemia are necessary in sera only when hyperprolactinemia is detected and the method of choice is pretreatment of sera with PEG *(27)*. The results of this study provide a standardized and validated PEG pretreatment protocol together with normative reference data for sera pretreated with PEG. These data are available to users and manufacturers of the most common immunoassay platforms and should serve to facilitate the more widespread introduction of macroprolactin screening to clinical laboratory practice.

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