Prolactin Results for Samples Containing Macroprolactin Are Method and Sample Dependent, Georges Gilson,1,2 Patrick Schmit,1 Jean Thix,1 Jean-Paul Hoffman,3 and René-Louis Humbel 1 (1 Laboratoire de Biochimie et d’Immunopathologie, Centre Hospitalier de Luxembourg, rue Barbé 4, 1210 Luxembourg, Luxembourg; 2 Laboratoire Clinique Sainte-Marie, rue Wurth-Paquet 7, 4350 Esch-sur-Alzette, Luxembourg; 3 Laboratoire National de Santé, Division de Biochimie, rue du Laboratoire 42, 1911 Luxembourg, Luxembourg; # author for correspondence: fax 352-457794, e-mail gilson.georges@chl.lu)

Prolactin (PRL) exists in human serum in several molecular forms that can be identified by gel-filtration chromatography (GFC). The 23-kDa monomer is the predominant form in the general population, but other circulating species include the 50-kDa form (big PRL) and the 150- to 170-kDa macroprolactin (big big PRL) (1, 2). The prevalence of macroprolactin in the general population is currently unknown, but this macromolecular form of PRL has been characterized as a complex of PRL with an IgG antibody (3) that has reduced bioactivity in vivo (4) and a variable reactivity with commercial immunoassays for PRL (4–8). Macroprolactin is cleared from the blood circulation more slowly than monomeric PRL, leading to an apparent hyperprolactinemia, depending on the immunoassay used for the measurement of PRL. Thus, identification of macroprolactin as a cause of high PRL in a patient sample is important because it can help resolve diagnostic confusion and avoid expensive pituitary investigations and inappropriate treatment (5). As a result of distribution of serum containing macroprolactin in the United Kingdom National External Quality Assessment Scheme, immunoassays for the measurement of PRL have been subdivided into three classes according to their reactivity with macroprolactin: low-, medium-, and high-reading methods (6).

We used the polyethylene glycol (PEG) precipitation method (4) to screen for the presence of macroprolactinemia in a population of 319 hyperprolactinemic samples, as previously described (4). In our population of 319 hyperprolactinemic samples with increased serum (4–8).

The difference plot for the Elecsys and the Immulite assays (Fig. 1B) shows that the Elecsys assay gave higher results than the Immulite assay and that the bias was influenced by the presence of macroprolactin. In the Elecsys assay, the sera containing only monomeric PRL gave results that were, on average, 44% higher (range, 44–79%) than in the Immulite assay, and the macroprolactinemic samples gave results that were 78% higher (range, 46–130%) on average. All samples presenting a difference >80% between the Elecsys and the Immulite results were macroprolactinemic samples, and all samples presenting a difference <45% were samples containing only monomeric PRL. The overlapping of the macroprolactinemic and the monomeric PRL populations was greater than for the Elecsys/ACS:180 comparison because...
56% (33 of 59) of the macroprolactinemic samples present a difference of the Elecsys and the Immulite results between 45% and 80%.

Fig. 1C compares the medium-reading Immulite method and the low-reading ACS:180 method. Although in some samples the results by the two methods were quite different, there was little overall bias attributable to the presence of macroprolactin. We obtained an average difference of $-5.1\%$ (range, $-51\%$ to $30\%$) for the monomeric PRL samples and an average difference of $2.4\%$ (range, $-50\%$ to $86\%$) for the macroprolactin-containing samples.

We confirmed in our study that, in general practice, macroprolactinemia is a common phenomenon in hyperprolactinemic samples. Of the three automated immunoassay systems tested, the Elecsys assay gave higher results than those obtained by the Immulite and the ACS:180 assays, and the bias was influenced considerably by the presence of macroprolactin. Our data confirmed that the reactivity with macroprolactin is dependent on the assay used for the PRL measurement, but we additionally showed that the PRL results obtained for samples containing macroprolactin are also sample dependent. Thus, even if the average difference between results of macroprolactinemic samples measured with the Elecsys and ACS:180 assays (78%) was much higher than the average difference for monomeric PRL samples (40%), we could identify a subpopulation of sera containing macroprolactin that behave like monomeric samples and present only a difference of 20–59%. Consequently, it is not possible to exclude the presence of macroprolactin by comparison of the results obtained by a high-reading method (Elecsys 2010; Roche) and a low-reading method (ACS:180; Bayer), as has been proposed by some authors (9). In such a comparison, 29% of the macroprolactinemic samples of our population would not have been recognized. The difference plot illustrating the Immulite/ACS:180 comparison (Fig. 1C) shows little overall bias attributable to the presence of macroprolactin, but the considerable range in the differences between the Immulite and the

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**Fig. 1.** Difference plots of PRL results using samples containing macroprolactin (●) or monomeric PRL (○).

(A), Elecsys/ACS:180 comparison. (B), Elecsys/Immulite comparison. (C), Immulite/ACS:180 comparison.
ACS:180 results obtained for macroprolactinemic samples (range, −50% to 86%) indicates a highly variable, sample-dependent response of the ACS:180 assay to macroprolactin. The great disparity of values observed when comparing results from macroprolactinemic samples measured by the Elecsys or the Immulite assay with the results obtained by a low-reading method such as ACS:180 may reflect variation in the structure of macroprolactin. Macroprolactin is most probably not one unique molecule but rather a heterogeneous family of PRL-IgG complexes that react differently depending on the type of immuneassay used for PRL determination.

In conclusion, our study reinforces the point that PRL assays from different manufacturers give highly variable prolactin results for samples containing macroprolactin (4–8). Our data additionally show that the reactivity of macroprolactin in a PRL immuneassay, be it a low-, medium-, or high-reading method, is not identical for all macroprolactinemic samples. This finding underscores the necessity of a systematic screening strategy for macroprolactin in all samples with increased PRL (4, 5, 10, 11). With the Elecsys PRL assay, PEG precipitation, with a cutoff value of 50%, was an efficient and easy-to-use screening tool for the presence of macroprolactin. Because of the interference of PEG in some commercially available PRL assays, the confirmation of macroprolactinemia may require time-consuming methods, such as centrifugal ultracentrifugation (9) and GFC.

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References