for glucose and 92%–104% for galactose. The CV of 5% (n = 3) for both sugars is attributable mainly to the irradiation step because of a lack of homogeneity in domestic ovens. The detection limit was 5 μmol/L (signal-to-noise ratio = 3) for both sugars without modification of the split ratio.

This study shows that a simple microwave oven can be used to accelerate sample preparation in GC analysis of monosaccharides in plasma. Flame ionization detection enables easy measurement of glucose and can achieve the detection limits necessary for galactose. The present procedure is an alternative for laboratories that lack automated glucose analyzers and for others that do galactose screening with methods such as thin-layer chromatography (4, 5).

Indirect Methods for Reference Intervals Based on Current Data

To the Editor:
Grossi et al. (1) recently reported an interesting project that used a sophisticated algorithm for the formulation of reference intervals based on ~15,000,000 records related to 197,350 individuals. We noted an important difference between their reference interval calculated for thyrotropin (TSH) based on results obtained with the Architect (Abbott) analyzer in women (0.28–4.45 mIU/L) and that recently reported by Kratsch et al. (2). Kratsch et al. selected a group of 870 blood donors with negative thyroid ultrasonography and thyroid autoantibodies, as recommended by criteria of the National Academy of Clinical Biochemistry, and found a reference interval of 0.4–3.77 mIU/L (2). The optimal serum TSH reference interval is strongly debated, and a lowering of the upper reference limit is advocated by some authors (3). Furthermore, the algorithm used by Grossi et al. (1) cannot be implemented easily in most institutions because it requires considerable hardware and software resources and statistical expertise that are not commonly available. In our opinion, indirect methods are much simpler and more practical tools for the calculation of reference values or health-related limits (HRLs), especially when the fraction of pathologic values is not too high (4–6).

We retrieved the results of thyroid panels (which included measurement of anti-thyroid peroxidase antibodies) from the records of 15,359 female and 3862 male patients stored in our laboratory information system (LIS) over a 30-month period (January 1, 2003, to June 26, 2005). We calculated the Advia Centaur (Bayer) TSH HRL, using the program GraphROCTM (Fig. 1A), and obtained an upper limit of 3.7 mIU/L. As shown in Fig. 1B, the upper limit of the HRL did not change substantially after we removed the repeat tests (2893) and the results obtained in individuals positive for thyroid antibodies (7995).

This limit confirms a previous study carried out in 2000 with the same analyzer and the same software in 40,095 and 26,001 results retrieved from the LIS of the laboratories of Vicenza and Verona hospitals, without any selection criteria; the HRLs were 0.28–3.5 and 0.22–3.6 mIU/L, respectively, and the test result distribution appeared unimodal (7).

The 3.7 mIU/L limit is also consistent with those reported in 2 multicenter studies carried out in Spain [144 reference individuals (8)] and in the United Kingdom [303 individuals (9)] with the same analyzer; the reference intervals obtained in those studies were 0.43–3.69 (8) and 0.48–3.63 mIU/L (9), respectively. Finally, the 97.5th centile of TSH concentration reported by the National Health and Nutrition Examination Survey (NHANES) III in the decades between 20 and 60 years was between 3.56 and 3.82 mIU/L (10). In conclusion, the retrieval of results from the

References

Fig. 1. Chromatogram of a plasma sample with 1 mmol/L glucose and 100 μmol/L galactose added.
LIS, followed by a simple treatment and visual inspection of the data, seem to yield results more consistent with clinical requirements than a much more demanding procedure such as the REALAB Project.

In our opinion, the somewhat bimodal distribution of TSH for men and women further reinforces our approach and our conclusion. Although our extraction criterion that a request for anti-thyroid peroxidase antibody testing be included may have led to the selection of an increased number of pathologic results, inducing a certain heterogeneity, we did not use any selection criteria in the study carried out in 2000, and we obtained an unimodal distribution for both the Vicenza and Verona results (7).

Fig. 1. HRLs for TSH calculated from records retrieved before (A) and after (B) removal of repeat tests and results obtained in individuals positive for thyroid antibodies.

References

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To the Editor:

We appreciate the interest of colleagues in our approach to the definition of reference values (1). They point out the complexity of our suggested statistical procedure compared with a simpler approach, and they highlight differences between our and their reference intervals for serum thyrotropin (TSH), with special reference to the upper reference limit (URL).

Concerning the first point, we recognized in our report that the application of our procedure needs the cooperation of an expert statistician and, of course, the availability of a large computerized database. However, our approach aimed to define the reference limits for several quantities commonly measured in serum (actually 36), based on the large non-diseased population of individuals referred to our center, without having detailed clinical information on each individual. To achieve this, we developed a method based on a specified rationale, including a multistep selection of nondiseased individuals, followed by statistical, nonparametric definition of percentile-based limits. This is not simple, of course, but it has a rational basis: simpler approaches are not applicable. By contrast, Giavarina et al. do not provide any rationale for their simple approach. Looking at their graphs, we presume that they locate the URL at the local minimum on the right side of the major peak of the distribution, where it appears as the cross-point of 2 underlying subdistributions (are these healthy and diseased individuals?). The method does not locate exactly the percentile-related URL. This criterion is hardly related to the theory of reference values—more complicated patterns might be very difficult to explore; therefore, we have concerns about the possibility of generalizing this technique. Moreover, the method is intrinsically univariate, wasting highly relevant information provided by other available quantities, as we showed with our correlation analysis.

Concerning the second point, it must be considered that, together with several variables (e.g., ethnic group and environment), sex and age distributions are very important factors in determining the population’s values (2). Actually, a simple linear regression of TSH values over age performed on our raw data shows slopes of 0.01229 (P < 0.0001) for females and 0.02324 (P < 0.0001) for males. To give just a crude interpretation of our regression data, for every year of increasing age, the TSH mean value increases by 0.01229 mIU/L in females and by 0.02324 mIU/L in males. Independently from age distribution, the different clinical settings should also be considered in comparing results. On one extreme there is a trial specifically aimed at estimating TSH reference limits (3), whereas on the opposite extreme there is our retrospective (a posteriori) analysis, based on opportunistic data. Results from settings so different can hardly be compared.

The report based on the National Health and Nutrition Examination Survey (NHANES) III study results (2) includes a long list of distribution parameters (2.5, 50, and 97.5 centiles) of TSH values by age class, sex, and ethnicity in 2 different populations: a disease-free population and a reference population (risk factors excluded). Leaving out ethnic group- and age-related differences (which are not insignificant), we found in the entire disease-free population that the 97.5 centile is 6.10 mIU/L for women and 4.81 mIU/L for men, whereas in the whole reference population the corresponding values are 4.09 and 4.12 mIU/L, respectively. These last values compare acceptably with our URL values (4.45 and 4.08 mIU/L, respectively), indirectly confirming the power of the multivariate algorithm in selecting the disease-free portion of the population. There is still controversy about the correct reference interval of serum TSH. A recent report (4) debated the need for a narrower TSH reference interval and recommended lowering the URL to 2.5 mIU/L. With this limit, the sensitivity of the test is increased, but its specificity is inevitably decreased.

References


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