Workshop report

The Third International Workshop on the Standardisation of Insulin Autoantibody Measurement

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on behalf of the Immunology and Diabetes Workshops and participating laboratories*

Previous insulin autoantibody (IAA) workshops have achieved important advances in the standardisation of IAA measurement [1, 2]. All but one of the participants now use a human rather than porcine or bovine insulin ligand, and the majority of radiobinding assays (RBA) employ a reverse HPLC A14 monoiodinated tracer of specific activity exceeding 300 ICi/ μ g. Only assays which incorporate a displacement/preabsorption step to identify specific binding are now included. Data from each laboratory is expressed in standard deviation scores, based on the displaced signals from normal sera. The

standard deviation score (SDS), a measure of the certainty with which a test serum can be deemed different from normal, is unprejudiced and has made it possible to compare individual assays, whatever their type.

Fig. 1. The coefficients of variance from each laboratory defined as standard deviation/mean $\times 100$, and based on 10 normal sera. Numbers below each bar indicate individual laboratories. RBA = radio-binding assay

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Fig. 2. The dilution curves obtained by each laboratory (ELISA above, radiobinding assay (RBA) below) from a reference insulin autoantibody (IAA) positive serum prediluted 1:1, 1:5 and 1:10. The data is presented so as to compare individual laboratories with the consensus of all laboratories using that assay type (those laboratories performing better than average on the left, others on the right). The dotted diagonal represents the line of equality and the boxed area encloses three standard deviation scores. Numbers in parentheses indicate individual laboratories. SDS = standard deviation score

Fig. 3. The signals obtained by each laboratory from three sera believed to be insulin autoantibody (IAA) positive, expressed in standard deviation scores (● = serum no. 5; ■ = no. 12; ▲ = no. 16). Numbers on the abscissa indicate individual laboratories. SDS = standard deviation score. RBA = radiobinding assay

The third IAA workshop involved 21 laboratories (10 RBA and 11 ELISA) and the measurement of 21 serum samples. Three of the samples were centrally prepared dilutions of a disclosed reference serum drawn from an islet cell antibody positive adult whose IAA had been detectable by most laboratories in the second workshop. The remaining 18 sera were undisclosed and supplied lyophilised in numbered tubes. Ten were from normal control subjects and eight from patients who were either newly Type 1 diabetic, but not yet insulin-treated, or high risk non-diabetic subjects who subsequently became diabetic.

The coefficients of variation (CV = standard deviation/mean) of individual laboratories, based on measurement of 10 normal sera, are shown in Figure 1, grouped according to assay type. There was no statistically significant difference in CV between ELISA laboratories and those using RBA ($t = 1.19$ $p > 0.05$), there being a wide range within each assay type and considerable overlap between the two.

Figure 2 shows the dilution curves obtained for the reference serum by individual laboratories, plotted against the mean (consensus) value for all laboratories using RBA or ELISA. The diagonal bisecting each panel represents the theoretical line of perfect agreement between the laboratory represented and all others using the same method. Each assay method is represented in two panels, the individual laboratories generating above "average" signals for this serum on the left, and signals at or below average on the right. The horizontal displacement of the dilution curve reflects the mean signal intensity obtained for the reference serum from the assay method used. The logarithmic scales are the same for both assay types and the boxed areas define three standard deviations. There were considerable differences in signal intensity, both between and within assays. A minority of both assay types was unable to detect the 1:10 dilution and some (both RBA and ELISA) were unable or barely able to detect the 1:1 dilution.

Most laboratories found five of the eight sera from patients with newly diagnosed Type 1 or "prediabetes" to be negative, but the remaining three sera (Nos 5, 12 and 16) were deemed positive by a sufficient number to allow the following observations (Fig. 3): 1. Serum 16 was frequently positive (≥ 3 SDS) in RBA (8/10) but was uniformly negative in ELISA (0/10); 2. But for this discrepancy, the more performant RBA's and ELISA's gave comparable signals when expressed in SDS units; 3. There was no clear relationship between clinical performance of the assays and the precision with which they measured normal sera (Fig. 1). Thus, for example, RBA 19 and 32 had an average or lower than average CV but were poorly able to detect the IAA positive sera, while others, such as RBA 1 and 38 had rather higher CV's but performed well clinically. Similar observations were true for ELISA laboratories 4 and 36 and 5 and 21, respectively. It should be noted that the scale in Figure 3 is

logarithmic to accommodate signal strengths ranging from 3 to 80 SDS.

Two factors contribute to a high standard deviation score: precision and sensitivity [3]. The data from this workshop indicate that each is present to very variable extents in different assays, independently of their type.

The difference in detection frequency between RBA's and ELISA's for certain sera such as No. 16 may be due to the particular orientation of the insulin molecule on solid phase which results in masking of particular binding epitopes, or to an iodination-induced amplification of binding affinity which has recently been demonstrated to occur occasionally in liquid phase [4].

With the system of precision units it has been possible to identify laboratories which perform well in the measurement of IAA, and others which perform less well. Insufficient numbers of diabetes-relevant sera have been analysed in the workshops performed to date to allow definitive conclusion regarding IAA to be reached. The next IAA workshop, to be presented in Israel in Spring 1990, will involve the measurement of IAA on a large number of blinded sera from patients with Type 1 diabetes at the time of diagnosis and from normal control subjects and should allow clinical performance of IAA assays to be meaningfully and statistically compared. Laboratories interested in participating should contact either Dr. J.P. Palmer, Medical Service (III), 1660 S. Columbian Way, Seattle, WA 98108, USA.

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Acknowledgements. TWJ is supported by the Wellcome Trust. We are grateful to Mrs. W. Couper for preparing the manuscript.

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