*Workshop report***Fifth International Serum Exchange Workshop for Insulin Autoantibody (IAA) Standardization**

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

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Insulin autoantibodies (IAA), first reported in 1983 [1], are measured in laboratories worldwide in investigations of the aetiology of Type 1 (insulin-dependent) diabetes mellitus. Annual serum exchange workshops have been held since 1987 under the auspices of the International Diabetes Workshops in order to improve the

standardization of IAA measurements worldwide [2–4]. In the fourth serum exchange workshop it was determined that IAA measured by radioimmunoassay (RIA) were more closely linked to the disease process of Type 1 diabetes than those measured by enzyme-linked immunosorbent assay (ELISA). It was recom-

Fig. 1. Percentage of laboratories ($n = 28$) reporting individual sera from control subjects (▨ $n = 26$), first degree relatives of Type 1 (in-

sulin-dependent) diabetic patients (■ $n = 6$), or Type 1 diabetic patients (▩ $n = 12$) ≥ 3 SD from the mean of the control sera

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Fig. 2. Representative example of standard curve obtained from five doubling dilutions of a monoclonal insulin antibody expressed as standard deviation score (SDS) and arbitrary insulin autoantibody "IAA units". — — — $y = 13.4 + 8.4 \times R = 0.99$

Fig. 3. Correlation between the slope of the standard curves and the final dilution of the serum used in individual insulin autoantibody assays ($p < 0.006$). — — — $y = 0.13 + 0.01 \times R = 0.68$

Fig. 4. Percentage coefficient of variation (C.V.) obtained among 25 laboratories for four sera (numbers 23, 25, 34, 44) when results expressed as delta % binding $\square$, standard deviation score $\blacksquare$, insulin autoantibody units $\blacksquare$, sera 25 units $\blacksquare$, sera 44 units $\square$

mended that only RIA assays or assays proven to perform equally as well should be used to measure IAA associated with Type 1 diabetes [4].

The aim of the fifth serum exchange workshop was to investigate the variation in IAA quantitation between laboratories, and to determine if this variation could be reduced by reference of a standard. This approach has improved the standardization of islet cell antibody (ICA) assays [5–7].

Sera from 26 control subjects, 12 newly-diagnosed Type 1 diabetic patients, and six first degree relatives of Type 1 diabetic patients which had been submitted by participating laboratories were distributed without identification as to their origin to 33 laboratories. Additionally, five doubling dilutions of a monoclonal antibody to human insulin were also sent in the same manner to each laboratory. Each laboratory was instructed to use an RIA assay with a human insulin ligand and a competitive displacement step. Data meeting these criteria were submitted by 28 laboratories.

Results were analysed in several ways. (1) The raw data was expressed as delta % binding for each sample and was calculated as $(\text{cpm without insulin competition} - \text{cpm with insulin competition}) \times 100 / \text{total counts}$. Each laboratory's delta % binding results for the 26 control sera were then used to calculate their mean and standard deviation. Using delta % binding data, four sera (numbers 23, 25, 34, 44) were found to be positive (greater than 3 SD above the mean of a each individual laboratory's control sera) by at least 25 laboratories (89–96%). Serum number 34 was from a first degree relative of a Type 1 diabetic patient, and sera numbers 23, 25 and 44 were from Type 1 diabetic patients (Fig. 1). These four sera were used for the concordance analyses. The three laboratories that were unable to find all four sera positive (> 3 SD above their control sera) were excluded from analysis. (2) Based upon each laboratory's standard deviation of control sera, a standard deviation score (SDS) was then calculated for each serum. $\text{SDS} = (\text{mean delta \% binding of test serum} - \text{mean delta \% binding of control sera}) / \text{standard deviation of control sera}$. The SDS provides a measure of the certainty with which individual sera can be deemed different from normal. (3) A standard curve was constructed for each laboratory using the SDS of the five doubling dilutions of the insulin monoclonal antibody. Arbitrary "IAA units" were assigned to these curves (Fig. 2). The SDS of the four positive sera were then plotted on these standard curves and the "IAA units" for each serum was determined. (4) The serum which had the highest signal in most of the laboratories (serum number 25) was used as a standard for the other three positive sera (number 23, 34, 44). The value of serum number 25 was set at 100 and the value of the other three sera relative to serum number 25, based on SDS, was determined. (5) Similarly, serum number 44 was used as a standard for the other three positive sera (number 23, 25, 34). The value of serum number 44 was set at 100 and the relative values of the other sera, based on SDS, were determined.

Most laboratories had an excellent correlation between dilution of the monoclonal antibody and corresponding SDS (mean r value for all laboratories was 0.96 ± 0.05), but the slopes of the relationships varied greatly between laboratories (range 0.76–30.5). The slope of this relationship was significantly correlated with the final dilution of serum used by each laboratory for their IAA assay (Fig. 3).

The variation between laboratories for each of the four sera (numbers 23, 25, 34, 44) was calculated using raw data expressed as delta % binding, SDS, "IAA units", and with serum number 25 or serum number 44 as reference (Fig. 4). Using delta % binding, the coefficient of variation (CV) was 57% for serum number 23, 62% for serum number 25, 61% for serum number 34, and 59% for serum number 44. Use of the SDS demonstrated no significant change in CV for sera numbers 23, 34, 33 (60, 62, 58% respectively) and a slight increase in CV for serum number 25 (79%). All other methods of analysis (i.e. "IAA units", serum 25 and serum 44 comparison units) resulted in higher variation between laboratories than use of delta % binding or SDS for all four sera.

In conclusion, the data from the fifth IAA serum exchange workshop demonstrate quite reasonable inter-assay variation for quantitation of IAA (mean $\pm$ SD CV of $59.8 \pm 2.2\%$ for the four positive

sera). The use of standards, either a monoclonal antibody or sera with high signals, does not improve the variation between laboratories, probably because of the marked differences in the slope of the relationship between dilution of a standard and signal strength. The use of raw data expressed as delta % binding or SDS produces similar CV for the positive sera.

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