Diabetes Antibody Standardization Program: First Assay Proficiency Evaluation

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The aims of the first proficiency evaluation of the Diabetes Antibody Standardization Program (DASP) were to assess general implementation of assay methods and to evaluate the new World Health Organization (WHO) reference reagent for autoantibodies to GAD and IA-2. Forty-six laboratories in 13 countries received coded sera from 50 patients with newly diagnosed type 1 diabetes and 50 blood donor control subjects, together with the WHO reference reagent and diluent serum. Results were analyzed using receiver operator characteristic (ROC) curves. Sensitivity was adjusted to 90% specificity in workshop controls. The median adjusted sensitivity for GADA (45 laboratories) was 84% (range 62–96%), for IA-2A (43 laboratories) was 58% (50–74%), and for insulin autoantibody (IAA; 23 laboratories) was 36% (13–66%). ROC curve analysis showed all GADA and IA-2A assays, and 18/23 IAA assays found significant differences between patients and control subjects. There was good concordance between laboratories in ranking of samples by GADA and IA-2A levels or if results were expressed in relation to the WHO reference reagent. Assays that achieved the highest sensitivity for IAA were also concordant in ranking samples, but overall concordance for IAA was poor. Differences in assay protocols between laboratories must be addressed so that all centers and kit manufacturers can perform to the same high standard. *Diabetes* 52:1128–1136, 2003

Autoantibodies against islet cell antigens are important markers of diabetes-associated autoimmunity. Their measurement is widespread and used to identify people at increased risk for developing type 1 diabetes (1) or requiring insulin treatment (2), to aid in the classification of diabetes (3), to study the natural history of diabetes (4,5), and to measure efficacy in intervention trials.

Concordance of the results obtained from different laboratories is essential for comparison of studies from different centers and is increasingly important in recruitment into multicenter studies. This issue has been addressed in a series of international workshops and antibody standardization and proficiency programs since the first islet cell antibody (ICA) standardization workshop in 1985 (6,7). Subsequent workshops examined other islet autoantibodies as they were described, and the combined islet autoantibody workshop in 1995 included antibodies to GAD, protein tyrosine phosphatase IA-2, and insulin, as well as ICA. (8). The major achievements of these activities have been the introduction of a reference standard preparation and units for ICA measurement, validation of diabetes-associated antibody markers and methods for their measurement, and an improved concordance between laboratory measurements (6,8). The ICA reference standard has recently been given World Health Organization (WHO) status and undergone preliminary evaluation in a small number of laboratories as a reference reagent for autoantibodies to GAD and IA-2 (9).

The Diabetes Antibody Standardization Program (DASP), an extension of Immunology of Diabetes Society autoantibody workshop activities, was established in collaboration with the U.S. Centers for Disease Control and Prevention to evaluate and improve general implementation of assay methods and to undertake extended evaluation of the new WHO international reference reagent for antibodies to GAD and IA-2. The aim of the first proficiency evaluation was to assess and improve comparability of islet autoantibody measurements between laboratories in preparation for multicenter studies. To achieve this, coded sera were used to evaluate individual laboratory performances for measurement of autoantibodies to GAD, IA-2, and insulin (IAA) and to determine how the GAD and IA-2 antibody reference standard preparation should be used to enable reporting of common international units.

RESEARCH DESIGN AND METHODS

Sera. Sera were obtained from 50 patients with newly diagnosed type 1 diabetes (median age, 17 years; range, 9–31). Of these, 19 were female and 31 were male, 49 were white and 1 was Hispanic. All samples were collected within 14 days of starting insulin treatment (median, 4.5 days). Control sera were obtained from 50 U.S. blood donors (median age 20 years; range 18–28). Sera were prepared in 100-μl aliquots and frozen. Coded sera were distributed to 46 laboratories in 13 countries (see APPENDIX for listing of participating laboratories). Each participating laboratory received 1–3 uniquely coded sets of sera depending on the volume requirements of their assays. Sera from different sets could not be combined, so only those assays that used <100 μl could be included in the evaluation.

Laboratories were asked to test the sera with whatever relevant assays were currently in use for detection of prediabetes. Of the participating laboratories, 45 reported results for GAD antibodies, 43 for IA-2/ICA512 antibodies, and 23 for IAA. One laboratory reported results for antibodies to

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AS_{90}, adjusted sensitivity$_{90}$; AUC, area under the curve; DASP, Diabetes Antibody Standardization Program; ELISA, enzyme-linked immunosorbent assay; IAA, insulin autoantibody; ICA, islet cell antibody; ROC, receiver operator characteristic; WHO, World Health Organization.

a GAD_{65}/$IA\text{-}2_{ic}$ fusion protein and two for ICA testing by indirect immunofluorescence.

In addition, each laboratory received a lyophilized aliquot of the new WHO international reference reagent for GAD and IA-2 antibodies (97/500) (9) together with negative diluent serum. Laboratories were asked to test the standard (250 units/ml) undiluted and 1:2, 1:4, 1:8, 1:16, and 1:64 dilutions as well as the negative diluent in every GAD and IA-2 antibody assay run.

Data analysis

Sensitivity and specificity. Laboratory-defined sensitivity for each assay was calculated as the percentage of sera from patients with newly diagnosed diabetes reported as positive using the laboratory's own cutoff. Laboratory-defined specificity was calculated as the percentage of healthy control sera reported as negative using the same threshold.

Receiver operator characteristic curves. Receiver operator characteristic (ROC) curve analysis was used to evaluate the performance of each assay in discriminating disease from nondisease. The area under the curve (AUC) with 95% CI was calculated assuming a nonparametric distribution. An AUC of 1.00 would indicate that the test achieved 100% accuracy in identifying disease, and an AUC of 0.00 would indicate that all disease and nondisease sera were misclassified on the basis of the test result. Each ROC curve was tested against a null hypothesis that the AUC was 0.5, which would indicate that the test achieved random assignment of disease/nondisease status.

To facilitate additional comparison between laboratories using very different thresholds for positivity, the coordinates of the ROC curve were used to identify the level of sensitivity achieved with 1-specificity of 0.1, which was defined as the adjusted $sensitivity_{90}$ (AS_{90}). This threshold was selected to minimize the effect of outlier antibody levels in the limited number of control sera tested. It is not suggested that this is a clinically appropriate threshold. The validity of these measures was assessed by relating them to other parameters, including laboratory-defined sensitivity and specificity and concordance of antibody levels.

Concordance of autoantibody levels in different assays. For comparing relative levels of autoantibody in different assays, the diabetic sera were ranked from highest to lowest for each assay. For each serum, the rank in each assay was then plotted on the y-axis, with the sera arranged in order of ascending median rank along the x-axis. Concordance was assessed by linear regression of individual assay rank versus the median rank for all sera from patients with type 1 diabetes. Differences in the concordance of ranking in subgroups of laboratories were analyzed by comparing the variance of the regression in an F test.

Signal:noise ratio. The signal:noise ratio for each GAD and IA-2 antibody assay was calculated as the median cpm in patient samples reported positive in >50% of laboratories divided by the median cpm in control samples reported negative in >75% of laboratories. This parameter could not be calculated for IAA assays, as many laboratories had reported only Δ cpm with and without cold insulin.

Estimation of limit of detection. The lower limit of detection in relation to the WHO standard was determined for each GAD and IA-2 antibody assay. The threshold was defined from the variability of measurement in control sera and calculated as (*median cpm or OD) + 2 * (SD of the difference between duplicates*). The limit of detection was defined as the dilution of the WHO standard with median cpm or OD immediately above the threshold. Three control sera that were reported as GAD antibody positive in >25% of laboratories were excluded from the calculation of limit of detection for these markers.

Evaluation of the use of the WHO standard in antibody quantification. The WHO international reference reagent for GAD and IA-2 antibodies (97/500) has been defined as containing 100 units per vial (250 units/ml) (9). WHO units for GAD and IA-2 antibodies were calculated as:

1) an index related to the reference material using the formula

$$Units = 250^{*}\frac{[cpm\ or\ OD\ (unknown) - cpm\ or\ OD\ (negative\ diluent\ serum)]}{[cpm\ or\ OD\ (WHO\ standard) - cpm\ or\ OD\ (negative\ diluent\ serum)]}$$

2) units derived from a logarithmic standard curve constructed from dilutions of the WHO reference reagent. Units assigned to individual dilutions were 125 units/ml for 1:2, 62 units/ml for 1:4, 31 units/ml for 1:8, 16 units/ml for 1:16, and 4 units/ml for 1:64. For comparing the two methods of unit derivation, the median and interquartile range of reported units for each of the 50 patient sera were compared using the Wilcoxon matched pairs test.

RESULTS

GAD antibodies. Results were reported from 46 assays (Table 1). Laboratory-defined sensitivity using each laboratory's own cutoff ranged from 58 to 88%, the majority of assays reporting very similar sensitivity (median, 77%; interquartile range, 74–80%). Laboratory-defined specificity ranged from 80 to 100% and again was similar in the majority of assays (median, 94%; interquartile range, 92–98%). Samples from 30 patients and no control subjects were reported positive in ≥95% of assays; an additional 8 patients and 2 control subjects were reported positive in >50% of assays, and another 2 patients and 1 control subject were positive in >25% of assays (Fig. 1*A*). Agreement in >95% of assays was observed for 77 samples (34 patient samples and 43 control samples). The ranking of the GAD antibody titer in the patient samples was highly concordant between laboratories ($r = 0.96$, $P < 0.0001$; Fig. 1*B*).

For comparing assay performances directly, results from each assay were represented as a ROC curve, and the AUC and the sensitivity achieved with specificity of 90% (AS_{90}) were calculated (Table 1). All laboratories reported significant differences between patients and control subjects (all $P < 0.0001$), and results were significantly higher in patients than in control subjects (all $P < 0.0001$; Mann-Whitney U test). The majority of assays had similar performance (median, AUC 0.93; interquartile range, 0.90–0.94; median, AS_{90} 84%; interquartile range, 79–87). AUC and AS_{90} correlated positively with the laboratory-defined sensitivity ($r = 0.48$, $P < 0.001$; $r = 0.45$, $P < 0.002$), assay specificity ($r = 0.36$, $P < 0.02$; $r = 0.42$, $P < 0.005$), and assay signal:noise ratio ($r = 0.47$, $P < 0.001$; $r = 0.52$, $P < 0.001$). Nine assays (laboratories 109, 113, 114, 116, 120, 121, 147a, 150, and 153) had both AUC and AS_{90} values in the upper 25th centile. One of these (laboratory 150) used a commercially produced GAD65 antigen that was labeled in-house with ^{125}I. The remaining eight assays with high AUC and AS_{90} used in-house assays with ^{35}S labeled in vitro transcribed/translated recombinant GAD65 or GAD65/67 chimera. Eleven assays (laboratories 110, 112, 117, 128, 131, 135, 141, 142, 147b, 149, and 151) had both AUC and AS_{90} values in the lower 25th centile; all but one of these (laboratory 110) also had laboratory-defined sensitivity or specificity in the lower 25th centile. These 11 assays included 8 using in-house methods with ^{35}S-labeled in vitro–transcribed/translated recombinant GAD65, one assay (laboratory 147b) using ^{125}I-labeled $GAD65_{46-585}$, one (laboratory 112) using Europium-labeled GAD65, and one commercial enzyme-linked immunosorbent assay (ELISA) (laboratory 151). Concordance as assessed by ranking of patient sera was lower between assays with AUC/AS_{90} performances in the lower 25th centile ($r = 0.94$, variance = 22.9) than between assays in 25th–75th centiles ($r = 0.96$, variance = 15.4; $P < 0.0001$) and between assays in the upper 25th centile ($r = 0.97$, variance = 13; $P < 0.0001$ vs. lower 25th centile, and $P < 0.02$ vs. 25th–75th centiles; F test).

IA-2 antibodies. Results were reported from 44 assays (Table 2). Laboratory-defined sensitivity ranged from 40 to 88%, the majority of assays reporting very similar sensitivity (median, 57%; interquartile range, 54–58%). Laboratory-defined specificity ranged from 28 to 100% and again was similar in the majority of assays (median, 99%; interquartile range, 96–100%). Samples from 23 patients and no control subjects were reported positive in ≥95% of assays; an additional 5 patients were reported positive in >50% of assays, and another 1 patient was positive in >25% of

TABLE 1
GAD autoantibody assays

Lab No.	Lab-defined sensitivity (%)	Lab-defined specificity (%)	Area under ROC curve (95% CI)	*P*	Sensitivity at specificity 90% (AS_{90} [%])	Signal: noise ratio	Threshold dilution	Assay type	Antigen	Label
147a	78	98	0.97 (0.93–1.00)	<0.0001	92	14	16	RBA	Human GAD_{67}(1–101)/ GAD_{65}(96–585)	^{35}S
150	88	96	0.97 (0.93–1.00)	<0.0001	96	32	64	RBA	Human GAD_{65}, Full	^{125}I
113	72	98	0.96 (0.93–1.00)	<0.0001	92	32	16	RBA	Human GAD_{65}, Full	^{35}S
114	76	96	0.96 (0.93–1.00)	<0.0001	92	37	64	RBA	Human GAD_{65}, Full	^{35}S
123	78	96	0.96 (0.93–0.99)	<0.0001	86	9	16	RBA	Human GAD_{65}, Full	^{35}S
116	84	92	0.96 (0.92–0.99)	<0.0001	86	23	64	RBA	Human GAD_{65}, Full	^{35}S
109	74	96	0.95 (0.91–1.00)	<0.0001	88	13	16	RBA	Human GAD_{65}, Full	^{35}S
120	78	96	0.95 (0.90–1.00)	<0.0001	93	22	16	RBA	Human GAD_{65}, Full	^{35}S
121	80	94	0.95 (0.90–1.00)	<0.0001	92	23	64	RBA	Human GAD_{65}, Full	^{35}S
137	78	100	0.94 (0.90–0.99)	<0.0001	86	36	16	RBA	Human GAD_{65}, Full	^{35}S
145	80	90	0.94 (0.90–0.99)	<0.0001	84	12	16	RBA	Human GAD, 46–586	^{125}I
153	82	92	0.94 (0.90–0.99)	<0.0001	88	37	64	RBA	Human GAD_{65}, Full	^{35}S
105	82	90	0.94 (0.89–0.99)	<0.0001	86	11	16	RBA	Human GAD, 46–586	^{125}I
132	84	90	0.94 (0.89–0.99)	<0.0001	84	15	16	RBA	Human GAD, 46–586	^{125}I
155	74	96	0.94 (0.89–0.99)	<0.0001	84	9	8	RBA	Human GAD_{65}, Full	^{35}S
140	70	98	0.94 (0.89–0.98)	<0.0001	80	4	4	RBA	Human GAD_{65}, Full	^{35}S
152	80	94	0.94 (0.89–0.98)	<0.0001	85	7	2	RBA	Human GAD_{65}, Full	^{35}S
126	72	94	0.94 (0.88–0.99)	<0.0001	87	11	8	RBA	Human GAD_{65}, Full	^{35}S
111	84	98	0.93 (0.88–0.99)	<0.0001	86	6	16	RBA	Human GAD_{65}, Full	^{35}S
133	84	96	0.93 (0.88–0.99)	<0.0001	90	8	16	RBA	Human GAD_{65}, Full	^{3}H
115	74	98	0.93 (0.88–0.98)	<0.0001	86	4	8	RBA	Human GAD_{65}, Full	^{35}S
124	78	90	0.93 (0.88–0.98)	<0.0001	80	8	64	RBA	Human GAD, 46–586	^{125}I
156	76	94	0.93 (0.87–0.99)	<0.0001	86	42	8	RBA	Human GAD_{65}, Full	^{35}S
138	82	96	0.93 (0.87–0.98)	<0.0001	86	14	64	RBA	Human GAD_{65}, Full	^{35}S
144	72	98	0.93 (0.87–0.98)	<0.0001	80	5	16	RBA	Human GAD_{65}, Full	^{35}S
154	64	100	0.93 (0.87–0.98)	<0.0001	85	8	8	RBA	Human GAD_{65}, Full	^{35}S
118	76	94	0.92 (0.87–0.98)	<0.0001	80	6	8	RBA	Human GAD_{65}, Full	^{35}S
148	82	92	0.92 (0.87–0.98)	<0.0001	84	11	16	RBA	Human GAD, 46–586	^{125}I
119	70	100	0.92 (0.86–0.98)	<0.0001	86	16	16	RBA	Human GAD_{65}, Full	^{35}S
129	76	98	0.92 (0.86–0.98)	<0.0001	78	11	16	RBA	Human GAD_{65}, Full	^{35}S
139	80	96	0.92 (0.86–0.98)	<0.0001	88	17	16	RBA	Human GAD_{65}, Full	^{35}S
146	82	88	0.92 (0.86–0.98)	<0.0001	81	6	8	RBA	Human GAD_{65}, Full	^{35}S
134	78	94	0.91 (0.85–0.97)	<0.0001	84	11	8	RBA	Human GAD_{65}, Full	^{35}S
136	82	92	0.91 (0.85–0.97)	<0.0001	83	6	16	RBA	Human GAD_{65}, Full	^{35}S
127	74	98	0.90 (0.84–0.97)	<0.0001	83	4	16	RBA	Human GAD_{65}, Full	^{125}I
142	80	84	0.90 (0.83–0.96)	<0.0001	76	23	16	RBA	Human GAD_{65}, Full	^{35}S
128	72	98	0.89 (0.83–0.96)	<0.0001	78	3	8	RBA	Human GAD_{65}, Full	^{35}S
149	76	84	0.89 (0.83–0.96)	<0.0001	74	11	8	RBA	Human GAD_{65}, Full	^{35}S
141	76	90	0.89 (0.82–0.95)	<0.0001	78	6	16	RBA	Human GAD_{65}, Full	^{35}S
110	74	94	0.86 (0.79–0.94)	<0.0001	74	6	8	RBA	Human GAD_{65}, Full	^{35}S
147b	76	84	0.86 (0.79–0.94)	<0.0001	62	9	16	RBA	Human GAD, 46–586	^{125}I
135	58	100	0.86 (0.78–0.94)	<0.0001	74	3	8	RBA	Human GAD_{65}, Full	^{35}S
131	66	94	0.85 (0.78–0.93)	<0.0001	68	7	4	RBA	Human GAD_{65}, Full	^{35}S
112	60	96	0.85 (0.77–0.93)	<0.0001	73	8	4	IFMA	Human GAD_{65}, Full	Europium
117	80	80	0.85 (0.77–0.93)	<0.0001	74	9	16	RBA	Human GAD_{65}, Full	^{35}S
151	72	92	0.82 (0.73–0.91)	<0.0001	76	6	16	ELISA	Human GAD_{65}, Full	HRP

assays (Fig. 2*A*). Agreement in >95% of assays was observed for 81 samples (41 patient samples and 40 control samples). The ranking of the IA-2 antibody titer in the patient samples was highly concordant between laboratories ($r = 0.89$; $P < 0.0001$; Fig. 2*B*). Samples with median rank <22 were those reported negative in the majority of assays, and the expected large scatter was observed. Two patient samples (median ranks 26 and 29) had clearly dichotomous results. These were reported as negative with ranks of 3–19 and 6–15 in all assays using the human IA-$2_{256–556/630–979}$ antigen and positive with ranks of 22–33 and 25–43 in assays using IA-2ic or full-length IA-2 antigen.

Using ROC curves, all laboratories reported significant differences between patients and control subjects (all $P <$ 0.0001), and results were significantly higher in patients than in control subjects (all $P < 0.0001$, Mann-Whitney *U* test). Again, the majority of assays had similar performance (median AUC, 0.77; interquartile range, 0.73–0.81; median AS_{90}, 59%; interquartile range, 56–65). Seven assays had both AUC and AS_{90} values in the upper 25th centile (laboratories 109, 113, 115, 132, 150, and 156). Five of these assays with high AUC and AS_{90} values were in-house radiobinding assays with ^{35}S-labeled in vitro transcribed/translated recombinant IA-2ic ($n = 3$), full-

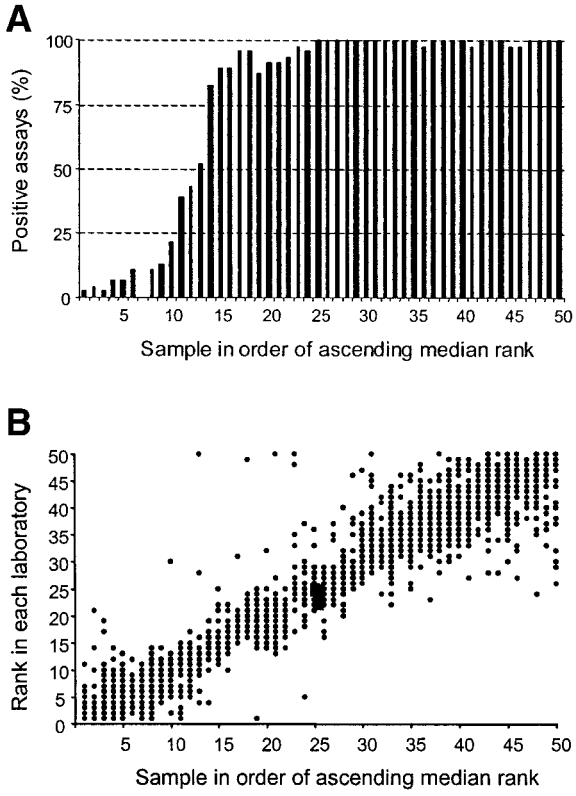

FIG. 1. GAD autoantibodies in samples from 50 patients with newly diagnosed type 1 diabetes in the DASP-1 proficiency evaluation. *A*: The proportion of assays reporting each sample as GAD autoantibody positive on the basis of the laboratory-defined cutoff. *B:* The rank of individual samples in each assay versus the median rank obtained for all 46 participating assays. Samples are arranged in the order of their median rank in all assays.

length IA-2, or IA-$2_{404–979}$. The remaining two assays used radiobinding assays with ^{125}I-labeled IA-2ic. Five assays (laboratories 110, 117, 119, 141, and 151) had both AUC and AS_{90} values below the 25th centile, and all had laboratory-defined sensitivity or specificity in the lower 25th centile. These included four radiobinding assays with in vitro transcribed/translated IA-$2_{256–556/630–979}$ and an ELISA with IA-2ic (laboratory 151). Low performance in IA-2 antibody assays was associated with low performance in GAD antibody assays: of the five IA-2 assays with both AUC and AS_{90} in the lower quartile, four were in laboratories that also had GAD antibody assays with performances below the 25th centile ($P < 0.05$). As with GAD antibodies, concordance as assessed by ranking of patient sera was less between IA-2 antibody assays with AUC and AS_{90} performance in the lower 25th centile ($r = 0.85$, variance = 59.8) than between assays in the 25th–75th centiles ($r = 0.9$, variance = 38.4; $P < 0.0001$) or between assays in the upper 25th centile ($r = 0.89$, variance = 43.5; $P < 0.01$).

IAA. Results were reported from 23 assays (Table 3). Laboratory-defined sensitivity ranged from 4 to 42% (median, 14%; interquartile range, 7–25%). Laboratory-defined specificity ranged from 89 to 100% (median, 100%; interquartile range, 98–100%). Only four assays reported IAA in >30% of patient samples (all four had laboratory-defined specificity of 98 or 100%). Samples from two patients and no control subjects were reported positive in >95% of assays (Fig. 3*A*). An additional four patients and no control subjects were reported positive in >50% of assays, and a further three patients were reported positive in >25% of assays. For 74 samples (26 patient samples and 48 control samples), >95% of assays were in agreement. Although the concordance of ranking of the IAA titer in the patient samples between laboratories was significant ($r = 0.56$; $P < 0.0001$), it was clearly inferior to that observed for GAD and IA-2 antibodies ($P < 0.0001$; Fig. 3*B*).

Using ROC curves, 5 of 23 laboratories did not find significant differences between patients and control subjects ($P > 0.05$). Performance characteristics varied substantially (median AUC, 0.67; interquartile range, 0.60–0.74; median AS_{90}, 36%; interquartile range, 26–47). AUC and AS_{90} were positively correlated with the assay-reported sensitivity ($r = 0.5$, $P < 0.02$; $r = 0.6$; $P < 0.005$). Six assays (laboratories 116, 120, 121, 126, 133, and 153) had both AUC and AS_{90} values in the upper 25th centile. All six assays used in-house microradiobinding assays with ^{125}I-labeled insulin as previously described (10–12). Laboratory-defined sensitivity in these six assays ranged from 14 to 36% (median, 31%). Five laboratories (laboratories 105, 117, 132, 135, and 136) had both AUC and AS_{90} in the lower 25th centile. These included two of the three participating laboratories using a commercially available radiobinding assay with ^{125}I insulin. Concordance in ranking of patient samples was markedly higher between the assays with AUC and AS_{90} in the upper 25th centile ($r = 0.8$, variance = 70.3) than between the remaining assays ($r = 0.56$, variance = 130; $P < 0.0001$; Fig. 3*B* and *C*).

Other assays. The radioimmunoassay for antibodies to GAD_{65}/IA-2_{ic} fusion protein achieved 88% sensitivity with 100% specificity. The AUC was 0.960 (95% CI, 0.92–1.00) and AS_{90} 0.90. Of the two ICA assays participating in the workshop, one (laboratory 137) achieved 90% sensitivity with 98% specificity, and the other (laboratory 120) achieved 68% sensitivity with 100% specificity.

WHO reference reagent. The limits of detection of GAD and IA-2 antibodies, expressed as dilutions of the WHO standard, are shown in Tables 1 and 2. The undiluted WHO standard (250 units/ml) was above the limit of detection of all GAD and IA-2 antibody assays. The median limit of detection of the participating GAD antibody assays was the 1:16 dilution (16 units/ml). In seven assays, the limit of detection was below the 1:64 dilution (4 units/ml). The median limit of detection of IA-2 antibody assays was below the 1:64 dilution (4 units).

Use of common units derived from the WHO standard. The range of GAD and IA-2 antibody levels expressed as WHO units/ml for the 50 patient samples is shown in Fig. 4. Units were slightly but significantly higher when calculated as an index than when derived from a standard curve for GAD antibodies (median 181 vs. 179 units/ml; $P = 0.0004$, Wilcoxon paired test) and IA-2 antibodies (median 78 vs. 67 units/ml; $P = 0.0002$, Wilcoxon paired test). For GAD antibodies, the interquartile ranges of reported units did not differ significantly between index- and standard curve–derived units ($P = 0.065$, Wilcoxon paired test). For IA-2 antibodies, the interquartile ranges of index-derived units were significantly lower than those of standard curve–derived units ($P = 0.005$, Wilcoxon paired test). Antibody levels measured in assays

TABLE 2
IA-2 autoantibody assays

Lab No.	Lab-defined sensitivity (%)	Lab-defined specificity (%)	Area under ROC curve (95% CI)	*P*	Sensitivity at specificity 90% (AS_{90} [%])	Signal: noise ratio	Threshold dilution	Assay type	Antigen	Label
115	62	98	0.90 (0.84–0.96)	<0.0001	74	37	64	RBA	hu IA-2 ic	^{35}S
150	58	100	0.86 (0.79–0.94)	<0.0001	72	13	64	RBA	hu IA-2 ic	^{125}I
128	56	98	0.84 (0.76–0.94)	<0.0001	62	15	16	RBA	hu IA-2 ic	^{35}S
133a	52	100	0.84 (0.75–0.92)	<0.0001	64	90	64	RBA	hu IA-2 bdc	^{35}S
144	54	100	0.83 (0.75–0.91)	<0.0001	58	64	64	RBA	hu IA-2 ic	^{35}S
132	58	100	0.83 (0.74–0.91)	<0.0001	66	22	64	RBA	hu IA-2 ic	^{125}I
113	60	92	0.83 (0.74–0.91)	<0.0001	68	141	16	RBA	hu IA-2 ic	^{35}S
136	58	100	0.82 (0.74–0.91)	<0.0001	68	9	8	RBA	hu IA-2 ic	^{35}S
156	57	100	0.82 (0.73–0.91)	<0.0001	67		64	RBA	hu IA-2 ic	^{35}S
135	58	94	0.82 (0.73–0.90)	<0.0001	62	24	16	RBA	hu IA-2 bdc/ic	^{35}S
109	52	98	0.81 (0.72–0.90)	<0.0001	66	17	64	RBA	Full length IA-2	^{35}S
145	58	100	0.81 (0.71–0.90)	<0.0001	72	20	2	RBA	hu IA-2 ic	^{125}I
124	58	100	0.81 (0.71–0.90)	<0.0001	68	12	16	RBA	hu IA-2 ic	^{125}I
147	72	60	0.80 (0.71–0.88)	<0.0001	56	4	8	RBA	hu ICA512, 1–979	^{35}S
105	58	98	0.80 (0.70–0.89)	<0.0001	68	11	4	RBA	hu IA-2 ic	^{125}I
138	60	98	0.79 (0.69–0.89)	<0.0001	68	74	64	RBA	hu IA-2 ic	^{35}S
152	60	94	0.79 (0.69–0.88)	<0.0001	62	21	8	RBA	hu IA-2 ic	^{35}S
126	56	100	0.79 (0.69–0.88)	<0.0001	61	56	64	RBA	hu IA-2 ic	^{35}S
111	56	100	0.78 (0.69–0.88)	<0.0001	62	106	64	RBA	hu IA-2 ic	^{35}S
155	50	100	0.78 (0.68–0.87)	<0.0001	58	11	64	RBA	Full length IA-2	^{35}S
137	54	100	0.77 (0.68–0.87)	<0.0001	58	138	8	RBA	hu IA-2 ic	^{35}S
140	56	100	0.77 (0.67–0.87)	<0.0001	56	101	64	RBA	hu IA-2 ic	^{35}S
129	54	100	0.77 (0.67–0.87)	<0.0001	58	46	64	RBA	hu IA-2 ic	^{35}S
133b	57	100	0.76 (0.66–0.86)	<0.0001	59		No standard curve	RBA	hu IA-2 ic	^{35}S
123	54	100	0.76 (0.66–0.86)	<0.0001	62	111	64	RBA	hu IA-2 ic	^{35}S
131	88	28	0.75 (0.66–0.85)	<0.0001	52	21	8	RBA	hu IA-2 ic	^{35}S
149	54	92	0.75 (0.65–0.85)	<0.0001	50	72	64	RBA	hu IA-2 bdc	^{35}S
148	58	100	0.75 (0.65–0.85)	<0.0001	58	105	64	RBA	hu IA-2 ic	^{35}S
134	56	98	0.75 (0.65–0.85)	<0.0001	58	25	16	RBA	hu IA-2 ic	^{35}S
118	54	96	0.75 (0.65–0.85)	<0.0001	54	47	16	RBA	hu IA-2 bdc	^{35}S
121	58	100	0.74 (0.64–0.85)	<0.0001	58	120	64	RBA	hu IA-2 ic	^{35}S
146	52	98	0.73 (0.63–0.84)	<0.0001	60	17	64	RBA	hu IA-2 bdc	^{35}S
107	50	94	0.73 (0.63–0.83)	<0.0001	52	35	8	ELISA	hu IA-2 ic	HRP
153	58	100	0.73 (0.62–0.83)	<0.0001	59	62	64	RBA	hu IA-2 ic	^{35}S
117	58	74	0.72 (0.62–0.82)	<0.0001	54	44	64	RBA	hu IA-2 bdc	^{35}S
151	54	90	0.71 (0.60–0.83)	<0.0001	54	27	4	ELISA	hu IA-2 ic	HRP
139	58	100	0.71 (0.60–0.82)	<0.0001	59	85	64	RBA	hu IA-2 ic	^{35}S
116	58	98	0.71 (0.59–0.82)	<0.0001	58	114	64	RBA	hu IA-2 ic	^{35}S
120	58	90	0.70 (0.59–0.81)	<0.0001	58	128	64	RBA	hu IA-2 ic	^{35}S
141	48	98	0.70 (0.59–0.80)	0.001	50	39	64	RBA	hu IA-2 bdc	^{35}S
142	50	96	0.70 (0.58–0.81)	0.001	56	118	64	RBA	hu IA-2 bdc	3H
114	58	100	0.68 (0.56–0.79)	0.003	58	132	64	RBA	hu IA-2 ic	^{35}S
119	50	100	0.65 (0.54–0.77)	<0.0001	52	27	16	RBA	hu IA-2 bdc	^{35}S
110	52	94	0.65 (0.53–0.77)	0.011	54	31	64	RBA	hu IA-2 bdc	^{35}S

using IA-$2_{256–556/630–979}$ antigen were lower than those from assays using IA-2ic or full-length IA-2 antigens, whether units were derived from an index or a standard curve ($P < 0.002$, Mann-Whitney *U* test).

DISCUSSION

The first DASP proficiency evaluation has demonstrated a remarkable degree of concordance between laboratories in GAD and IA-2 antibody measurement using radiobinding assays. This has been achieved much more quickly than by previous efforts to standardize ICA assays. The format and size of the workshop, together with the methods of analysis used, allowed fuller evaluation of assay performance than has been possible in small-scale proficiency schemes. Concordance between laboratories was directly related to their capacity to distinguish patient and control samples as defined in the ROC analysis. This was in turn related to assay type, antigen, and signal:noise ratio. For both GAD and IA-2 antibodies, only radiobinding assays achieved high sensitivity and specificity. For IA-2 antibodies, radiobinding assays that used the IA-$2_{256–556/630–979}$ antigen had lower sensitivity than assays that used intact intracellular IA-2. Samples from two patients had antibodies against the intracellular IA-2 but not against the IA-$2_{256–556/630–979}$ spliced form. For GAD antibodies but not for IA-2 antibodies, high sensitivity was

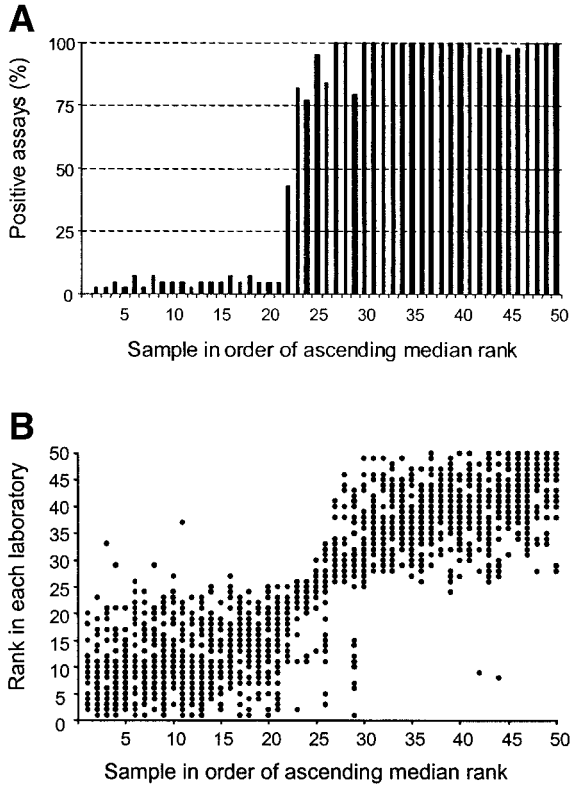

FIG. 2. IA-2 autoantibodies in samples from 50 patients with newly diagnosed type 1 diabetes in the DASP-1 proficiency evaluation. *A*: The proportion of assays reporting each sample as IA-2 autoantibody positive on the basis of the laboratory-defined cutoff. *B*: The rank of individual samples in each assay versus the median rank obtained for all 44 participating assays. Samples are arranged in the order of their median rank in all assays.

also associated with higher assay signal:noise ratio. This difference may be attributable to higher signal:noise ratios in IA-2 antibody assays, which makes this variable less likely to be the limiting factor in determining sensitivity. Finally, the laboratories that were least able to distinguish patient and control samples were often the same for both GAD and IA-2 antibody assays.

An important aspect of this study was the evaluation of general introduction of common units for measurement of GAD and IA-2 antibodies, based on the WHO reference preparation. The value of introducing a common standard to allow laboratories to express GAD and IA-2 antibody results in common units was confirmed, and the Immunology of Diabetes Society now recommends that results be expressed as WHO units/ml. Two methods for deriving units were compared: an index calculated from the ratio of test sample to a single standard and units derived from a standard curve of multiple dilutions over high and low antibody levels. Small but statistically significant differences were observed using these two methods. Index-based units were consistently marginally higher than those derived using a standard curve. For GAD antibodies, between laboratory variation, measured as the interquartile range, was not significantly different between units derived from an index or from a standard curve. For IA-2 antibodies, however, the interlaboratory variation in units derived from an index was significantly less than in units derived from a standard curve. Supplies of the WHO reference reagent are limited, and laboratories will not be able to include this as a standard in all assays but will need to calibrate their own laboratory standards to the reference reagent. As comparability between laboratories was not improved by use of a standard curve, except perhaps at very low antibody levels (data not shown), it is concluded that laboratory standards should be calibrated to

TABLE 3
Insulin autoantibody assays

Lab no.	Lab-defined sensitivity (%)	Lab-defined specificity (%)	Area under ROC curve (95% CI)	*P*	Sensitivity at specificity 90% (AS_{90} [%])	Assay type	Antigen	Label
116	36	100	0.80 (0.71–0.90)	<0.0001	66	RBA	Human insulin	^{125}I
120	32	100	0.77 (0.68–0.86)	<0.0001	52	RBA	Human insulin	^{125}I
133	14	100	0.77 (0.68–0.86)	<0.0001	47	RBA	Human insulin	^{125}I
153	34	98	0.77 (0.68–0.87)	<0.0001	64	RBA	Human insulin	^{125}I
121	30	98	0.75 (0.66–0.85)	<0.0001	47	RBA	Human insulin	^{125}I
126	16	100	0.75 (0.65–0.88)	<0.0001	51	RBA	Human insulin	^{125}I
156	6	100	0.73 (0.62–0.83)	<0.0001	56	RBA	Human insulin	^{125}I
148	42	98	0.72 (0.62–0.82)	<0.0001	50	RBA	Human insulin	^{125}I
118	10	98	0.70 (0.60–0.81)	<0.0001	36	RBA	Human insulin	^{125}I
138	4	100	0.63 (0.58–0.79)	0.002	32	RBA	Human insulin	^{125}I
140	14	100	0.68 (0.57–0.78)	0.003	40	RBA	Human insulin	^{125}I
115	16	100	0.67 (0.57–0.78)	0.003	42	RBA	Human insulin	^{125}I
113	12	100	0.66 (0.55–0.77)	0.006	30	RBA	Human insulin	^{125}I
123	30	90	0.65 (0.54–0.76)	0.012	26	RBA	Human insulin	^{125}I
137	8	96	0.64 (0.53–0.75)	0.014	29	RBA	Human insulin	^{125}I
149	4	98	0.62 (0.51–0.73)	0.033	21	RBA	Human insulin	^{125}I
145	8	100	0.62 (0.50–0.73)	0.044	38	RBA	Human insulin	^{125}I
114	14	100	0.59 (0.48–0.70)	0.121	26	RBA	Human insulin	^{125}I
117	16	98	0.58 (0.47–0.70)	0.155	25	RBA	Human insulin	^{125}I
132	8	100	0.58 (0.44–0.67)	0.324	19	RBA	Human insulin	^{125}I
135	6	100	0.55 (0.43–0.66)	0.404	19	RBA	Human insulin	^{125}I
136	20	94	0.52 (0.40–0.63)	0.746	21	RBA	Human insulin	^{125}I
105	6	100	0.32 (0.21–0.42)	0.002	13	RBA	Human insulin	^{125}I

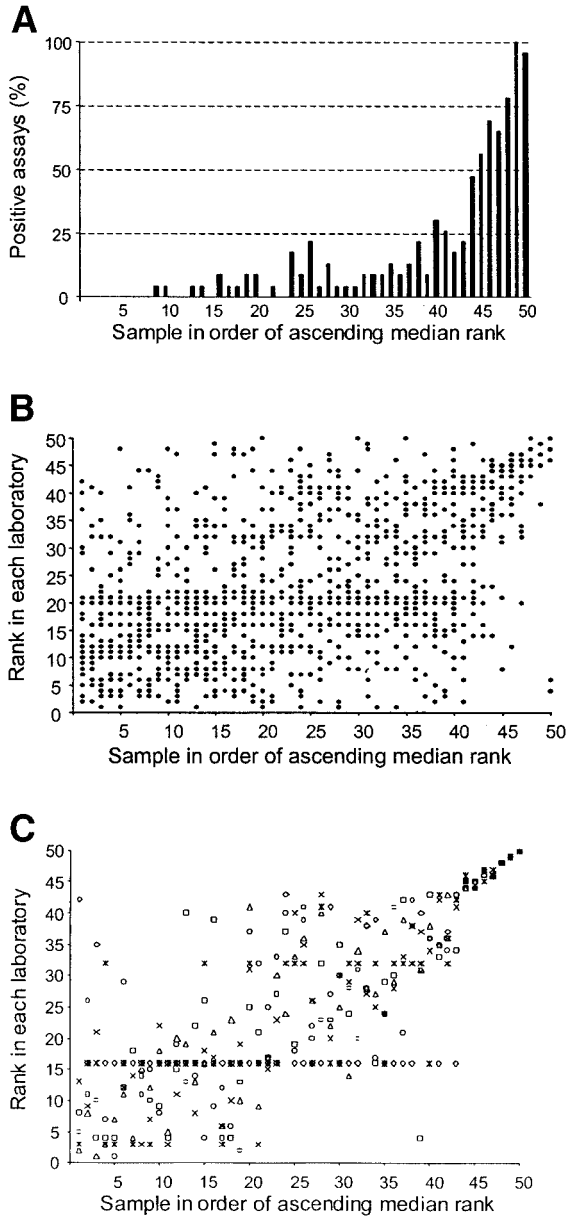

FIG. 3. Insulin autoantibodies in samples from 50 patients with newly diagnosed type 1 diabetes in the DASP-1 proficiency evaluation. *A*: The proportion of assays reporting each sample as insulin autoantibody positive on the basis of the laboratory-defined cutoff. *B*: The rank of individual samples in each assay versus the median rank obtained for all 23 participating assays. *C*: The rank of individual samples in each of the 6 assays that achieved the highest sensitivity versus the median rank obtained for all 22 assays. Concordance between these 6 assays was markedly higher than that of the other 16 assays ($P < 0.0001$). Samples are arranged in the order of their median rank in all assays. The results from each laboratory are indicated by a different symbol.

the undiluted WHO reference reagent using the more simple index method. It should be noted, however, that the use of multiple-point standard curves would be expected to be of greatest benefit in reducing variation within a laboratory over time and is likely to be useful in this context, although it has not been possible to address this issue in this workshop.

In contrast, there was wide variation between IAA assays in this workshop, and although a number of sensitive assays were identified, the overall performance of IAA assays was poor. In previous workshops, the majority of IAA assays were based on polyethylene glycol precipitation of immune complexes and used serum volumes of up to 600 μl (13), whereas this workshop was limited to assays that used a maximum of 100 μl per test sample. As a consequence, the majority of laboratories used a version of the recently introduced IAA microassays using protein A precipitation (10). Of the IAA microassays in the workshop, six had sensitivity >30% and these assays were highly concordant in their ranking of samples. The workshop demonstrated, however, that most laboratories have yet to optimize the IAA assay performance and that significant effort is needed to transfer the IAA microassays successfully to a larger number of laboratories. The introduction of a reference standard preparation, as has been done for ICA, GAD, and IA-2 antibodies, will be important.

In this set of samples, GAD antibody determination by radiobinding assays achieved higher sensitivity than testing for IA-2 antibodies or, as expected in patients of this age, IAA (Fig. 5). Sensitivity could be maximized by testing for both GAD and IA-2 antibodies. Of the patient samples, 88% were defined as GAD and/or IA-2 antibody positive in at least 80% of assays. One laboratory, using a radiobinding assay for antibodies to a GAD_{65}/$IA\text{-}2_{ic}$ fusion protein, achieved 88% sensitivity with 100% specificity and the highest area under the ROC curve overall. The potential of ICA determination by indirect immunofluorescence to be the most sensitive single test was confirmed in this workshop, although this was achieved by only one laboratory. Previous workshops, however, have demonstrated the great problems of standardizing this assay.

Most of the laboratories that participated in this evaluation are primarily involved in research. Islet antibody testing, however, is likely to be increasingly used in clinical practice and may need to be performed in nonspecialist laboratories. It is therefore of interest that, particularly for IA-2 antibodies, some assay kits achieved good discrimination between cases and controls with AUC and AS_{90} above the 75th centile. The format of this proficiency evaluation allows much fuller blinded evaluation of the performance of a kit than is generally possible. It is therefore hoped that more kit manufacturers will participate in DASP activities in the future.

In summary, the first DASP proficiency evaluation and the format of analysis have shown that, in the majority of participating laboratories, GAD and IA-2 antibody assays perform well. It has been possible to identify GAD antibody, IA-2 antibody, and IAA assays that achieve high sensitivity and specificity so that the characteristics associated with good levels of discrimination between disease and nondisease can be defined. This should allow laboratories that are not yet performing to the highest standards to identify areas for improvement. The validity of using both the index and multiple-point methods for deriving common units from the WHO standard preparation has been demonstrated, and it has been shown that good interlaboratory concordance is achieved when antibody levels are expressed in terms of WHO units. On the basis of these results, the Immunology of Diabetes Society now recommends that all GAD and IA-2 antibody results be expressed in WHO units/ml. The evaluation has also demonstrated that although the recently introduced IAA microassays can achieve high levels of sensitivity and

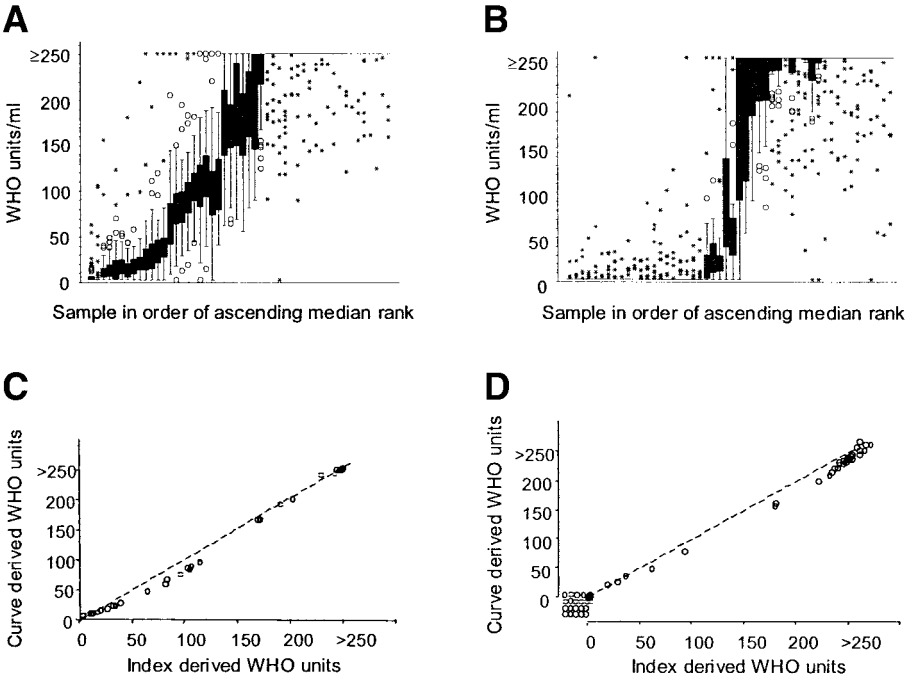

FIG. 4. GAD autoantibodies (*left*) and IA-2 autoantibodies (*right*) in samples from 50 patients with newly diagnosed type 1 diabetes expressed as WHO units/ml. *A* and *B*: Box and whisker plots showing interquartile range (■), median (horizontal line), upper and lower 90th centile (error bars), and outliers (circles and asterisks) for each sample. Index-derived WHO units/ml are shown. Samples are arranged in the order of their median rank in all assays. *C* and *D*: The median index-derived versus standard curve–derived WHO units for each sample. The line of identity is represented by a dashed line.

good interlaboratory concordance; the sensitivity of these assays as implemented in most laboratories is low and must be improved. These results will be invaluable in the design, implementation, and interpretation of multicenter trials and studies in type 1 diabetes.

APPENDIX: PARTICIPATING LABORATORIES

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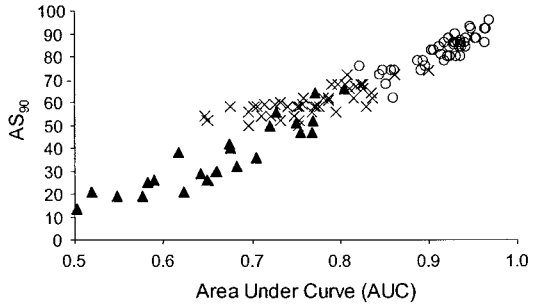

FIG. 5. ROC curve analysis. The area under the ROC curve versus the AS_{90} calculated from the ROC curve for each GAD antibody assay (○), IA-2 antibody assay (X), and IAA assay (▲).

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