Clinical Chemistry 43:5
779–785 (1997)

Endocrinology and Metabolism

Measurement of antibodies against glutamic acid decarboxylase 65 (GADA): two new ^{125}I assays compared with [^{35}S]GAD 65-ligand binding assay

Henrik Borg,[1]* Per Fernlund,[2] and Göran Sundkvist[1]

Recently, 65-kDa glutamic acid decarboxylase (GAD 65) antibodies (GADA) have been introduced as autoimmune markers in blood to confirm the diagnosis of insulin-dependent diabetes mellitus (IDDM). In this study, to evaluate two new assays that use ^{125}I-labeled GAD 65, we assayed samples from 100 children with recent onset of diabetes and 100 control children; the results were compared with those of a [^{35}S]GADA assay and with results for islet cell antibodies (ICA), the conventional autoimmune marker. Receiver operating characteristic (ROC) curve analysis showed one of the new assays (from RSR) to be more sensitive ($P = 0.01$) than the comparison ([^{35}S]GADA) assay, whereas the second new assay (from Elias) was less sensitive ($P < 0.001$). The GADA frequency at 97.5% specificity was greatest in the comparison assay: 63 of 100 vs 41 of 100 ($P < 0.01$) and 53 of 100 ($P = 0.16$) in the RSR and Elias assays, respectively. Almost all GADA-positive patients had ICA, but one-third of the ICA-positive patients was GADA-negative. Accordingly, adding GADA analysis results to ICA testing increased the frequency of detection of autoimmune markers only slightly (from 81% to 85%). In conclusion, at 97.5% specificity the [^{35}S]GADA assay seemed to be more efficient than the ^{125}I assays, although the difference was significant only for the Elias ^{125}I assay. Antigen-specific antibodies other than GADA may explain the difference in GADA and ICA frequencies.

INDEXING TERMS: diabetes • autoimmunity • islet cell antibodies • method comparison • ROC curve analysis

Diabetes mellitus can be separated into two main types: insulin-dependent diabetes mellitus (IDDM) and non-insulin-dependent diabetes mellitus (NIDDM).[3] The initiation of IDDM is thought to be related to an autoimmune attack on the insulin-producing pancreatic β cells, leading to their destruction and thereby to loss of endogenous insulin secretion *[1]*; in contrast, the pathogenesis of NIDDM is unrelated to autoimmunity. Although IDDM usually affects young patients and NIDDM mostly obese patients, it is often difficult to separate IDDM from NIDDM in the clinical situation; IDDM occurs at all ages, and one-third of the NIDDM patients in a Swedish population were not obese *[2]*.

In IDDM, markers of the autoimmune process may appear in blood. Islet cell antibodies (ICA; antibodies reacting against the islets of Langerhans), are the standard among such markers. Although not all ICA antigens have been defined, the ICA assay is at present the most reliable test for identifying autoimmune diabetes. ICA can be demonstrated in ~80% of the children at the time of diagnosis with diabetes *[3]*. ICA may also be detected in ~10% of patients diagnosed as NIDDM and in such cases indicate an on-going progressive β cell destruction leading to insulin dependency within 3 years; i.e., these patients must be regarded as having a slow-onset form of IDDM *[4]*. Accordingly, analysis for autoantibodies is a valuable tool in the classification of diabetes.

Although regarded as the standard for detecting the autoimmune process of IDDM, the ICA test, which utilizes indirect immunofluorescence, is laborious and difficult to standardize. A need for simpler alternatives is apparent. Glutamic acid decarboxylase antibodies (GADA) are another autoimmune marker. These antibodies are associated with β cell failure and IDDM in patients

Departments of [1] Endocrinology and [2] Clinical Chemistry, University of Lund, Malmö University Hospital, S-205 05 Malmö, Sweden.

*Address for correspondence: Wallenberg Laboratory, Entrance 46, 2nd Fl., Malmö University Hospital, S-205 05 Malmö, Sweden. Fax +46 40 337041; e-mail Henrik.Borg@medforsk.mas.lu.se.

Received August 21, 1996; revised and accepted December 26, 1996.

[3] Nonstandard abbreviations: IDDM, insulin-dependent diabetes mellitus; NIDDM, non-insulin-dependent diabetes mellitus; ICA, islet cell antibodies; GADA, glutamic acid decarboxylase antibodies; and GAD 65, 65-kDa glutamic acid decarboxylase.

with adult-onset diabetes *[5–7]* but are also often detected at the time of diagnosis of diabetes in children *[8]*. After the 65-kDa glutamic acid decarboxylase antigen (GAD 65) was characterized *[9]*, radioligand assays were developed to measure antibodies to this antigen. ^{35}S-labeled GAD 65, synthesized by in vitro translation, has been established as the preferred reagent for GADA assays, showing high sensitivity *[10, 11]*. These assays, however, also have drawbacks, being expensive and technically demanding. For routine analysis, therefore, an assay using ^{125}I-labeled GAD 65 would be more convenient.

To evaluate two new commercial assays that use ^{125}I-labeled GAD 65 as antigen, we tested the assays on samples from 100 children with newly diagnosed diabetes and on samples from 100 control children. Although the primary aim of this study was to evaluate the two ^{125}I assays of GADA in relation to the ^{35}S assay, the nature of the test samples also gave us the opportunity to examine the association between GADA and ICA among children with recently diagnosed diabetes mellitus.

Materials and Methods

SUBJECTS

One hundred patients' samples were collected from consecutively diagnosed children [all but one were Caucasians, 52 were girls, and mean ± SD ages were 9 ± 4 years (range 1–15 years)] recently diagnosed with diabetes mellitus. The samples were obtained within 2 weeks of diagnosis in 95 of the patients, and 81 of the 100 were ICA positive. The control samples were obtained from 100 subjects randomly selected from 1031 apparently healthy school children *[12]*; their ages were 11 ± 2 years (range 7–13; slightly higher than the patients, $P < 0.01$), 49 were girls, and 2 of the 100 were ICA-positive.

REAGENTS

The GAD 65 cDNA used in the comparison assay was obtained from the University of Washington, courtesy of Thomas Dyrberg, Catherine E. Grubin, Allan E. Karlsen, Åke Lernmark, and Karen L. Deyerle. It was provided as a plasmid (pEx9) in DH5 224 *Escherichia coli* cells. pEx9 is a recombinant of the pcDNAII vector (from Invitrogen, Carlsbad, CA), with the GAD 65 cDNA inserted between the *Bam*HI and *Xba*I restriction sites *[11]*. The construction of the cDNA has been described before *[13]*. Minipreps of the plasmid were done with accepted methods *[14]*, and the plasmid DNA obtained was checked by cutting with *Bam*HI and *Xba*I, which gave a fragment of the expected size (1807 bp), and by sequencing the insert from both ends with vector-specific primers (Universal and M13 reverse primer; Pharmacia, Uppsala, Sweden). The sequence was in accordance with the published cDNA sequence for human GAD 65 *[15]* except for a modification at the 5′-end; this did not change the amino acid sequence but made the DNA more suitable for in vitro expression *[11]*. Because the cDNA is positioned downstream of the Sp6 promoter, the mRNA formed should thus give a complete protein, including the signal peptide.

TNT Coupled Reticulocyte Lysate System was obtained from Promega (Madison, WI), RNAsin from Appligen (Illkirch, France), L-[^{35}S]methionine from Amersham Ltd. (Bucks, UK), Protein A–Sepharose CL-4B from Pharmacia, and a Multiscreen 96-well filtration system from Millipore (Bedford, MA). Buffers used in the comparison assay were "plain" buffer (NaCl 150 mmol/L, Tris 20 mmol/L, pH 7.4, and NaN_3, 2 g/L), coating buffer (plain buffer plus bovine serum albumin, 10 g/L), and washing buffer (plain buffer plus bovine serum albumin, 1 g/L, and Tween 20, 1.5 mL/L).

ASSAY METHODS

^{35}S assay. The comparison assay was a slight modification of the assay described by Petersen et al. *[11]*. The ^{35}S-labeled human recombinant GAD 65 was synthesized with circular pEx9 in the TNT Coupled Reticulocyte Lysate System. The yield of the translation product, determined by precipitating the product with trichloroacetic acid and measuring the radioactivity of the precipitate, was 20–30% of the total [^{35}S]methionine added. We did not separate the translation product from the remaining free labeled-methionine. Overnight incubations at 4 °C with [^{35}S]GAD 65 were made in duplicate for each sample. Two aliquots from each incubation were then further incubated with Protein A–Sepharose on a 96-well filtration plate to collect the immunocomplexes formed. After the filtration and washing, the bottom of each well was punched into a scintillation bottle, and the radioactivity was counted with a Wallac 1410 (Pharmacia) liquid scintillation counter.

Sera pooled from three blood donors served as a negative control, whereas plasma from a patient with high concentrations of GADA (diluted in negative control serum to give a more suitable concentration) served as a positive control. The controls were stored as single-use aliquots at −70 °C. The results obtained with the [^{35}S]GADA assay are presented as a GADA index: $100 \times (u - n)/(p - n)$, where u = counts per minute (cpm) of the unknown sample, n = cpm of the negative control, and p = cpm of the positive control. (For cpm values, we used the mean activity of all four measurements for a sample.)

At the time of the study, the assay was evaluated in the International Diabetes Workshop GADA65 Proficiency Program (#2) and showed a sensitivity and a specificity of 100% (24 samples evaluated).

^{125}I assay 1. The first new assay tested was the GAD 65-antibody assay from RSR (Cardiff, UK), which included ^{125}I-labeled human recombinant GAD 65. The GAD 65 had been produced by expression in yeast (*Saccharomyces cerevisiae*) and was purified by ion-exchange chromatography (on DEAE) and affinity chromatography with a monoclonal antibody to GAD 65. The assay kit also included solid-phase Protein A; GADA

assay buffer; assay calibrators with GADA concentrations of 0, 1, 3, 10, 30, and 300 arbitrary units (A1-units) per liter; and two assay control sera.

The assay was carried out according to the instructions of the manufacturer. Briefly: Sample and ^{125}I-labeled GAD 65 were incubated for 120 min at room temperature to form immune complexes, which were adsorbed onto solid-phase Protein A. After addition of assay buffer and centrifugation, the radioactivity of the sediment was counted in a gamma-counter. Each sample was assayed in duplicate. Results were read from a calibration curve constructed in the same run with the calibrators (also in duplicate), and expressed in A1-units. The total time for the assay was 6 h.

^{125}I assay 2. Assay 2, the GAD II antibody IRMA from Elias (Freiburg, Germany), included ^{125}I-labeled human recombinant GAD 65. The GAD 65 had been obtained by expression of human full-length cDNA in a baculovirus/Sf9 insect cell system and had 6 histidyl residues at the carboxy-terminus (to facilitate purification of the antigen on Ni^{2+}-resin). The assay kit also included anti-human IgG; assay buffer; assay calibrators with GADA concentrations of 25, 100, 250, 500, 1000, and 5000 arbitrary units (A2-units) per liter; positive and negative controls; and dilution sera.

The assay was carried out according to the instructions of the manufacturer. Briefly: Sample and ^{125}I-labeled GAD 65 were incubated for 120 min at room temperature to form immune complexes, which were then precipitated by anti-human IgG. After addition of assay buffer and centrifugation, the radioactivity of the sediment was counted in a gamma-counter. Each sample was assayed in duplicate. Results were read from a calibration curve constructed in the same run with the calibrators (also in duplicate), and expressed in A2-units. Total time for this assay was also 6 h.

Assay performance. The precision of the assays was monitored by including in each run two control samples in duplicates, one with a low concentration of GADA and one with a high concentration. The intra- and interassay variation was estimated by two-way analysis of variance of the pooled values for the controls.

STATISTICS

The nonparametric Mann–Whitney test was used to analyze differences in antibody concentrations between patient and control groups, the nonparametric Spearman test to analyze the degree of correlation between the different GADA assays, and the Fisher test to analyze differences in frequency. ROC (receiver operating characteristic) curves were constructed with SAS (Cary, NC) 6.10/OS/2 Warp Connect software. The significance of differences of areas under various ROC curves was calculated as described elsewhere *[16]*.

Results

The results obtained by the three assays are shown in Fig. 1. There was a pronounced difference between GADA results for the patients and the controls in all three assays. The mean GADA index obtained with the comparison

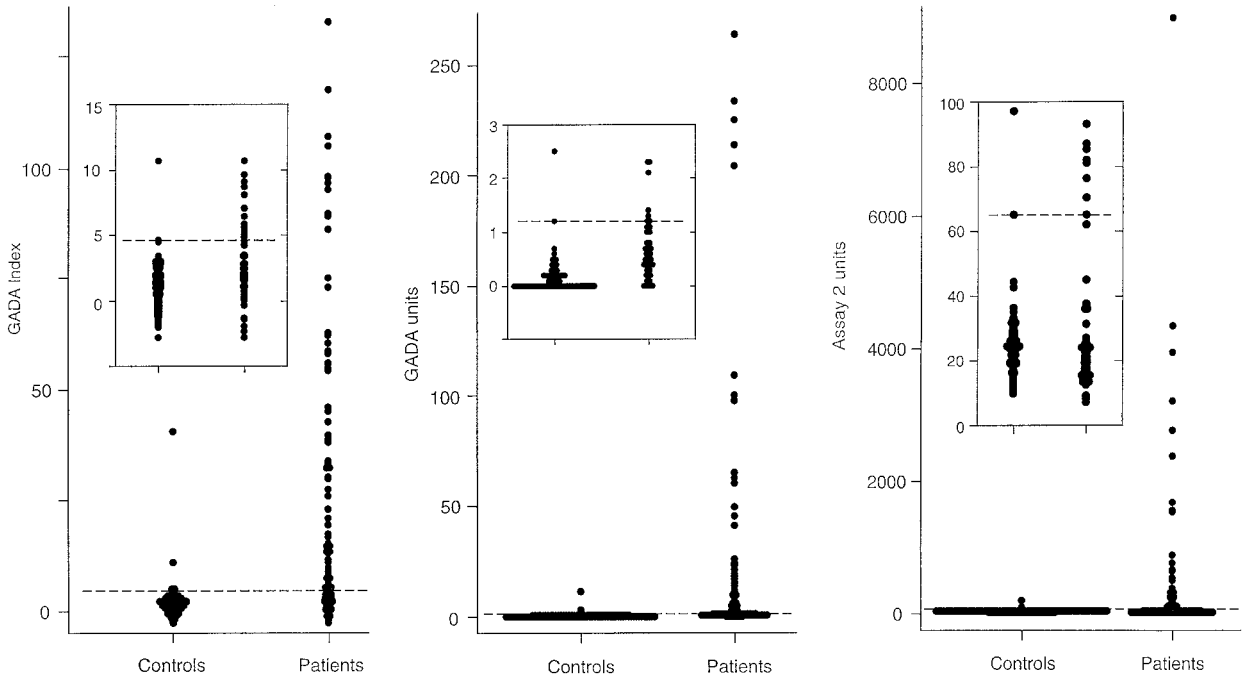

Fig. 1. GADA results for the comparison assay (*left*), assay 1 (*middle*), and assay 2 (*right*) in 100 patients and 100 controls.
Horizontal bars indicate the cutoff value corresponding to 97.5% specificity. *Inserts* are magnifications of the *y*-axis. *Middle panel: y*-axis is GADA A1-units.

assay was 26.5 ± 33.2 (median 10.2) in diabetic children vs 1.5 ± 4.3 (median 1.2) in the control children ($P < 0.001$). The corresponding values obtained with assay 1 were 21.8 ± 52.7 A1-units (median 1.2) and 0.1 ± 0.3 A1-units (median 0.0) ($P < 0.001$), respectively. With assay 2, the respective values were 399.5 ± 1161.0 A2-units (median 26.0) and 24.9 ± 10.5 A2-units (median 23.4) ($P < 0.01$). Fig. 1 demonstrates that the comparison assay results for the patients were more evenly distributed between the highest and the lowest results than were the results of assays 1 and 2.

Figure 2 (top) shows the ROC curves obtained for the three methods when the sensitivities were determined from the results for the diabetic children (n = 100) and specificities were based on the control children's results (n = 100). To compare the three assays, we took the area under the curve as a measure of assay efficiency, i.e., 0.83 for the ^{35}S assay, 0.90 for assay 1, and 0.66 for assay 2. By this measure, the comparison assay and assay 1 were clearly more efficient than assay 2 ($P < 0.001$). However, the difference between the comparison assay and assay 1 was also statistically significant ($P = 0.01$). Including ICA results to define patients and controls (i.e., excluding ICA-negative patients and ICA-positive controls) increased the efficiency (area under the curve) of all three assays (Fig. 2, bottom) but did not change the relations between the assays.

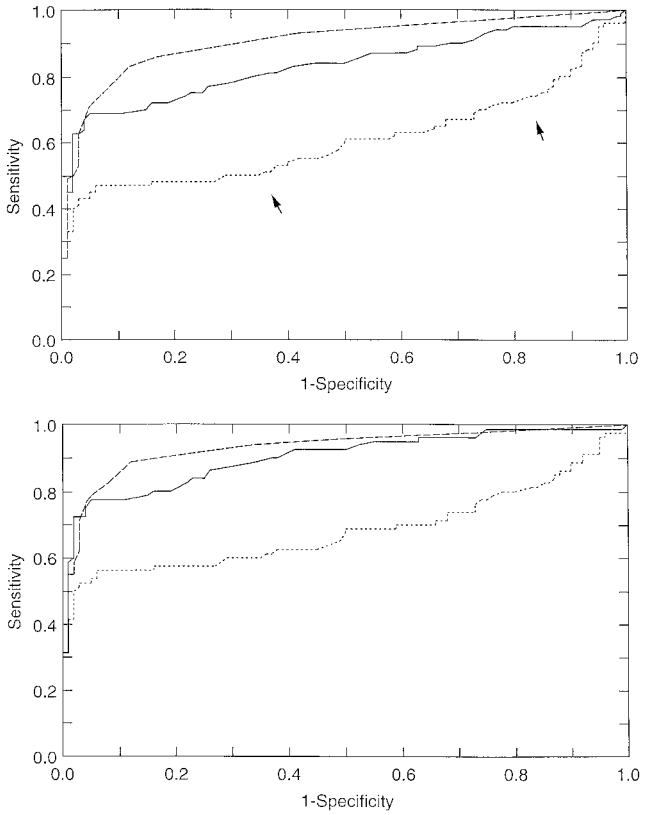

Fig. 2. ROC curves for the comparison assay (——), assay 1 (- - -), and assay 2 (- - -), with specificities based on results for the control children (n = 100) and sensitivities based on (*top*) results for the diabetic children (n = 100) or (*bottom*) results for the ICA-positive diabetic children only (n = 81).

Arrows indicate upward concave sections (see *Results*).

Because ROC curves compare methods over the whole range of specificities, most of which are not relevant in clinical work, we also calculated the sensitivities of the three methods at a few selected clinically relevant specificities (Table 1). At specificities of 97.5% and 99%, respectively, the sensitivity of the comparison assay appeared to be higher than those of assay 1 and assay 2; however, the difference was significant only with regard to assay 2 ($P < 0.01$). For assay 1, the manufacturer suggested a cutoff limit of 1.0 A1-units. In the current subject material, this cutoff value corresponded to a specificity of 97% and a sensitivity of 59%. For assay 2, the manufacturer's suggested cutoff was 70 A2-units, corresponding to a specificity of 97.7% and a sensitivity of 40% in the current study material.

The ROC curve for assay 2 deviates from the usual shape, showing a constant upward convexity (Fig. 2, both panels). The sections with upward concave shapes (one major and one minor) were attributable to a subgroup of the patients—mainly those who were ICA-negative, who gave very low GADA results, even in comparison with the majority of the control subjects (Fig. 3).

In any case, the correlations between the different GADA assays were high: comparison assay vs assay 1, $r_S = 0.93$; comparison assay vs assay 2, $r_S = 0.85$; assay 1 vs assay 2, $r_S = 0.88$.

The frequency of ICA and GADA positivity at 97.5% specificity for GADA are shown in Table 2. All but four GADA-positive patients were also ICA-positive. Therefore, at this specificity (97.5%) for GADA, ICA was more sensitive than GADA: 81 of 100 ICA-positive vs 63 of 100 GADA-positive by the comparison assay ($P < 0.01$) and 53 of 100 GADA-positive by assay 1($P < 0.001$) (Table 3).

Table 1. Sensitivities at selected specificities for three GADA assays.[a]

	Sensitivity, %		
Specificity, %	**^{35}S assay**	**Assay 1**	**Assay 2**
Total population (n = 200)[b]			
90.0	69 (3.0)	82 (0.4)	47 (33)
95.0	69 (3.4)	69 (0.6)	43 (40)
97.5	63 (4.6)	53 (1.2)	41 (65)
99.0	37 (25.4)	32 (6.8)	26 (150)
Selected population (n = 181)[c]			
90.0	78 (3.0)	90 (0.4)	56 (33)
95.0	78 (3.4)	79 (0.6)	52 (40)
97.5	72 (4.6)	62 (1.2)	51 (65)
99.0	46 (25.4)	40 (6.8)	32 (150)

[a] Cutoff limits are shown in parentheses.

[b] 100 newly diagnosed children (81 ICA-positive) and 100 healthy control children.

[c] Excluding the 19 ICA-negative newly diagnosed diabetic children.

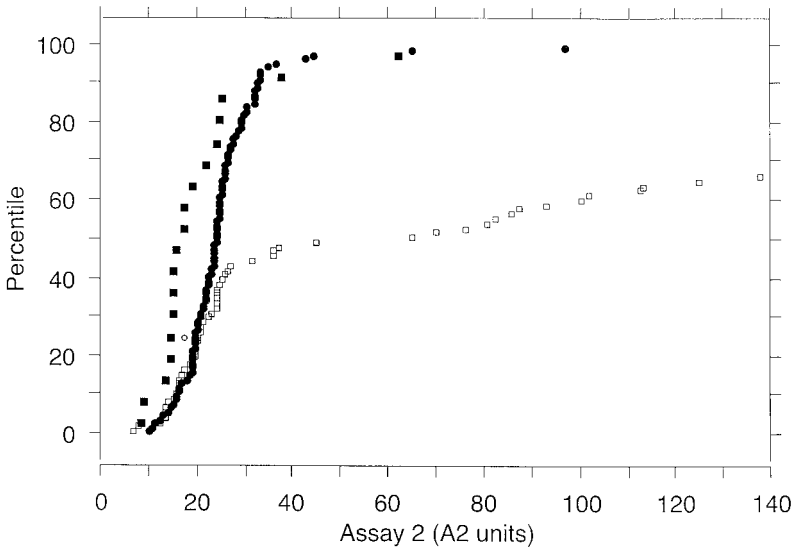

Fig. 3. Percentiles plot of GADA results with assay 2, for ICA-negative patients (■), ICA-positive patients (□), ICA-negative controls (●), and ICA-positive controls (○).

The reproducibility of the assays was determined from the results for two control samples run in duplicate in each run of each assay. In the comparison assay, the intraassay CV was 17% at the low concentration and 20% at the high concentration. The interassay CV for the comparison assay at both values seemed small, because the estimate by analysis of variance gave negative values for the variances. In assay 1, the intraassay CV was 13% at both the low and the high concentrations. This assay also led to a negative value for the variance when estimating the interassay CV at the low concentration; at the high concentration, the intraassay CV was 4%. In assay 2, the intraassay CV was 12% at the low concentration and 8% at the high concentration. The estimated interassay CV was 15% at the low concentration and 4% at the high concentration.

Table 2. Comparison of the frequency of GADA (at 97.5% specificity) in 100 diabetic children by the three GADA assays: comparison assay (comp.) and assays 1 and 2.

	No. of patients		
	All	ICA positive	ICA negative
Total	100	81	19
Positive for all three assays:	41	41	0
Positive for two assays:			
Comp. and 1	12	10	2
Comp. and 2	0	0	0
1 and 2	0	0	0
Positive for one assay:			
Comp.	10	8	2
1	0	0	0
2	0	0	0

Table 3. Frequency of ICA and (or) GADA in diabetic children (n = 100) for the comparison assay and assay 1.

	Prevalence, %	
	Comp. assay	Assay 1
ICA- and GADA-positive	59	51
ICA-positive, GADA-negative	22	30
ICA-negative, GADA-positive	4	2
ICA- and GADA-negative	15	17

Discussion

As with most other methods for detecting autoimmune markers in patients with diabetes mellitus, none of the three GADA assays evaluated achieved 100% sensitivity and specificity, although the results of all three correlated well. We do not know at present whether the sensitivity of <100% was the result of too high a detection limit for the GADA methods, the fact that antibodies to GAD do not always appear in patients with autoimmune diabetes mellitus, or the pathogenesis of IDDM in some cases being nonautoimmune. Also, of course, the properties and quality of the GAD 65 antigen used are of major importance for the performance of the assay. Given that the assays used three different preparations of the antigen, steric antigen differences might be involved. Whatever the reason, we conclude that there are indeed differences between the assays in their capacity to detect GADA.

Assay 2 was less efficient than both the comparison assay and assay 1, but the reason for the discrepancy is unclear. Perhaps the technique for the collection of the immune complexes has an effect. Anti-human IgG might be less efficient than Protein A in capturing all of the different types of autoantibodies that could appear and that might differ between patients. Also, assay 2 used a smaller sample volume than the other two assays, which might influence the limit of detection.

Assay 2 gave very low GADA values for those patients who were ICA-negative, values that were quite low even in comparison with the majority of the values for the control subjects. We had similar findings in preliminary

experiments using the same GAD 65 antigen as in assay 2 in a radioligand assay developed in our laboratory. We found (Fernlund, Borg, Sundkvist, unpublished) a subgroup of IDDM patients with lower GADA values than the majority of the control subjects. We were unable to explain these findings but believe this indicates that the antigen used in assay 2 is in some respect more similar to the antigens displayed in the ICA assay than are the antigens in the two other GADA assays.

In studies comparing the clinical performance of various assays, one must select the subjects carefully, both the patients for the sensitivity determination and the controls for the specificity determination. Ideally, the test subjects should not differ from those with whom the assay is intended to be used. It is also important to establish well-defined criteria for how the between-assays comparison should be done. One general approach is to construct ROC curves for each assay and let the areas under the curves show which assay is the best, as we have done here. This means, however, that the comparison is integrated over all possible specificities, many of which are of minor interest in clinical situations. Therefore, we have also given the sensitivities of the assays when the cutoff values correspond to some of the conventional specificities, i.e., 95%, 97.5%, and 99%. What cutoff specificity to use depends on the purpose of the investigation in which the assay is to be applied. At the specificities given (95%, 97.5%, and 99%), the comparison assay had the highest sensitivity (although this was not significantly different from that of assay 1), whereas assay 2 seemed to be the least efficient.

Almost all GADA-positive patients were ICA-positive as well. On the other hand, one-third of the ICA-positive patients were GADA-negative. Thus, adding GADA measurements to ICA measurements does not seem to increase the frequency of detection of autoimmunity markers in young diabetic patients. The observation that GADA-positive patients almost always also are ICA-positive is in accordance with the concept that GAD 65 is just one of the antigens ICA react with. Further studies of patients who were ICA-positive but GADA-negative might reveal new ICA antigens, one putative candidate being IA2 *[17]*.

Our results in this study are in accordance with previous studies of GADA and ICA in large populations of Caucasians with recent-onset IDDM. The studies have shown a frequency of 60–80% for GADA, whereas the ICA frequency has generally been higher, 80–90% (Table 4). In the past, children with diabetes have almost always been regarded as having IDDM, but the increasing incidence of NIDDM in adolescents *[18]* is making the differential diagnosis between IDDM and NIDDM more uncertain. In fact, ICA-negative children with diabetes have features (e.g., no high-risk HLA haplotypes) that are inconsistent with IDDM *[19]*. ICA and GADA may be important also for the differential diagnosis of diabetes in children, not just in adults *[4, 8]*.

We thank Thomas Dyrberg, Catherine C. Grubin, Allan Karlsen, Åke Lernmark, and Karen L. Deyerle for providing GAD cDNA; RSR and Elias for the kind gift of assay kits; Lis-Britt Granberg, Ingegerd Larsson, Hayan Li, Ann Radelius, Christina Rosborn, and Tiina-Maija Tuomi for valuable assistance; and Jan-Åke Nilsson for help with ROC curve constructions. This study was supported by grants from the Child Diabetes Fund, Lundström Foundation, Malmö Diabetes Association, Novo-Nordic Foundation, Research Funds Malmö University Hospital, Swedish Diabetes Association, Swedish Medical Research Council (7507 and 5913), and University Funds Lund University.

Table 4. Frequency of GADA and ICA in studies of patients with recently diagnosed IDDM.

	Patients				Controls			
			% positive				% positive	
Study	Age, years	n	GADA	ICA	Age, years	n	GADA	ICA
Bonifacio et al. [*20*]	1–15	69	65	93	1–40	83	1	1
	15–40	31	81	77				
Grubin et al. [*10*]	0–14	105	77	88	0–14	157	2	2
Hagopian et al. [*8*]	0–14	495	70[a]	84	0–14	417	4[b]	4
Lühder et al. [*21*]	2–24	51	59	—	18–58	54	0	—
Myers et al. [*22*]	≤20	89	65	61	10 ± 5	36	6	0
	>20	40	68	60	43 ± 17	70	0	0
Petersen et al. [*11*]	0–60	100	77	54[c]	24–52	100	4	—
Vandewalle et al. [*23*]	0–39	312	76	—	18 ± 13	163	1	—
Velloso et al. [*24*]	5–44	50	80	84	22–65	20	0	0

[a] 7 patients not included.
[b] 5 controls not included.
[c] 19 patients not included.

References

1. Eisenbarth GS. Type 1 diabetes mellitus. N Engl J Med 1986;314: 1360–8.
2. Gottsäter A, Landin-Olsson M, Lernmark Å, Fernlund P, Sundkvist G. Islet cell antibodies are associated with β-cell failure also in obese adult onset diabetic patients. Acta Diabetol 1994;31:226–31.
3. Landin-Olsson M, Karlsson A, Dahlquist G, Blom L, Lernmark Å, Sundkvist G. Islet cell and other organ-specific autoantibodies in all children developing type 1 (insulin-dependent) diabetes mellitus in Sweden during one year and in matched controls. Diabetologia 1989;32:387–95.
4. Gottsäter A, Landin-Olsson M, Fernlund P, Lernmark Å, Sundkvist G. β-cell function in relation to islet cell antibodies during the first 3 years after clinical diagnosis of diabetes in type II diabetic patients. Diabetes Care 1993;16:902–10.
5. Hagopian WA, Karlsen AE, Gottsäter A, Landin-Olsson M, Grubin CE, Sundkvist G, et al. Quantitative assay using recombinant human islet glutamic acid decarboxylase (GAD-65) shows 64 K autoantibody positivity at onset predicts diabetes type. J Clin Invest 1993;91:368–74.
6. Tuomi T, Groop L, Zimmet PZ, Rowley MJ, Knowles W, Mackay IR. Antibodies to glutamic acid decarboxylase reveal latent autoimmune diabetes mellitus in adults with a non-insulin-dependent onset of disease. Diabetes 1993;42:359–62.
7. Niskanen LK, Tuomi T, Karjalainen J, Groop LC, Uusitupa MIJ. GAD antibodies in NIDDM. Diabetes Care 1995;18:1557–65.
8. Hagopian WA, Sanjeevi CB, Kockum I, Landin-Olsson M, Karlsen AE, Sundkvist G, et al. Glutamate decarboxylase-, insulin-, and islet cell- antibodies and HLA typing to detect diabetes in a general population-based study of Swedish children. J Clin Invest 1995; 95:1505–11.
9. Baekkeskov S, Jan-Aanstoot H, Christgau S, Reetz A, Solimena M, Cascalho M, et al. Identification of the 64K autoantigen in insulin dependent diabetes as the GABA synthesizing enzyme glutamate decarboxylase. Nature 1990;347:151–6.
10. Grubin CE, Daniels T, Toivola B, Landin-Olsson M, Hagopian WA, Li L, et al. A novel radioligand binding assay to determine diagnostic accuracy of isoform-specific glutamic acid decarboxylase antibodies in childhood IDDM. Diabetologia 1994;37:344–50.
11. Petersen J, Hejnæs R, Moody A, Karlsen A, Marshall M, Høier-Madsen M, et al. Detection of GAD65 antibodies in diabetes and other autoimmune diseases using a simple radioligand assay. Diabetes 1993;43:459–67.
12. Samuelsson U, Ludvigsson J, Sundkvist G. Islet cell antibodies (ICA), insulin autoantibodies (IAA), islet cell surface antibodies (ICSA) and C-peptide in 1031 school children in a population with a high background incidence of IDDM. Diab Res Clin Pract 1994;26:155–62.
13. Sambrook J, Fritsch EF, Maniatis T. Molecular cloning. A laboratory manual. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 1989.
14. Karlsen AE, Hagopian WA, Petersen JS, Boel E, Dyrberg T, Grubin CE, et al. Recombinant glutamic acid decarboxylase (representing the single isoform expressed in human islets) detects IDDM-associated 64,000-M_r autoantibodies. Diabetes 1992;41: 1355–9.
15. Karlsen AA, Hagopian WA, Grubin CE, Dube S, Disteche CM, Adler DA, et al. Cloning and primary structure of a human islet isoform of glutamic acid decarboxylase from chromosome 10. Proc Natl Acad Sci U S A 1991;88:8337–41.
16. Hanley JA, McNeil BJ. A method of comparing the areas under receiver operating characteristic curves derived from the same cases. Radiology 1983;148:839–43.
17. Bonifacio E, Lampasona V, Genovese S, Ferrari M, Bosi E. Identification of protein thyrosine phosphatase-like insulin dependent-related 37/40K autoantigen and a target for islet-cell antibodies. J Immunol 1995;155:5419–26.
18. Pinhas-Hamiel O, Dolan LM, Daniels SR, Standiford D, Khoury PR, Zeitler P. Increased incidence of non-insulin-dependent diabetes mellitus among adolescents. J Pediatr 1996;128:608–15.
19. Babu SR, Walravens P, Wang T, Rothman R, Lumlung M, Verge C, et al. Disease heterogeneity at the onset of diabetes in children evaluated with multiple "biochemical" autoantibody screening in DQ typing [Abstract]. Diabetes 1996;45:201A.
20. Bonifacio E, Genovese S, Braghi S, Bazzigaluppi E, Lampasona V, Bingley PJ, et al. Islet autoantibody markers in IDDM: risk assessment strategies yielding high sensitivity. Diabetologia 1995;38: 816–22.
21. Lühder F, Woltanski K-P, Mauch L, Haubruck H, Kohnert K-D, Rjasanowski I, et al. Detection of antibodies to the 65-kD isoform of glutamate decarboxylase by radioimmunoassay. Eur J Endocrinol 1994;130:575–80.
22. Myers AM, Rabin DU, Rowley MJ. Pancreatic islet cell cytoplasmic antibody in diabetes is represented by antibodies to islet cell antigen 512 and glutamic acid decarboxylase. Diabetes 1995;44: 1290–5.
23. Vandewalle CL, Falorni A, Svanholm S, Lernmark Å, Pipeleers DG, Gorus FK, et al. High diagnostic sensitivity of glutamate decarboxylase autoantibodies in insulin-dependent diabetes mellitus with clinical onset between age 20 and 40 years. J Clin Endocrinol Metab 1995;80:846–51.
24. Velloso LA, Kämpe O, Hallberg A, Christmanson L, Betsholtz C, Karlsson FA. Demonstration of GAD-65 as the main immunogenic isoform of glutamate decarboxylase in type 1 diabetes and determination of autoantibodies using a radioligand produced by eucharyotic expression. J Clin Invest 1993;91:2084–90.