# Improved Specificity of ICA Assays in the Fourth International Immunology of Diabetes Serum Exchange Workshop

CARLA J. GREENBAUM, JERRY P. PALMER, S. NAGATAKI, Y. YAMAGUCHI, J.L. MOLENAAR, W.A.M. VAN BEERS, NOEL K. MACLAREN, ÅKE LERNMARK, AND PARTICIPATING LABORATORIES

**The goal of the Fourth International Workshop for Standardization of ICA Measurements was to determine the specificity of ICA assays and their ability to distinguish between control sera ($n$ = 57) and sera from IDDM-related individuals—representing relatives of IDDM patients ($n$ = 21), healthy individuals who later developed IDDM ($n$ = 8), or newly diagnosed IDDM patients ($n$ = 23). Results from 28 laboratories were analyzed. The mean specificity (percentage of control sera reported as negative) among 27 laboratories was 91%, including 6 laboratories with 100% specificity. Nevertheless, 78% of laboratories found at least one control sample >0 JDF U. Among samples from first-degree relatives, the mean concordance was 86%, including three sera found negative (0 JDF U) by all laboratories. Among individuals who later developed diabetes, the mean concordance was 93%, with two sera found positive by 100% of laboratories. In sera from newly diagnosed IDDM patients, the mean concordance was 82%. Three sera were found positive and one serum negative by all laboratories. The JDF U of the sera considered to be positive were significantly greater than each laboratory's average for the controls. In conclusion, the results from laboratories participating in the Fourth International ICA Workshop demonstrated excellent specificity, good concordance, and an ability to separate control sera from defined, IDDM-related subjects. *Diabetes* 41:1570–74, 1992**

From the Department of Medicine, University of Washington, Seattle, Washington; the Nagasaki University School of Medicine, Nagasaki, Japan; the Klinik Immunologie, Kelft, The Netherlands; and the University of Florida, Gainesville, Florida.

Address correspondence and reprint requests to Carla J. Greenbaum, MD, Veterans Affairs Medical Center, 1660 South Columbian Way, No. 111, Seattle, WA 98108.

Received for publication 13 March 1992 and accepted in revised form 16 July 1992.

ICA, islet cell antibody; IDDM, insulin-dependent diabetes mellitus; JDF U, Juvenile Diabetes Foundation units; IDW, Immunology of Diabetes Workshop.

ICAs, first described in 1974 (1), are measured in numerous laboratories worldwide, primarily in studies of the etiology and pathogenesis of diabetes but also lately as markers for identifying individuals at risk for developing IDDM (2–6). Other possible uses of ICA measurements include the classification of IDDM in unusual presentations (7) and monitoring the progress of patients on various therapeutic regimens (8–10). The importance of ICA measurements has prompted four international IDW serum exchange workshops, in an attempt to improve the comparability of assays worldwide. After the first workshop showed wide variations in results (11), standard curves constructed from a reference serum (JDF standard) were used, and improved concordance was seen between laboratories in the second workshop (12,13). The third workshop indicated that the interlaboratory variation decreased when participating laboratories had access to the JDF standard (14,15). However, too few control sera were used in the third workshop to determine the specificity (negativity in health) of assays.

The goal of the fourth workshop, therefore, was to determine the specificity of ICA assays and to determine the ability of assays to distinguish between control sera and disease-related sera from relatives of IDDM patients, healthy individuals who later IDDM diabetes, and newly diagnosed IDDM patients. Each laboratory was given the JDF standard serum and asked to report their results in JDF U on a total of 109 coded serum samples.

## RESEARCH DESIGN AND METHODS

**Serum exchange.** Sera from 57 healthy, nondiabetic control subjects, 21 first-degree relatives of IDDM patients, 8 healthy individuals who later developed IDDM, and 23 newly diagnosed IDDM patients, were lyophilized in the laboratory of N. Maclaren (University of Florida at

TABLE 1
Results of immunofluoresence or peroxidase staining of human, monkey, rat, and baboon pancreases

| Laboratory | Stain | Pancreas type | Incubation time (min)* | Specificity (%) |
|---|---|---|---|---|
| 1 | I | H | 30 | 66 |
| 2 | I | H | 30 | 79 |
| 4 | I | H | 30 | 88 |
| 5 | I | H | 18–24 H* | 100 |
| 6 | I | H | 30 | 91 |
| 7 | I | H | 30 | 91 |
| 8 | I | H | 75 | 84 |
| 9 | P | R | 30 | 74 |
| 10 | I | H | 30 | 96 |
| 11 | I | M | 30 | 100 |
| 12 | I | H | 30 | 96 |
| 13 | I | H | 30 | 98 |
| 14 | P | R | 60 | 89 |
| 15 | I | H | 18–24 H* | 100 |
| 16 | N/A | N/A | N/A | 98 |
| 17 | I | H | 30 | 96 |
| 18 | I | H | 20 | 88 |
| 19 | I | H | 30 | 88 |
| 20 | I | H | 20 | 96 |
| 21 | I | H | 18–24 H* | 98 |
| 22 | I | H | 180 | 100 |
| 23 | I | B | 30 | 65 |
| 24 | I | H | 60 | 88 |
| 25 | P | R | 30 | 100 |
| 26 | P | R | 30 | 27 |
| 27 | I | H | 30 | 98 |
| 28 | I | H | 30 | 100 |
| 29 | I | H | 30 | 96 |

(I), immunofluoresence; (P), peroxidase; (H), human; (R), rat; (M), monkey; (B), baboon.
*Time is expressed in minutes, except for data from laboratories 5, 15, and 21.

Gainesville), coded, and sent to 47 laboratories worldwide. In addition, the JDF standard serum was sent noncoded for use in constructing a standard curve. This serum is arbitrarily set to contain 80 JDF U of ICA.

**Assays.** Complete and analyzed results of immunofluoresence (23) or peroxidase (4) staining of human (22), monkey (1), rat (4), or baboon (1) pancreases were received from 28 laboratories. The time of incubation varied, with 19 laboratories incubating for 20 or 30 min, four incubating for 1–3 h, and four incubating overnight (18–24 h) (Table 1).

**Analysis.** Each laboratory was asked to generate a standard curve (ranging from 0 to 80 JDF U) from the JDF standard serum and to assign JDF U to ICA titers for each individual serum tested. Except for results >80 JDF U, which were considered to be 80 JDF U, each laboratory's assigned JDF U were used in this analysis.

Specificity was defined as the percentage of control sera reported with 0 JDF U. The mean ± SD JDF U in the 57 control sera was computed for each individual laboratory.

It was expected that no group of the disease-related sera would all be positive, and, therefore, no group could be used as positive controls. Nonetheless, it was observed that within each group, concordance occurred among most laboratories as to whether a serum was positive (>0 JDF U) or not. For control sera, the average concordance was 91.3 ± 7.8%. If we required that 83% (mean concordance of controls ± SD) of laboratories be in agreement as to whether a serum sample was positive or negative, then 10 control sera would be termed equivocal. Similarly, if we required that 67% (mean concordance of controls ± 3 SD) of laboratories agree as to whether a serum is positive or negative, then only one control sample would be equivocal. We constructed a table listing how many sera from each group would be termed positive, negative, or equivocal, depending on the percentage of concordance required (Table 2). Using these data, we selected the more stringent criteria and used 3 SD to define sera in which ≥67% of laboratories were in agreement as positive or negative. Sera in which less concordance occurred between laboratories were termed equivocal.

**Statistical analysis.** Results were expressed as mean ± SD. Comparisons between groups were assessed with the nonparametric Mann-Whitney *U* test. Significance was accepted at 0.05.

## RESULTS

**Control sera (*n* = 57).** The mean specificity (percentage of control sera reported as 0 JDF U) among 27 laboratories was 91%, including 6 laboratories with 100% specificity. Three of the four laboratories that incubated overnight had 100% specificity (Table 1). One laboratory (no. 26) with poor specificity (27%) was excluded from further analysis. The specificity of laboratories did not

TABLE 2
Sera defined as positive, negative, or equivocal from each group depending on concordance required among laboratories

| | Positive sera | | | | Negative sera | | | | Equivocal sera | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | I (%) | R (%) | P (%) | C | I (%) | R (%) | P (%) | C (%) | I (%) | R (%) | P (%) | C |
| Concordance required (%)* | | | | | | | | | | | | |
| 83 | 6 (26) | 1 (5) | 5 (63) | 0 | 3 (13) | 13 (62) | 2 (25) | 47 (82) | 14 (61) | 7 (33) | 1 (13) | 10 (18) |
| 75 | 11 (48) | 2 (10) | 5 (63) | 0 | 8 (35) | 15 (71) | 3 (38) | 55 (96) | 4 (17) | 4 (19) | 0 | 2 (4) |
| 67 | 11 (48) | 3 (14) | 5 (63) | 0 | 9 (39) | 16 (76) | 3 (38) | 56 (98) | 3 (13) | 2 (10) | 0 | 1 (2) |
| 52 | 11 (48) | 5 (24) | 5 (63) | 0 | 12 (52) | 16 (76) | 3 (38) | 57 (100) | 0 | 0 | 0 | 0 |

Values are *n* (%). (I), IDDM patients; (R), first-degree relatives; (P), healthy individuals who later developed IDDM; (C), control subjects.
Percentage of concordance among laboratories for control sera mean ± 1 SD = 83%; mean ± 2 SD = 75%; mean ± 3 SD = 67%; and mean ± 5 SD = 52%.

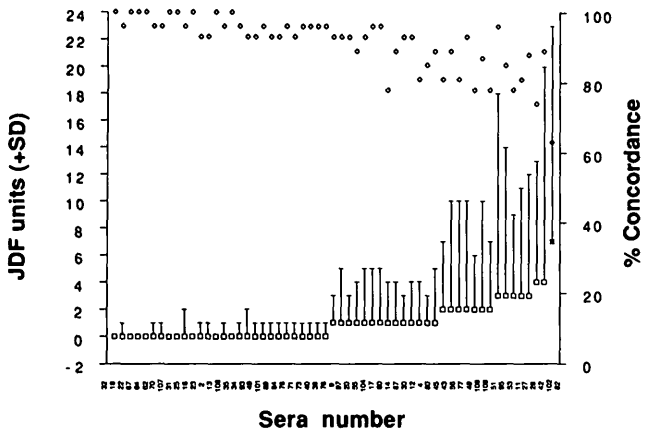


FIG. 1. Individual control sera ($n$ = 57) from 27 laboratories—mean ± SD JDF U and percentage of concordance. (□), negative sera; (*), equivocal sera; (◇), percentage of concordance.

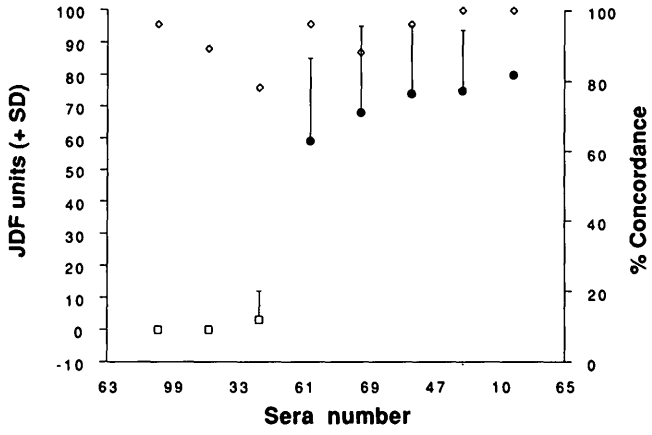


FIG. 3. Sera from 8 healthy individuals who later developed clinical IDDM from 27 laboratories—mean ± SD JDF U. (□), negative sera; (*), equivocal sera; (●), positive sera; (◇), percentage of concordance.

correlate with type of pancreas used, incubation time, or method of staining (Table 1).

Of control sera, 98% (56/57) were found negative by at least 74% of laboratories. Of the serum samples (no. 13, 16, 22, 31, 32, 35, 64, 67, and 107), 9 (16%) were reported negative by all laboratories. Only one serum sample (no. 82) with 7 ± 16 JDF U was found negative by <67% of laboratories (63%), and, therefore, was termed equivocal (Fig. 1).

**First-degree relatives of IDDM patients ($n$ = 21).** Of the sera from first-degree relatives of IDDM patients, 16 of 21 were negative (>67% of laboratories reported 0 JDF U) (Fig. 2). Three of these samples (no. 52, 54, 7) were reported negative by all laboratories. Two (no. 105, 86) were equivocal with 56 and 63% of laboratories reporting them to be >0 JDF U.

**Healthy individuals who later developed clinical IDDM ($n$ = 8).** Three sera (no. 63, 99, 33) were negative, that is, were reported with 0 JDF U by 96, 89, and 78% of laboratories, respectively (Fig. 3). Five sera (no. 61, 69, 47, 10, 65) were positive (>67% of laboratories reporting >0 JDF U) and of these, two sera (no. 10, 65) were found to be positive (75 ± 19 and 80 ± 0 JDF U, respectively) by all laboratories.

**Newly diagnosed IDDM patients ($n$ = 23).** Eleven sera from newly diagnosed IDDM patients were positive (>67% of laboratories reporting >0 JDF U). Three of these sera (no. 68, 83, 85) were reported to have >0 JDF U (73 ± 21, 71 ± 18, and 68 ± 23 JDF U, respectively) by all assays. Only one sample (no. 8) was reported negative (0 JDF U) by all laboratories, and three (no. 103, 57, 74) were equivocal with 46, 45, and 42% of laboratories reporting >0 JDF U (Fig. 4).

**Combined positive sera.** Three sera from first-degree relatives, 5 from healthy individuals who later developed IDDM, and 11 from newly diagnosed IDDM patients, were identified as positive (>67% of laboratories reporting >0 JDF U). In all laboratories (100%), the mean JDF U for these positive sera in each group were significantly greater than the mean JDF U of the 57 controls reported from each individual laboratory (Fig. 5).

## DISCUSSION

The aim of the Fourth International IDW Serum Exchange Workshop was to determine the specificity (negativity in

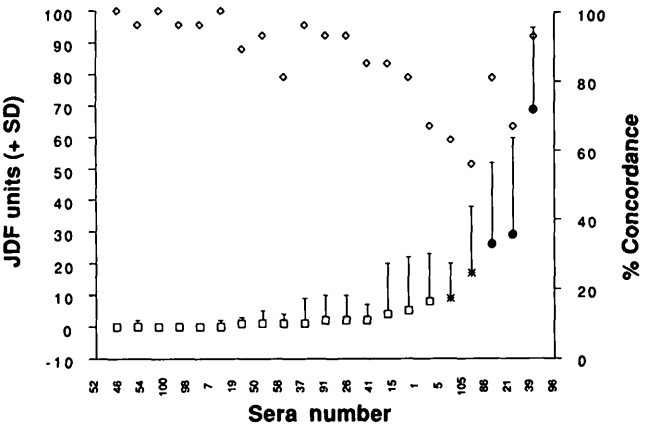


FIG. 2. Sera from 21 first-degree relatives of IDDM patients from 27 laboratories—mean ± SD JDF U. (□), negative sera; (*), equivocal sera; (●), positive sera; (◇), percentage of concordance.

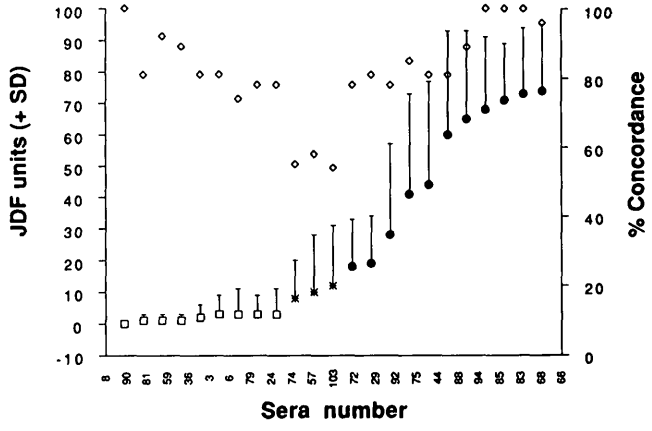


FIG. 4. Sera from 23 newly diagnosed IDDM patients from 27 laboratories—mean ± SD JDF U. (□), negative sera; (*), equivocal sera; (●), positive sera; (◇), percentage of concordance.

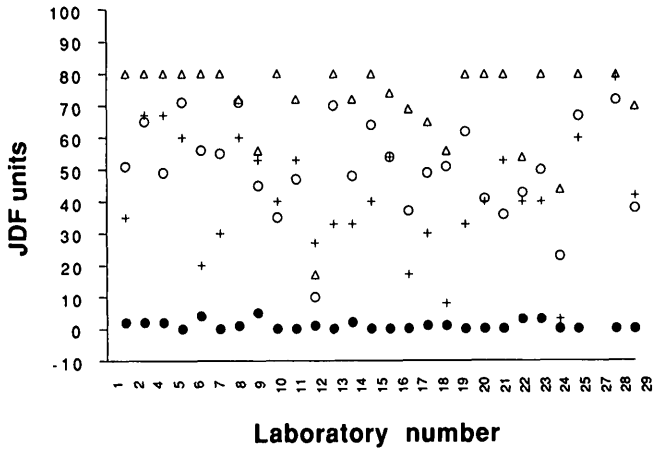


**FIG. 5. Negative sera from control subjects (●, *n* = 56) and positive sera from first-degree relatives of IDDM patients (+, *n* = 3), newly diagnosed IDDM patients (○, *n* = 11), and healthy individuals who later developed clinical IDDM (△, *n* = 5), in each laboratory—mean JDF U.**

health) of various assays and to test the ability of the laboratories to distinguish control sera from IDDM-relevant sera (that is, sera from first-degree relatives of IDDM patients, healthy relatives who later developed IDDM, and patients with newly diagnosed IDDM). The workshop was designed to allow each laboratory to use the JDF standard serum to generate their results in JDF U. The JDF U reported by each of the 28 laboratories for the 109 samples were used in this analysis.

In this workshop, laboratories were instructed to use the JDF standard serum, which had been assigned 80 JDF U, to construct their own standard curve. However, because several laboratories reported titers >80 JDF U (data not shown), some samples (no. 65, 66, 10) were identified as containing higher titers of ICA. One of these sera could, for example, be used as a high titer JDF standard in future workshops to compare results among laboratories over a large titer range. This may be particularly important in light of studies that suggest that the higher the titer, the greater the likelihood of IDDM development among selected first-degree relatives (16).

These results show that specificity (91%) was excellent among laboratories that participated in this workshop. The exception was one laboratory (no. 26) with a specificity of 27% that was excluded from further analysis. Also, no significant correlation was noted between time of incubation and specificity, although it was observed that three of four laboratories that incubated sera overnight had 100% specificity.

Currently, much IDDM research focuses on the ability of ICA to predict the onset of clinical IDDM. Prediction of IDDM may improve our understanding of the etiology and pathogenesis of IDDM and also may enable us to test the efficacy of therapeutic intervention to prevent progression of disease. The use of ICA as a predictive marker of IDDM requires concordance between laboratories and the ability to distinguish healthy control sera from disease-relevant sera. Prospective studies have demonstrated a high predictive value of ICA in certain situations, that is, specific genetic groups (relatives of IDDM patients), JDF titer >20 U, and association with abnormal β-cell function or high titers of insulin autoantibodies (1–4). The use of ICA as a predictive marker of IDDM outside of these situations remains to be studied.

In this workshop, the ability of all of the laboratories to find nearly all of the controls negative was excellent. Nevertheless, 78% of laboratories found at least one control sample positive. If it is assumed that only one serum (no. 82) is equivocal, 56 control sera are left. The specificity of these 56 sera would vary from 100% (all negative) in eight laboratories to 67 and 66% in laboratories no. 1 and 23.

This workshop also demonstrated that the JDF U for control sera as a group were significantly different from the positive sera in the disease-relevant groups in all laboratories. Nonetheless, these data must be interpreted with the understanding that sera analyzed in this workshop may not be representative of individuals in these groups.

In conclusion, the Fourth International IDW ICA Serum Exchange Workshop demonstrated that good specificity among control sera was seen among participating laboratories. In addition, all of the laboratories could distinguish controls from positive sera among groups newly diagnosed IDDM patients, healthy individuals who later developed IDDM, and first-degree relatives of IDDM patients.

## ACKNOWLEDGMENTS

This workshop study was supported by the Juvenile Diabetes Foundation International.

## APPENDIX: PARTICIPATING LABORATORIES

A. Arnaiz Villena (Madrid, Spain), J. Barbosa (Minneapolis, MN), C. deBeaufort (Luxembourg), D. Becker (Pittsburgh, PA), F. Becker (Giessen, Germany), C. Betterle (Padova, Italy), E. Bosi (Milan, Italy), G.F. Bottazzo (London, UK), G. Bright (Jacksonville, FL), P. Colman (Melbourne, Australia), R.L. Dawkins (Perth, Australia), R.B. Elliot (Auckland, New Zealand), Y. Feng (Shanghai, China), F. Gorus (Brussells, Belgium), C. Howard (Beaverton, OR), R. Jackson (Boston, MA), J. Karjalainen (Oulu, Finland), T. Kobayashi (Tokyo, Japan), M. Koelle (Buffalo, NY), H. Kuzuya (Kyoto, Japan), J. Kwan (Toronto, Canada), C. Levy Marchal (Paris, France), U. di Mario (Rome, Italy), R.C. McEvoy (New York, NY), K.M. Reinauer (Tubingen, Germany), W. Scherbaum (Ulm, Germany), G.J.P. Singh (Newport Beach, CA), C. Thovolet (Lyon, France), B. Vialettes (Marseilles, France), and A. Ziegler (Munich, Germany).

## REFERENCES

1. Bottazzo GF, Lorin-Christensen A, Doniach D: Islet cell antibodies in diabetes mellitus with autoimmune polyendocrine deficiencies. *Lancet* 2:1279–83, 1974
2. Tarn AC, Thomas JM, Dean DM, Ingram D, Schwarz G, Bottazzo GF, Gale EAM: Predicting insulin-dependent diabetes. *Lancet* 1:845–50, 1988
3. Srikanta S, Ganda OP, Tabizadeh A, Soeldner SJ, Eisenbarth GS: First-degree relatives of patients with type I diabetes mellitus: islet-cell antibodies and abnormal insulin secretion. *N Engl J Med* 313:461–64, 1985
4. Riley WJ, Maclaren NK, Krischer J, Spillar RP, Silverstein JH, Schatz

DA, Schwartz S, Malone J, Shah S, Vadheim C: A prospective study of the development of diabetes in relatives of patients with insulin-dependent diabetes. *New Engl J Med* 323:1167–72, 1990
5. Karjalainen JK: Islet cell antibodies as predictive markers for IDDM in children with high background incidence of disease. *Diabetes* 39:1144–50, 1990
6. McCulloch DK, Klaff LJ, Kahn SE, Schoenfeld SL, Greenbaum CJ, Mauseth RS, Benson EA, Nepom GT, Shewey L, Palmer JP: Nonprogression of subclinical β-cell dysfunction among first-degree relatives of IDDM patients: 5-yr follow-up of Seattle Family Study. *Diabetes* 39:549–56, 1990
7. Groop LC, Bottazzo GF, Doniach D: Islet cell antibodies identify latent type I diabetes in patients aged 35–75 yr at diagnosis. *Diabetes* 35:237–41, 1986
8. Mandrup-Poulsen T, Nerup J, Stiller CR, Marner B, Bille G, Heinrichs D, Martell R, Dupre J, Keown PA, Jenner MR, Rodger NEW, Wolfe B, Graffenried BC, Binder C: Disappearance and reappearance of islet cell cytoplasmic antibodies in cyclosporin-treated insulin-dependent diabetics. *Lancet* 1:499–506, 1985
9. Boitard C, Feutren G, Castano L, Debray-Sachs M, Aisan R, Hors J, Bach JF: Effect of cyclosporin A treatment on the production of antibody in insulin-dependent (type I) diabetic patients. *J Clin Invest* 80:1607–12, 1987
10. Mandrup-Poulsen T, Mulvig J, Andersen HV, Helqvist S, Spinas GA, Munck M, and the Canadian-European Randomized Control Trial Group: Lack of predictive value of islet cell antibodies, insulin antibodies, and HLA-DR phenotype for remission in cyclosporin-treated IDDM patients. *Diabetes* 39:204–10, 1990
11. Bottazzo GF, Gleichmann H: Immunology and Diabetes Workshops: report of the First International Workshop on the standardization of cytoplasmic islet cell antibodies. *Diabetologia* 29:125–26, 1986
12. Bonifacio E, Dawkins RL, Lernmark E: Immunology and Diabetes Workshops: Report of the Second International Workshop on the standardization of cytoplasmic islet cell antibodies. *Diabetologia* 30:273, 1987
13. Bonifacio E, Lernmark E, Dawkins RL: Serum exchange and use of dilutions have improved precision of measurement of islet cell antibodies. *J Immunol Methods* 106:83–88, 1988
14. Boitard C, Bonifacio E, Bottazzo GF, Gleichmann H, Molenaar J: Immunology and Diabetes Workshop: Report on the Third International Workshop on the standardization of cytoplasmic islet cell antibodies. *Diabetologia* 31:451–52, 1988
15. Bonifacio E, Boitard C, Gleichmann H, Shattock MA, Molenaar JL, Bottazzo GF: Assessment of precision, concordance, specificity, and sensitivity of islet cell antibody measurement in 41 assays. *Diabetologia* 33:731–36, 1990
16. Bonifacio E, Bingley PJ, Shattock M, Dean BM, Dunger D, Gale EA, Bottazzo GF: Quantification of islet-cell antibodies and prediction of insulin-dependent diabetes. *Lancet* 335:147–49, 1990