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The World Health Organization International Collaborative Study for Islet Cell Antibodies

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Abstract

Aims/hypothesis. Islet cell autoantibodies are a specific marker for Type 1 (insulin-dependent) diabetes mellitus. Standardisation of islet cell antibodies and the uniform reporting in International units is critical to research and the development of assays for islet cell autoantibodies as diagnostics.
Methods. The suitability of a candidate serum to serve as the international standard for islet cell antibodies was studied by 19 participants in 8 countries. In addition, the purpose was to investigate whether the serum could also serve as a standard for antibodies to the 65 000 M_r isoform of glutamic acid decarboxylase (GAD65) and islet antigen-2 (IA-2). Control sera were included in the study to assess the validity of the various assay systems. The sera were lyophilized to World Health Organization criteria and the candidate serum assigned the ampoule code number 97/550.
Results. The use of 97/550 was shown to notably reduce inter laboratory variability in the measurement of islet cell antibodies. In addition, there was a pronounced reduction in inter laboratory variability in the measurement of GAD65 and IA-2 antibodies.
Conclusions/interpretation. On the basis of the results reported here and with agreement of the participants, the preparation 97/550 has been established by the World Health Organization Expert Committee on Biological Standards for establishment as the first international standard for islet cell antibodies, with an assigned potency of 20 international units. In addition, 97/550 can serve as an international reference reagent for specific GAD65 antibodies, with an assigned potency of 100 units. It can also serve as a National Institute of Biological Standards and Control (NIBSC) reference reagent for IA-2 antibodies for evaluation of assays for this material. [Diabetologia (2000) 43: 1282–1292]

Keywords Standards, ICA, GAD65, IA-2, diabetes, diagnostics, autoantibodies, islet cells.

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Abbreviations: ICA, islet cell antibodies; JDF, juvenile diabetes foundation; IDW, immunology of diabetes workshops; IA-2, islet antigen-2; WHO, World Health Organization; NIBSC, National Institute of Biological Standards and Control; ECBS, expert committee for biological standadization; IDS, Immunology of Diabetes Society; IS, international standard; GCV, geometric coefficient of variation; GM, geometric mean.

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It has been known for two decades that patients with Type 1 (insulin-dependent) diabetes mellitus develop autoantibodies to pancreatic islet cell antigens [1–3] often many years before the clinical onset of the disease. On diagnosis of Type 1 diabetes there is little that can be done to cure the disease, as the autoimmune damage to the pancreas has already occurred. Screening of islet cell antibodies (ICA) in people related to Type 1 patients has proved a valuable tool in predicting the onset of the disease [4–7] in attempts to develop measures to prevent islet damage.

The appropriate detection of ICA has been the subject of many years work supported by the Juvenile

Table 1. Materials included in the study

Assigned ampoule code	Ampoule code in study	Residual moisture content[a]	Filling variation[b]	Nominal antibody content of ampoule[c]
97/550	A and C	1.01 %	0.44 %	20–40 JDF Units ICA
ICA Low	B	0.713 %	0.35 %	GAD65ab at 1:25 titre 0
ICA High	D	0.649 %	0.38 %	GAD65ab at 1:25 titre 1.0
ICA Medium	E	0.820 %	0.41 %	GAD65ab at 1:25 titre 0.4

[a] Residual moisture as measured by the Karl-Fisher method expressed as a percentage of dry weight of ampoule content [21].

[b] The coefficient of variation of filling the ampoules is defined as: CV = standard deviation ÷ mean of liquid mass before lyophilization [19].

[c] Measured as described in [22]

Diabetes Foundation (JDF) and organised by several international immunology of diabetes workshops (IDW) [8–11]. The IDW and later the Immunology of Diabetes Society (IDS) carry out serum exchanges to standardize ICA and a proficiency testing programme to monitor assay performance in different laboratories [12]. The current method of detection of ICA is using the immunohistochemical assay by indirect immunofluorescence binding on human pancreatic tissue sections.

It has been shown from these studies that it is vital that a reference serum calibrated in arbitrary units be included if estimates of ICA concentrations are to be comparable and hence of value both within and between laboratories [13]. One batch of serum has been shown to be the most reliable calibrator and has been assigned JDF units [13–15]. The standard is used increasingly to screen subjects at risk for Type 1 diabetes to participate in immune intervention trials [16]. This material is used worldwide to standardize the ICA assay [15] as well as assays for autoantibodies to the 65 000 M_r isoform of glutamic acid decarboxylase (GAD65) [12, 17, 18] and islet antigen-2 (IA-2) [12] (subclasses within ICA) for diagnostic and prognostic purposes. The use of such assays as diagnostics will most likely increase over the next few years and therefore, it is imperative that correctly standardized ICA, GAD65 and IA-2 antibody values are obtained.

The material currently used as the JDF standard [13, 15] is of great value but it is vital that the serum is ampouled in the most stable form and distributed worldwide in a controlled manner to appropriate laboratories. It is proposed that after the completion of this study, and on the agreement of the WHO Expert Committee for Biological Standardization (ECBS), 3500 ampoules of the remaining ICA standard serum having been prepared to WHO guidelines at the National Institute of Biological Standards and Control (NIBSC) and subject to a WHO International Collaborative Study, will be distributed worldwide by NIBSC.

In agreement with the workshops of the IDS, the WHO Standardization Programme has, through many extensive and well-reported international collaborative studies, shown that the adoption of a single international standard (IS) with an appropriate international unitage, results in a greatly reduced variability between potency estimates for biological preparations produced or assayed in different laboratories. Thus, the adoption of a single IS for ICA as well as for GAD65 and IA-2 antibodies is a desirable goal.

Materials and methods

Participants of the study. The WHO international collaborative studies are intended to represent an international geographical range and should include all types of laboratories that represent the users of the materials to be tested. In the case of ICA, a range of research and hospital laboratories routinely test for ICA and this was reflected in the choice of participants. A total of 19 participants in 8 countries were recruited to study the suitability of the JDF serum to serve as the international standard for islet cell antibodies. Throughout this report each participant has been identified only by a code number assigned at random and not related to the order of listing under the heading ‘Participants of the study’ at the end of the paper. Where the same participant has used different assay systems of the same broad type, e. g. two different GAD antigens, these have been further denoted by upper case letters A, B and so on and described as ‘assay information’ in the tables.

Materials included in the study. The preparations included in the study are listed in Table 1. They were lyophilised into glass ampoules in 400 µl aliquots containing 100 µl serum, 300 µl sterile distilled water and 0.4 mg trehalose. Lyophilisation was carried out at NIBSC and followed WHO guidelines [19]. The coefficient of variation of filling and the residual moisture content of the ampoules is indicated in Table 1. Several laboratories were initially requested to test the biological potency of the lyophilised preparations in comparison with the starting material, and confirmed that no gross loss of activity had taken place.

Stability of materials. Ampoules of 97/550 which had been stored at increased temperatures for more than 1 year were compared with ampoules of 97/550 stored continuously at –20 °C in ICA, GAD65 and IA-2 antibody assays. Samples stored at 4 °C, 20 °C or 37 °C did not show a detectable loss of activity and did not differ from one another. Samples stored at 56 °C showed a loss of activity which could also reflect greater difficulty in reconstitution after extended storage at this temperature.

Table 2. List of assays contributed to the study

Laboratory Code	Antigen of antibody measured	Type of assay
1	ICA	Immunofluorescence
	GAD	RIA
	IA2	RIA
2	GAD	RIA
	IA2	RIA
3	ICA	Immunofluorescence
	GAD	RIA
	IA2	RIA
4	IA2	RIA
	GAD	RIA
5	GAD	RIA
	IA2	RIA
6	GAD	RIA
	IA2	RIA
7	GAD (Antigen 1)	RIA
	GAD (Antigen 2)	RIA
	GAD (Antigen 3)	RIA
	ICA	Immunofluorescence
	IA2	RIA
8	GAD/IA2	RIA
	GAD	RIA
	IA2	RIA
9	GAD	RIA
	IA2	RIA
10	ICA	Immunofluorescence
	GAD	RIA
	IA2	RIA
11	ICA	Immunofluorescence
12	GAD	RIA
	GAD (New)	RIA
	IA2	RIA
	IA2 (New)	RIA
13	ICA	Immunofluorescence
	GAD	RIA
	IA2	RIA
14	ICA	Immunofluorescence
	GAD	RIA
	IA2	RIA
	IA2 beta	RIA
15	GAD	RIA
	IA2	RIA
	IA2 beta	RIA
16	GAD	RIA
	IA2	RIA
17	ICA	Immunofluorescence
	GAD	RIA
18	ICA	Immunofluorescence
	GAD	RIA
	IA2	RIA
	IA2 beta	RIA
19	ICA	Immunofluorescence

Study protocol. Participants were asked to assay the preparations in their own immunohistochemical assays for ICA and, if available, in specific immunoassays. Furthermore, they were requested to include their own in-house standards for these assays. Participants should have included all the preparations in each individual assay if possible.

For each assay method used, participants were asked to carry out at least two independent assays, each including, as far as possible, all of the preparations to be tested. Assays were considered independent if the dilutions of the various materials were from a freshly prepared ampoule or fresh dilutions from the stock solution and the assays carried out on different occasions. Participants were requested to include no less than four dilutions of each preparation in the linear portion of the dose-response curve. Each preparation should have been included at least in duplicate in the individual assays. It was preferable to use a freshly reconstituted ampoule in each assay.

All raw data was supplied by participants (such as scintillation counter read-outs or light microscopic estimates). Direct analysis of the raw data provided by the assays from all participants allows uniform procedures for calculations to be done.

Assays contributed to the study. Details of assay methods are shown in Table 2 as extracted from participants submissions.

Statistical analysis. For immunofluorescence assays for ICA, tests were generally carried out on dilution series of the samples and the result for each dilution in the series was reported as positive (+) or negative (–). The ‘effective end point’ was taken to be the geometric mean of the smallest dilution giving a positive response and the largest dilution giving a negative response. A few participants recorded some responses as ± . In these cases the dilution of sample giving that end point was taken as the ‘effective end point’; the smallest dilution giving a positive response was determined as the value halfway between (on a log scale) the dilutions giving + and ± and largest dilution giving a negative response similarly between the dilutions giving – and ± responses.

For assays giving a quantitative response, dose–response lines were plotted and examined both graphically and using analysis of variance to verify any apparent anomalies and to assess consistency of dose-response relations. Approximately 0.5 % of the total raw responses were omitted as contributing excessively to the between replicate variability or as giving a pronounced discontinuity of the dose-response line. For some preparations a range of the dilutions tested did not show a statistically significant regression of response on dose and response values did not differ significantly from control responses. These dilutions have been omitted from analysis, with consequent omission of the preparation in most cases, although in a few instances where the smallest dilution (largest dose) appeared to give a slight response it has been included in the analysis to give an approximate indication of relative activity.

For the majority of these assays, the dose-response lines could be satisfactorily described using a four-parameter logistic function. The responses, transformed to logits using the fitted asymptotes, have been analysed as parallel line assays using weighted regression and an in-house program (WRANL) [20] to give an assessment of linearity and parallelism and estimates of the relative potency of the various preparations.

Assessment of assay variability and combination of estimates. In our experience, the within assay replication is not always representative of the total assay variation. The collaborative study design thus included two ampoules of one ICA preparation which were identical except for code, namely A and C. The differences between these two identical preparations provided an additional measure against which the differences between non-identical preparations could be assessed. Thus assays which showed apparent differences between two preparations, but in which the difference between the two identical preparations was as large as any other, would not necessarily be taken to indicate differences between preparations.

Table 3. Laboratory weighted geometric mean end-point dilutions obtained for the various ampouled samples and the in-house standard (JDF units except in Lab07) using ICA immunofluorescence

Laboratory code	A	C	Ratio of duplicates C/A	B	D	E	In-house	Assay weight
Lab01	62	54	0.87	6	49	48	256	881
Lab03	23	11	0.50	3	11	23	57	33
Lab07	20	20	1.02	2	12	16	136	42
Lab10	8	8	1.00	1	8	4	32	8
Lab11	2	2	0.89	.	2	2	16	17
Lab13	28	36	1.30	3	16	32	256	67
Lab14	6	14	2.23	7	7	19	28	25
Lab14a[a]	3	4	1.36	2	3	8	10	132
Lab14b[a]	2	3	1.76	2	2	3	6	50
Lab17	11	12	1.09	2	10	10	362	573
Lab18	57	35	0.61	7	17	17	.	33
Lab19	104	98	0.95	.	45	73	.	364
Unweighted geometric mean of Laboratory means (Excluding [a])								
	19	18	0.97	3	13	16	85	
% GCV	231 %	209 %	50 %	105 %	151 %	196 %	228 %	

The assay weight provides an indication of the precision of these estimates. The ratio of the coded duplicate preparations A and C also provides an indication of the accuracy and precision of these assay systems. Estimates from Lab14 a and Lab14 b were measured using serum as diluent, and have been excluded from calculation of means

Estimates of relative potency have been combined as geometric means (GMs) and comparisons among them have been made using analysis of variance of the logs of the estimates. Fiducial intervals about mean estimates have been based on the variance of the logs of the estimates combined. The geometric coefficient of variation (GCV, determined as (s)–1, where s is the standard deviation of the log potency estimates, multiplied by 100 to give per cent) has been used to provide a summary measure of the precision of estimates for rapid comparison.

Results

Immunofluorescence assays. The different laboratories used different dilution factors and a number of participants carried out initial assays with large dilution factors but used smaller dilution factors in subsequent assays. The precision with which the end point is estimated depends on the dilution factor used. For example, an end point determined, using a dilution factor of 2, to lie between 32 and 64 is less precisely determined than an endpoint determined, using a dilution factor of 1.33, to lie between 48 and 64.

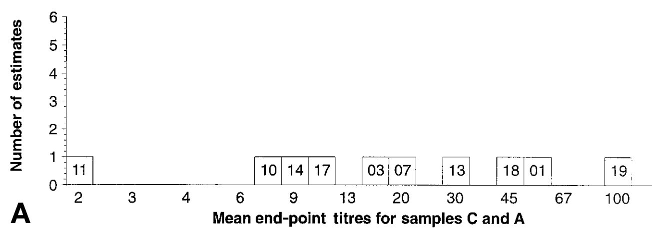
The end points for the participating laboratories have therefore been estimated as weighted geometric means over all assays in the laboratory, with weight proportional to the reciprocal of the log of the dilution factor squared, i. e. the smaller the dilution factor, the larger the weight (Table 3, Figs. 1, 2). The direct comparison of the mean estimates for the coded duplicate preparations A and C also provides a measure of the assay variability and the ratio of these two estimates can also be considered as a dilution factor which cannot be distinguished.

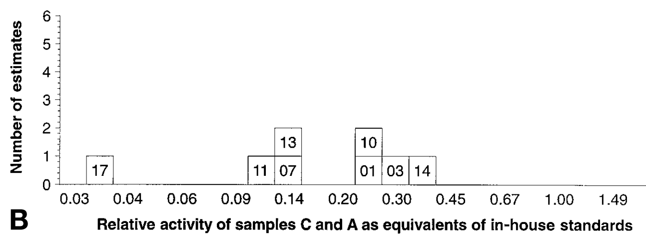
The assay weight given in Table 4 is the minimum of weights determined using the ratio of the duplicate preparations and of the end point estimates for A and C. It indicates the precision of the estimates. For example, a single measurement using a dilution factor of 2 would give an end point with a weight of 33, whereas a single measurement using a dilution factor of 4 would give an end point with a weight of 8.

Estimates of relative potency for the serum samples, given by the immunofluorescence assays and calculated as ratios of the laboratory geometric mean end points for each laboratory, are given in Table 4, in terms of both the in house standard (JDF units) and in terms of the proposed standard 97/550 coded A.

Estimates expressed as end points giving a defined response show the greatest between laboratory variability, as reflected by the magnitude of the GCV (Table 3). Estimates expressed relative to the in-house standard show greater between laboratory agreement than the end point estimates. Estimates relative to the common standard A show between laboratory agreement which is similar to the agreement seen between the duplicate samples in the same laboratory (Table 4). That is, within laboratories estimates for C, a coded duplicate of A, relative to A covered a fourfold range from 0.5 to 2.2 whereas estimates for D relative to A covered a range of less than fourfold from 0.4 to 1.1. Estimates of relative activity for B were more variable than end-point estimates because this sample was consistently found to give a negative response at a small and therefore limited dilution.

Dose-response lines for assays giving quantitative responses. For the majority of these assays the log dose-logit transformed response lines, for preparations giving a significant regression of response on dose, did not deviate consistently from linearity and parallelism (data not shown).





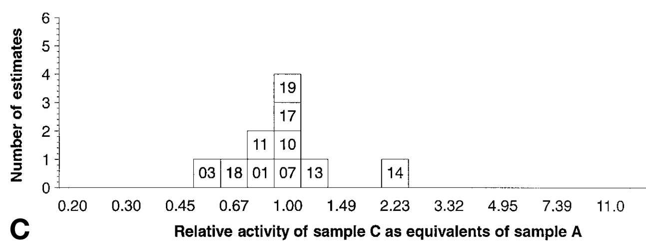
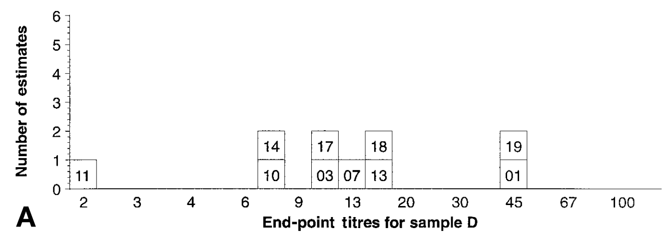
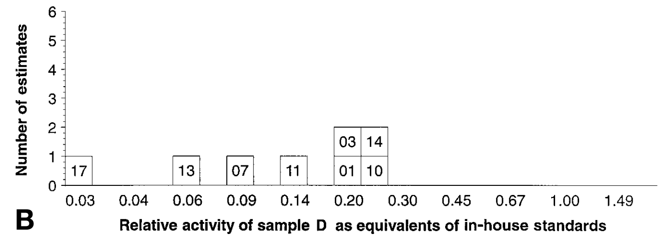


Fig. 1 A–C. Histograms showing (**A**) geometric mean end-point titres for samples C and A, samples identical except for code, (**B**) relative activity of samples C and A expressed as equivalents of the in-house standard and (**C**) relative activity of sample C expressed as equivalents of sample A, for which the expected value is 1. Each square denotes the estimate for one participant. The label in the square is the laboratory code





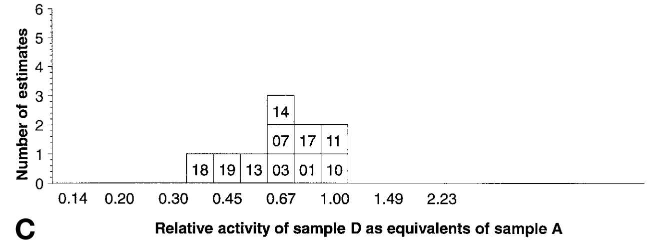


Fig. 2 A–C. Histograms showing (**A**) the geometric end-point titres for sample D, (**B**) the relative activity of sample D expressed as equivalents of the various in-house standards and (**C**) the relative activity of sample D expressed as equivalents of sample A used as a common reference preparation. Each square denotes the estimate for one participant. The label in the square is the laboratory code

In a few cases, consistent differences in slope were noted for one or two preparations, which apparently resulted from testing of these preparations over a different response range from the other preparations. Preparation B did not give a statistically significant regression of response on dose in a number of the GAD antibody assays and was omitted from analysis. In the GAD antibody assays of laboratories 09 and 18 the log dose-logit response lines for preparation E appeared to be less steep than lines for the other preparations although the response range was similar.

Preparation D did not give a statistically significant regression of response on dose in the IA-2 antibody assays of laboratories 07, 09 and 12 and was omitted from analysis and in the assays of laboratories 15 and 18 it appeared to give flatter dose-response lines than the other preparations. Preparation D also gave flatter dose-response lines than the other preparations in the GAD/IA-2 antibody assays of laboratory 08.

Preparation A, and its coded duplicate C, did not give a statistically significant regression of response on dose in the IA-2 antibody assays of laboratories 06 and 16 and these preparations were omitted from further analysis. Preparations B and D did not give a statistically significant regression of response on dose in the IA-2 beta antibody assays and have been omitted.

Assessment of assay variability for assays giving quantitative responses. The inclusion of preparation C, which was identical to preparation A except for code, provides a direct within assay measure of the accuracy and precision of the assay systems. The potency of C relative to A is expected to be 1 apart from imprecision or inaccuracy introduced by the various steps in the reconstitution and assay proce-

Table 4. Laboratory estimates of potency using immunofluorescence for ICA

Laboratory Codes	C/A	A/IH	B/A	B/IH	D/A	D/IH	E/A	E/IH
Lab01	0.87	0.23	0.10	0.02	0.85	0.19	0.84	0.19
Lab03	0.50	0.28	0.18	0.05	0.71	0.20	1.41	0.40
Lab07	1.02	0.15	0.10	0.01	0.62	0.09	0.78	0.12
Lab10	1.00	0.25	0.13	0.03	1.00	0.25	0.50	0.13
Lab11	0.89	0.12	.	.	1.06	0.13	1.06	0.13
Lab13	1.30	0.12	0.11	0.01	0.51	0.06	1.02	0.13
Lab14	2.23	0.34	0.70	0.24	0.71	0.25	1.92	0.66
Lab14a[a]	1.36	0.37	0.44	0.17	0.67	0.25	2.04	0.76
Lab14b[a]	1.76	0.41	1.09	0.44	0.86	0.35	1.51	0.61
Lab17	1.09	0.03	0.13	0.0 0	0.88	0.03	0.89	0.03
Lab18	0.61	.	0.16	.	0.39	.	0.39	.
Lab19	0.95	.	.	.	0.45	.	0.72	.
Unweighted geometric mean values of laboratory estimates ([a] excluding)								
	0.97	0.16	0.15	0.03	0.68	0.12	0.87	0.15
% GCV	50 %	112 %	92 %	253 %	40 %	115 %	59 %	154 %

The potency for ICA was estimated as the ratio of laboratory geometric mean end-point dilutions, relative to the geometric mean of A and C for ampouled preparations B (denoted BA), D (denoted DA) and E (denoted EA) and relative to the in-house standard (JDF units except in Lab07) for the mean of A and C (denoted AIH), B (denoted BIH), D (denoted DIH) and E (denoted EIH). The relative potency of the coded duplicate preparation C in terms of A (C/A) is also shown for comparison. Estimates from Lab14 a and Lab14 b were obtained using serum as diluent and have been excluded from calculation of means

Table 5. Laboratory estimates of potency using GAD antibodies

Laboratory code and assay information		C	D	E	B	IHS1
01	A	1.04	4.26	0.20	0.010	15.99
02	A	0.90	6.37	0.28	0.076	.
03	A	1.02	5.67	0.23	0.049	7.91
04	A	1.01	3.76	0.19	0.014	15.58
05	A	1.06	4.04	0.31	0.070	4.28
06	A	1.12	3.05	0.16	.	0.08
07	A	1.02	2.54	0.16	0.009	1.41
07	B	0.80	3.01	0.16	0.028	46.32
07	C	0.84	3.15	0.17	0.038	42.48
08	A	1.05	7.80	0.45	0.288	8.31
09	A	0.94	9.64	0.17	.	.
10	A	0.97	1.65	0.65	0.582	0.03
12	A	0.99	2.40	0.12	0.017	24.38
12	B	1.02	3.37	0.16	.	19.15
13	A	1.00	4.26	0.24	.	2.62
14	A	1.01	3.29	0.30	.	.
15	A	1.00	4.96	0.24	.	2.69
16	A	0.98	3.20	0.22	0.054	0.08
17	A	0.91	4.73	0.23	.	451.9
18	A	0.97	4.42	0.19	.	.
Unweighted geometric mean of laboratory means						
		0.98	3.94	0.22	0.04	
GCV		8 %	51 %	48 %	259 %	

GAD antibody assay laboratory geometric mean estimates of the potency of the ampouled preparations coded B, C, D and E relative to the ampouled preparation coded A are shown followed by the preparation used as standard and of the various in-house reference preparations (ISH1). The data is expressed as number of ampoules of A equivalent in activity to 1 ampoule of the ampouled preparations or to the various units of the in-house preparations. Where the same participant has used different assay systems of the same broad type, eg. two different GAD antigens, these have been further denoted by upper case letters A, B, and so on and described as 'assay information' in the table

Table 6. Laboratory estimates using radioimmunoassay for IA2 antibodies

Laboratory code and assay information		C	D	E	B	IHS1
01	A	1.05	0.05	0.23	0 .082	12.72
02	A	0.89	0.22	0.43	1.879	.
03	A	1.04	0.01	0.25	0.203	3.33
04	A	0.94	0.06	0.17	0.110	12.86
05	A	0.96	0.15	0.31	0.175	2.56
06	A [a]D	.	*	.	.	0.19
07	A	0.70	0.02	0.13	0.042	1.41
08	A	0.81	0.01	0.22	0.147	4.78
09	A	1.10	.	0.19	0.124	.
10	A	1.01	0.61	0.51	0.440	0.02
12	A	1.00	.	0.53	1.965	12.57
12	B	0.93	0.05	0.32	0.103	1.65
13	A	0.95	0.06	0.24	0.116	0.83
14	A	0.94	0.11	0.25	0.146	.
15	A	1.04	0.05	0.20	0.061	2.04
16	A [a]B	.	5.67	0.74	*	1.13
18	A	0.94	0.05	0.22	0.040	.
Unweighted geometric mean of laboratory means (excluding [a])						
		0.95	0.05	0.26	0.16	
GCV		12 %	277 %	48 %	221 %	

IA-2 antibody laboratory geometric mean estimates of the potency of the ampouled preparations coded B, C, D and E relative to the ampouled preparation coded A, except in assays marked *, followed by the preparation used as standard and of the various in-house reference preparations (ISH1). The data is expressed as number of ampoules of A equivalent in activity to 1 ampoule of the ampouled preparations or to the various units of the in house preparations. Where the same participant has used different assay systems of the same broad type, e. g. two different IA-2 antigens, these have been further denoted by upper case letters A or B and described as 'assay information' in the table

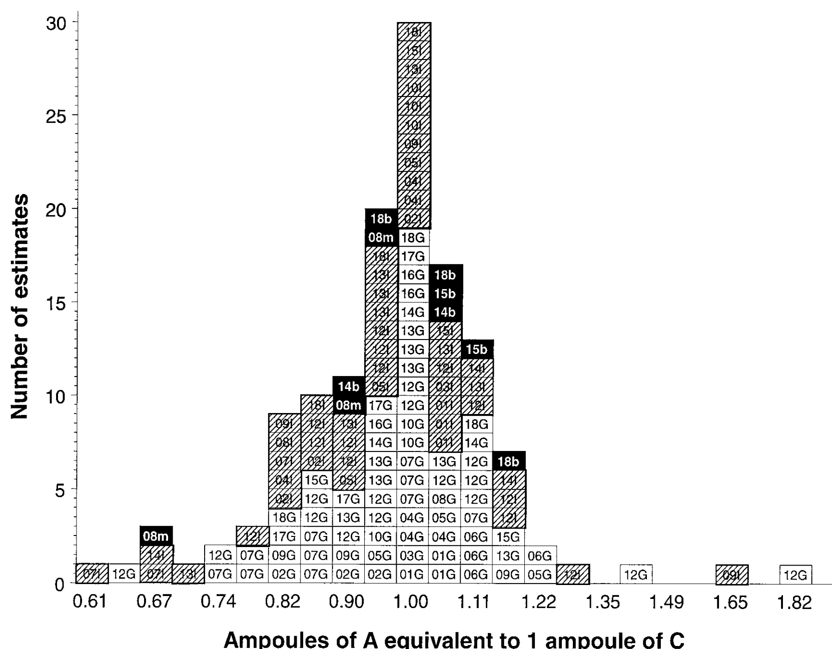


Fig. 3. Histogram showing estimates of the activity of preparation C expressed as equivalent ampoules of preparation A, identical to C except for code. Each square represents an estimate from an individual assay. The label in the square denotes the laboratory code followed by I to indicate IA-2 antibody assays, G to indicate GAD antibody assays, b to indicate IA-2beta antibody assays or m to indicate the IA-2/GAD antibody combined assay. The shading also indicates the assay type. As can be seen from the horizontal scale, estimates cover approximately a threefold range irrespective of assay type. As expected for two identical preparations, the estimates from each assay type centre about the value of 1, confirming the equivalence of the two preparations

Table 7. Laboratory estimates, using radioimmunoassays for IA-2beta and one assay for mixed GAD/IA-2 antibodies

Laboratory code and assay information		C	D	E	B	IHS1
14	beta	0.97	.	0.06	.	.
15	beta	1.08	.	0.05	.	0.84
18	beta	1.06	.	0.04	.	.
Unweighted geometric mean of laboratory means						
		1.04	.	0.05		
GCV		6%		20%		
08	IA2/GAD	0.83	0.81	0.21	0.096	9.15

Laboratory geometric mean estimates of the potency of the ampouled preparations coded B, C, D and E relative to the ampouled preparation coded A, except in assays marked *, followed by the preparation used as standard and of the various in-house reference preparations (IHS-1). The data is expressed as number of ampoules of A equivalent in activity to 1 ampoule of the ampouled preparations or to the various units of the in house preparations

dures. The observed potency is similar to its expected value of 1 both within each method separately, and in all laboratories (Tables 5, 6 and 7), with an overall mean of laboratory means (95% limits) of 0.97 (0.94 to 1.00) (Fig. 3).

This indicates that the assay systems are performing accurately to the extent that identical materials are measured to give 'identical' activities. The within assay variability based on the deviation from its expected value of 1 of the potency of C relative to A gives a geometric coefficient of variation (GCV) of 17%. That is, 70% of comparisons of two identical preparations within an assay should give an observed potency within the range 1/1.17 (= 0.85) and 1.17.

Variability between assays within the same laboratory and variability between laboratories were similar in magnitude to the within assay variability. That is, for identical preparations, these assay systems appear to be both accurate and to have a precision of the order of magnitude commonly seen for such systems.

Comparison of the ampouled samples, coded B, D and E, with 97/550 coded A by GAD antibody assays. Estimates of the activity for the ampouled samples are summarized in Table 5 and individual estimates for each assay are given in Figures 4–6.

Sample B was found to have a low, or not detectable, concentration of GAD antibody in the majority of these assay systems, with the exception of laboratories 10 and 08. All laboratories found sample D to contain the greatest concentration of GAD antibody, although the ratios of GAD antibody activity to the activity of the proposed standard coded A varied by more than fivefold, from 1.65 to 9.64 equivalents of A, between laboratories. Sample E was found by all laboratories to have GAD antibody activity less than

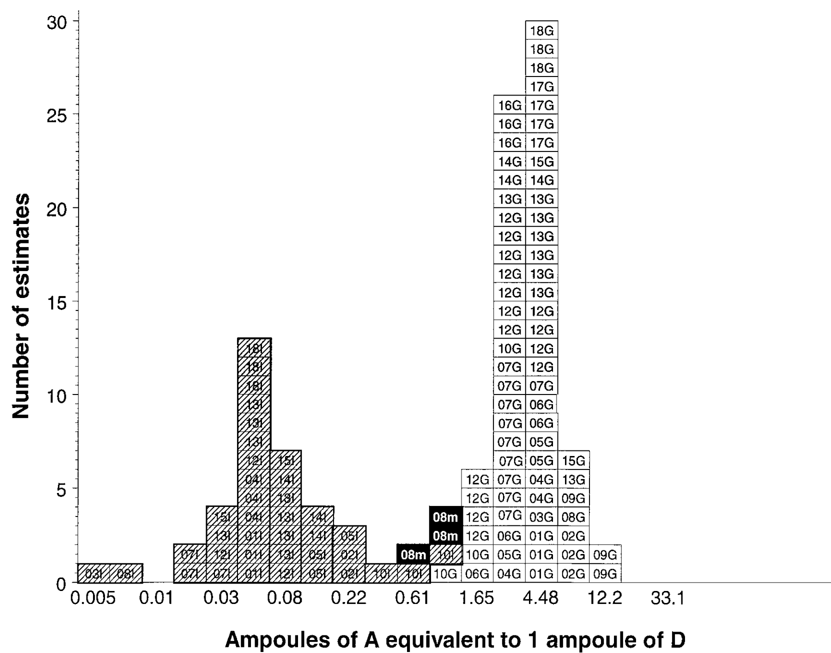


Fig. 4. Histogram showing estimates of the activity of preparation D expressed as equivalent ampoules of preparation A. Each square represents an estimate from an individual assay. The label in the square denotes the laboratory code followed by I to indicate IA-2 antibody assays, G to indicate GAD antibody assays, b to indicate IA-2beta antibody assays or m to indicate the IA2/GAD antibody combined assay. The shading also indicates the assay type. As can be seen from the horizontal scale, estimates for GAD or IA-2 antibody assays cover approximately a tenfold range. Preparations D and A are clearly dissimilar, i. e. contain different ratios of GAD and IA-2 antibodies and are distinguished by the two different assay systems

that of the proposed standard A, although the ratio of activity to that of A varied between laboratories from 0.12 to 0.65 equivalents of A.

Direct comparison of samples D and E showed greater variability between laboratories than comparison of either D or E with A. The between laboratory variability for estimates relative to A of each of these samples was greater than that for estimates of C. That is, between laboratory variability for comparisons of different serum samples (D or E with A, D with E) was larger than that for identical serum samples (C with A).

Comparison of the ampouled samples, coded B, D and E, with 97/550 coded A by IA-2 antibody assays. Estimates of the activity for the ampouled samples are summarized in Table 6 and individual estimates for each assay are given in Figures 4–6.

There is inconsistency between these assay systems, with each sample, including the proposed standard, found to give undetectable IA-2 antibody activity by at least one laboratory.

No IA-2 antibody activity was found for the proposed standard (and its duplicate C) in laboratories 06 and 16; samples B and E were found to be undetectable in laboratory 06; sample D was found to be undetectable in laboratories 09 and 12 and gave relatively low levels of activity in laboratories 03, 07 and 08. The proposed standard A was found to have greater IA-2 antibody activity than the other three samples with the exceptions of laboratories 06 and 16 where no IA-2 antibody activity was detected and of laboratories 02 and 12 where sample B was found to give about twice the IA-2 antibody activity of A.

The between laboratory variability for estimates relative to A of each of these samples was greater than that for estimates of C, identical to A, relative to A. Direct comparison of samples D and E showed greater variability between laboratories than comparison of either D or E with A. This variability reflects the crucial differences between the assay systems.

Comparison of the ampouled samples, coded B, D and E, with 97/550 coded A by IA-2beta antibody and other assays. Estimates of the activity for the ampouled samples are summarized in Table 7 and individual estimates for each assay are given in Figures 4–6. The three assays for IA-2beta antibody gave consistent results for the samples. Samples D and B were found to be undetectable and sample E was found to have a low but consistent activity of about 5 % of A. The GAD/IA-2 combined antibody assay gave estimates for samples E and B broadly similar to those given by both the GAD and IA-2 antibody assays. In contrast, sample D was found to give a similar activity to that of A, although in the GAD anti-

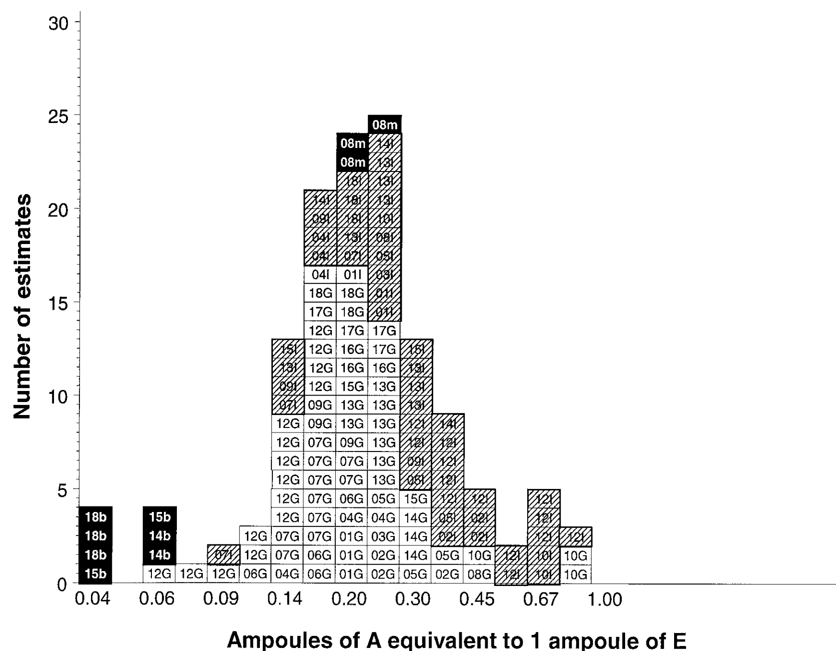


Fig. 5. Histogram showing estimates of the activity of preparation E expressed as equivalent ampoules of preparation A. Each square represents an estimate from an individual assay. The label in the square denotes the laboratory code followed by I to indicate IA-2 antibody assays, G to indicate GAD antibody assays, b to indicate IA-2beta antibody assays or m to indicate the IA2/GAD antibody combined assay. The shading also indicates the assay type. As can be seen from the horizontal scale, estimates for GAD or IA-2 antibody assays cover approximately a tenfold range. These estimates indicate that preparations E and A are dissimilar, i. e. contain different ratios of GAD, IA-2 and IA-2beta antibodies, and are distinguished by assay type

body assays D gave considerably larger activity than A and in the IA-2 antibody assays D gave substantially smaller activity than A.

Comparison of the ampouled preparations with the in-house standards. There were a variety of in-house reference preparations used for these assays, with unitages which were in several cases arbitrarily assigned. This is reflected in the range of estimates seen for the in-house reference preparations in Tables 6, 7 and 8. In the absence of clear characterization of the in-house preparations, we have not attempted to rationalize these estimates.

Discussion

The proposed standard, 97/550, and the serum samples coded B, D and E have been examined using a range of assay systems by 19 participants from 8 countries. The serum contained in 97/550, currently widely used as a reference preparation [12, 13, 15], was defined on the basis that the JDF units assigned gave the dilution factor of the serum expected to give the assay end point. The average JDF unitage of the in-house standard in this study is 85 and the potency of 97/550 relative to the in-house standards is 0.2. Thus, if the various in-house standards are assumed to be on average 85 units, then assignment of about 20 units to 97/550 would maintain overall continuity with the in-house standard.

It is therefore suggested that assignment of 20 international units per ampoule of 97/550 as far as possible maintains broad continuity with units for ICA currently in use. Moreover, estimates of the serum samples in terms of the proposed standard are less variable between laboratories than either estimates of the serum samples in terms of the in-house standards or directly measured end points.

Comparisons among the proposed standard and the serum samples in the GAD antibody assays show a broadly consistent ordering of these preparations, with D having greatest GAD antibody content, 97/550 having intermediate GAD antibody content, E having a small but clearly detectable GAD antibody content and B having a GAD antibody content which was smaller than any of the other preparations and undetectable in some assays.

Laboratory 10 showed, however, a strong exception to this broad pattern, and the observed GAD antibody content of B in laboratory 08 was also notably large. Moreover, the ratio of GAD antibody content for samples D and E in terms of A varied by more than fivefold, suggesting that there are considerable differences in assay specificity between these systems. It is suggested that use of 97/550 as a reference reagent for these assays might provide some reduc-

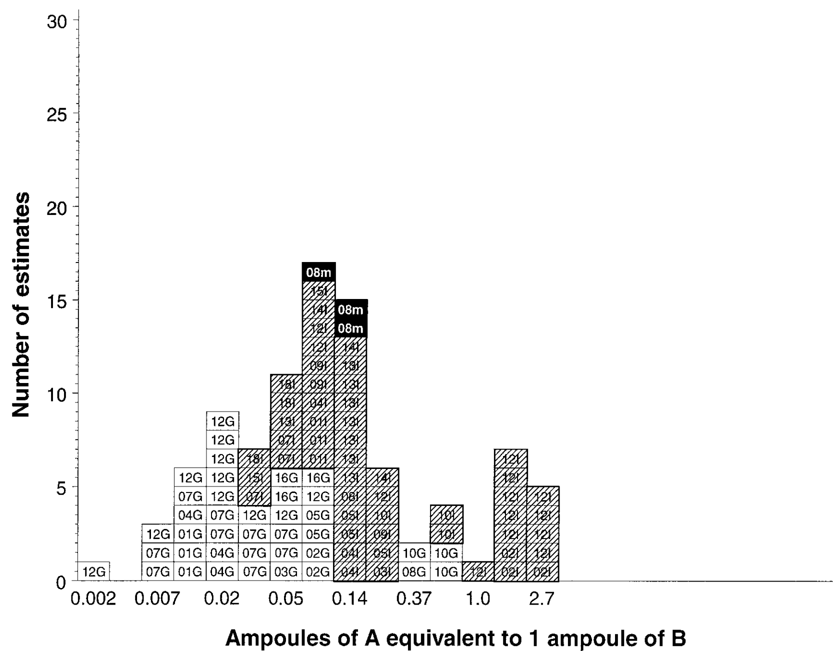


Fig. 6. Histogram showing estimates of the activity of preparation B expressed as equivalent ampoules of preparation A. Each square represents an estimate from an individual assay. The label in the square denotes the laboratory code followed by I to indicate IA-2 antibody assays, G to indicate GAD antibody assays, b to indicate IA-2beta antibody assays or m to indicate the IA-2/GAD antibody combined assay. The shading also indicates the assay type. As can be seen from the horizontal scale, estimates for GAD or IA-2 assays cover approximately a sevenfold range. These estimates indicate that preparations B and A are dissimilar, i. e. contain different ratios of GAD and IA-2 antibodies and can be broadly distinguished by assay type

tion in between-laboratory variability and would at least provide a common unitage in terms of which estimates could be compared between laboratories. This would contribute to the further characterization and understanding of these assay systems.

Comparisons among the proposed standard and the serum samples in the IA-2 antibody assays showed large inconsistencies in ordering of these preparations between different laboratories. For example, although 97/550 was found to contain the largest IA-2 antibody content in most laboratories, it was found to be undetectable in two laboratories and to be less than B in two laboratories. The decision of the WHO ECBS not to approve the 97/550 reference sample as a standard for IA-2 antibodies is explained by the variability of the IA-2 antibody assays. An additional study focussed on the suitability of the 97/550 preparation to serve as a standard for IA-2 antibodies will be needed. Use of 97/550 as a reference reagent in these assays should contribute to their characterization and understanding.

It was therefore recommended that the preparation in ampoules coded 97/550 be established as the international standard for islet cell antibodies and be assigned a unitage of 20 international units per ampoule. It was also recommended that the preparation in ampoules coded 97/550 be established as the first international reference reagent for GAD65 antibodies and be assigned a unitage of 100 units per ampoule. It was further recommended that the preparation in ampoules coded 97/550 be made available as an NIBSC reference reagent for use in further evaluating assays of IA-2 antibodies.

These recommendations were accepted by the WHO Expert Committee on Biological Standardization at its 51st meeting in October 1999.

Auxilliary information. The standard is available from the National Institute for Biological Standards and Control, Blanche Lane, South Mimms, Potters Bar, Hertfordshire EN6 3QG; Tel: 01707–654753. Fax: 01707–646730, email standards@nibsc.ac.uk.

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