A Research Standard for Human Serum Immunoglobulin D

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The concentration of immunoglobulin D in human serum is usually studied by immunochemical methods based on the precipitation of antigen–antibody complexes in agar gel. The single-radial-diffusion technique, originated by Mancini et al. (1965), is widely used. Techniques such as this could best be quantified by the inclusion of a biological standard for IgD in each titration run. The concentration of IgD as antigen in the solution under test would then be expressed as a potency in relation to the defined concentration of IgD antigen in the standard. A research standard for IgD has therefore been prepared and is described in this paper.

A research standard for human serum immunoglobulins G, A and M, coded 67/86, has previously been described (Rowe, Anderson & Grab, 1970). At the time of preparation of that material, the research standard for human serum immunoglobulin D, 67/37, was also prepared by the Division of Biological Standards, National Institute for Medical Research, London. Plasma was obtained by selection for higher than average IgD levels among samples from 761 healthy adult male blood donors in Great Britain: 34 samples were obtained in this way. The collection and storage of this material, and the removal of fibrinogen by recalcification, were carried out in a similar way to that described for preparation 67/86. On 5 April 1967 the material was distributed into glass ampoules and freeze-dried. During the filling, 51 of the ampoules were weighed. The mean wet weight of the contents was 1.015 g, with a total variation of $\pm 0.79\%$. The ampoules were further dried over phosphorus pentoxide, filled with pure dry nitrogen and then sealed by fusion of glass. They were stored at $-20°C$ in the dark. The dry weights of the contents of 6 ampoules were determined. The mean was 81.88 mg, and the range 81.65 mg–82.25 mg.

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Stability of IgD in the research standard

An estimate was made of the probable stability of the IgD present in 67/37. An accelerated degradation test was carried out by holding ampoules at $-70°C$, $-20°C$, $+4°C$ and $+37°C$ for periods of 6 months and 1 year. The ampoule contents were then reconstituted by the addition of 1 ml of distilled water, and the amount of IgD antigen in each solution was compared directly by the single-radial-diffusion test as previously described for other immunoglobulins in 67/86. A separate dilution series was prepared for each of 3 ampoules held at each temperature. Each dilution was tested twice in the same antibody-in-agar plate; the response was taken as the mean of two orthogonal diameters of the area of precipitation. The specific antiserum IgD was prepared by immunizing a sheep with a single D-myeloma protein emulsified in complete Freund's adjuvant. The antiserum was rendered specific for IgD on gel-diffusion analysis by absorption with normal human serum in liquid form which was deficient in IgD. The edges of the precipitation rings with 67/37 were satisfactorily sharp. The results of the titrations indicated that material stored at 37°C for either 6 or 12 months had lost 10% of its activity as antigen. There was no significant loss on storage at $+4°C$ or $+20°C$ as compared with material stored at $-20°C$ (see accompanying table). Any change of potency at the lower temperatures was thus too small to permit calculation of the stability of IgD at $-20°C$, but it may be assumed that the rate of degradation is so low as to be negligible for practical purposes, at least over the next 10 or 20 years.

The IgD content of the research standard

It is recognized that the current practice is to express immunoglobulin concentrations in terms of mg/ml. No definitive value for the IgD content of 67/37 in such terms is given here. The concentration of immunoglobulin D in a preparation such as 67/37 might be estimated by immunochemical comparison with representative solutions of isolated IgD of known concentration. However, a variety of factors may affect the values esti-

ACCELERATED DEGRADATION STUDY OF THE RESEARCH STANDARD FOR IgD 67/37 [a]

Time of storage	Temperature of storage			
	−70°C	+4°C	+20°C [b]	+37°C
6 months	95 (89–101)	95 (89–101)	—	90 (85–96)
1 year	—	102 (93–112)	99 (90–109)	90 (82–99)

[a] Results are expressed as mean percentage potencies (with 95% confidence limits) relative to freeze-dried material stored at −20° C.
[b] Room temperature.

mated.[1] The use of a D-myeloma protein as a reference protein in such a titration system could further reduce the reliability of the estimate. Nevertheless, by the use of the single-radial-diffusion technique, 67/37 has been compared with a purified D-myeloma protein. The protein concentration of the solution of the purified myeloma protein was calculated from its optical density at 280 nm assuming $E^{1\,cm} = 13.5$. Two specific anti-IgD antisera were used, each prepared by immunizing rabbits with a different myeloma protein, which was different from the myeloma protein used in the calibration titrations. Both antisera yielded similar results. On the basis of these tests the weight of IgD corresponding to 1 unit of activity was calculated to be 1.41 μg. This value is given to provide an approximate indication of the IgD content of the research standard. It is recommended that results obtained by the use of the research standard should *not* at present be expressed in terms of mass.

Unitage of the research standard 67/37

The material 67/37 has been established in the United Kingdom as the British research standard for IgD and a unitage has been assigned to it such that 1 unit of activity of IgD is the activity present in 0.8188 mg of the freeze-dried powder. Since the mean weight of contents of each ampoule is 81.88 mg each ampoule contains on average 100 units of activity of IgD.

Availability of the research standard

The research standard for IgD is now generally available to investigators wishing to use it in estimating concentrations of IgD in their own laboratories. Workers in North and South America should request 67/37 from Dr J. L. Fahey, National Cancer Institute, Immunoglobulin Reference Centre, Bethesda, Md. 20014, USA. Workers in the United Kingdom of Great Britain and Northern Ireland should request 67/37 from The Director, Division of Biological Standards, National Institute for Medical Research, Mill Hill, London N.W.7, England. Workers elsewhere should request 67/37 from The Director, WHO International Reference Centre for Immunoglobulins, 21 rue du Bugnon, 1011 Lausanne, Switzerland.

Recommendations for the use of the research standard

(1) On receipt the research standard should be stored in the dark at −20°C.

(2) One ampoule of the research standard may conveniently be reconstituted by the addition of 1.0 ml of distilled water. The powder should dissolve readily on standing for 1 hour at room temperature to give a slightly turbid solution. An appropriate series of dilutions of this material should be prepared and used on the same day that the material is reconstituted. (It may be found convenient to use a volume of water less than 1.0 ml: the powder will dissolve in 0.5 ml of water.)

(3) The total volume of the solution of the preparation made by the addition of 1.0 ml of water will exceed 1.0 ml. It has been calculated that the average volume of the solution will be 1.06 ml. This solution will therefore contain 94.3 units of IgD in 1.00 ml.

(4) Since the research standard is not available in sufficient quantity for routine inclusion in every titration run it is suggested that each investigator should prepare for himself a stable preparation

[1] Rowe, D. S., Anderson, S. G. & Grab, B. Paper in preparation.

of serum containing IgD and use this as a working local standard in each titration. Such a local standard might be formed by fresh serum of suitable IgD content frozen in small aliquots and held at −20°C or colder. This local standard should periodically be carefully assayed against the research standard, using techniques which have been validated statistically, and the results should be analysed statistically.

(5) The relative potency of IgD in a solution of a standard or in any other solution should be expressed as units of activity of IgD per ml of solution.

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A Research Standard for Human Serum Immunoglobulin E

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Immunoglobulin E has recently been recognized as a distinct class of human immunoglobulin. The concentration of IgE in serum is usually measured either by a radio-immunoadsorbent test (Johansson, Bennich & Wide, 1968) or by the radioactive single-radial-diffusion technique (Rowe, 1969). Methods such as these could best be quantified by the inclusion of a biological standard for IgE in each titration run. The activity of IgE as antigen in the solution under test would then be estimated as a potency in relation to the activity of IgE antigen in the standard. A research standard for IgE has therefore been prepared, in a way similar to that previously described for research standards for IgG, IgA and IgM (Rowe, Anderson & Grab, 1970), and IgD (Rowe, Anderson & Tackett, 1970).

As source material, sera containing high concentrations of IgE from 91 adult West African donors, bled in Gambia in February 1968, were chosen. The blood was allowed to clot and the serum was separated under sterile conditions. Each serum gave a precipitate in a double-diffusion analysis in agar gel against an antiserum specific for IgE. A total volume of 1400 ml of serum was obtained. It was transferred to the WHO International Reference Centre for Immunoglobulins at Lausanne, Switzerland, for further testing, and then to the Division of Biological Standards, National Institute for Medical Research, London, England, for freeze-drying. When in the laboratory, the serum was stored at −20°C; it was transported by air in insulated containers with solid carbon dioxide.

On 13 August 1968 the samples were thawed, pooled, and passed through a glass fibre pre-filter (designated by Millipore as type AP25) in order to clarify the material. On 14 August 1968 the material was distributed into ampoules and freeze-dried. (During the filling, 24 ampoules were weighed. The mean wet weight of contents was 1.013 g with a range from 1.011 g to 1.014 g.) The ampoules were further dried over phosphorus pentoxide, filled with pure dry nitrogen and sealed by fusion of glass. The ampoules were coded 68/341 and stored in the dark at −20°C. The dry weight of the contents of each of 6 of the ampoules was determined. The mean was 92.84 mg and the range 92.64 mg–93.18 mg. The water content was determined on 6 ampoules. The mean content was 0.66% and the range was 0.38%–1.34%. The oxygen content of the gas in each of 3 ampoules was estimated using the mass spectrometer. The average was was 0.56% and the range was 0.1%–0.8%.

Stability of IgE in the research standard

An estimate was made of the probable stability of the IgE present in 68/341. An accelerated degradation test was carried out by holding ampoules at

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