NIDDK calibrators.

1. Stock NIDDK standard.
Blood samples (minimum 60 mls) from 21 patients with type 1 diabetes and who had moderate to high titer GAD and IA-2 antibodies were collected. After confirmation of autoantibody titer and of non-infectious status the sera were pooled. The serum pool was cleared by high speed centrifugation and filtration through gauze to remove lipid. A total of 795 ml was obtained and this became the Stock TEDDY standard.

2. NIDDK negative pool.
Serum (230 mls each) from 12 blood donors was provided by the Munich blood donation center. All were negative for GAD and IA-2 antibodies. The 12 sera were pooled and then cleared by high speed centrifugation and filtration through gauze to remove lipid. A total of 2600 mls was obtained and became the stock TEDDY negative pool.

3. Preparation of NIDDK Calibrators from stocks.

A. 1/3.85	166 ml of stock plus 473 ml of negative	Total final vol: 394 ml
B. 1/10	245 ml of A plus 392 ml of negative	Total final vol: 397 ml
C. 1/26	240 ml of B plus 384 ml of negative	Total final vol: 392 ml
D. 1/67.6	232 ml of C plus 371.2 ml of negative	Total final vol: 393.2 ml
E. 1/176	210 ml of D plus 336 ml of negative	Total final vol: 394 ml
F. 1/457	152 ml of E plus 243.2 ml of negative	Total final vol: 395.2 ml

These represent sequential 1/2.6 dilutions.

4. Calibration.

The Calibrators were tested by NIDDK core laboratories against original NIDDK calibrators and assigned the following values:

DK Standard	GADA DK units/ml	IA-2A DK units/ml
A	457	224
B	227	101
C	91	41
D	35	15
E	12	5.8
F	3.5	1.9

5. Suggested use.

To calculate results laboratories should plot Log2 of the DK units for the standards (y-axis) against the mean reading obtained for those standards after subtraction of reading of negative calibrator (Microsoft Excel program can be used). A logarithmic trendline is then fitted to the points of the curve with the curve fit and R^2 value displayed on the chart.

The equation should take the form:

$$Y= A*Ln(x)-B$$

The values of A and B are specified by the curve fit while the value of x is the mean reading for the sample after subtraction of the mean reading of the negative calibrator.

The equation of the curve fit is then inserted into the column for calculating the DK units. The result for each sample is expressed in DK units/ml by raising 2 to the power of Y (ie. to the value generated by the curve fit for that sample). If the mean reading for the sample is less than the mean reading for the DK negative serum a value of zero DK units/ml is assigned to that sample.

The worksheets provided for the standard methods include columns to calculate the mean reading (Column D) and mean minus negative (Column E). The file contains examples for the construction of the standard curve and calculation of results in DK units/ml.