INSULIN AUTOANTIBODIES (IAA): EVALUATION OF ASSAY METHOD

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1 Background

Assessment of the presence of insulin autoantibodies (IAA) contributes to the predictability of the likelihood of developing Type 1 diabetes mellitus (T1D). The Finnish Type 1 Diabetes Prediction and Prevention Study, comprising a large population of 2448 genetically at-risk children (1), showed that IAA are usually the first islet autoantibody to appear in the natural history of T1D. With the use of high-quality assays, antibody testing in early childhood can identify individuals destined to develop the disease (2). Identifying the combination of autoantibodies to glutamic acid decarboxylase (GADAs) and IAA is useful in risk estimation, in fact, the presence of both indicates a high disease risk in unaffected individuals (3).

2 Aim of the study

Was to assess a chemiluminescence immunoassay for insulin autoantibodies (IAA).

3 Methods

The evaluation of IAA (Snibe Diagnostic) was performed with the chemiluminescence immunoassay (CLIA) analyzer MAGLUMI 600. The intra and inter run precision (CVA%) was determinated from the mean of three replicates for five separate run, each with two samples at different concentrations. CV obtained in the laboratory were compared with those provided by the manufacturer. We performed a test of dilution in order to highlight the presence of a systematic error. Control sera were obtained from 68 blood donors (median age 20 years; range 18–28 years). In all subjects were measured the autoantibodies to GAD 65 (anti-GAD65, Snibe Diagnostic) with the analyzer MAGLUMI 600. The statistical evaluation of Reference Intervals (RI) has provided the identification of possible outliers.

4 Results

The analytical variation and the test of dilution results are shown in Tables 1a and 1b.
The correlation between the values expected and values measured is reported in Table 2 and Figure 1.
Table 3 and Figures 2a-2b show the distribution of IAA values in the study population. Table 4 and Figure 3 report the concentrations of IAA and anti-GAD65 in the same group of subjects.

5 Conclusion

Islet autoantibody testing allows prediction of type 1 diabetes and definition of the latent autoimmune diabetes in adults subgroup of non-insulin-treated patients. The data obtained show suitable analytical performances comparable to those of the manufacturer. Our studies indicate the complete cross-validation and interpretation of results of GAD65 and Insulin autoantibody (IAA) assays in the normal subjects evaluated, using RIs, calibrators and protocols reported by the manufacturer.

ANALYTICAL VARIATION	
Precision	**CVA %**
Calculate	
Pool 1 (x=13,3 IU/mL)	
within run	2,67
between run	2,43
Pool 2 (x=27,5 IU/mL)	
within run	3,14
between run	0,62
Manufacturer	
Control 1 (x=10,5 IU/mL)	
within run	6,89
between run	9,74
Control 2 (x=20,4 IU/mL)	
within run	5,92
between run	9,01

Table 1a

RECOVERY				
Sample	**Dilutions**	**Expected (IU/ml)**	**Measured (IU/mL)**	**Recovery %**
A		106,44		
	1/2	53,22	59,12	111,09
	1/4	26,61	33,50	125,89
	1/8	13,31	16,87	126,79
	1/16	6,65	8,47	127,32
	1/32	3,33	4,62	138,90
	1/64	1,66	2,42	145,51
B		39,86		
	1/2	19,93	19,07	95,68
	1/4	9,97	10,00	100,35
	1/8	4,98	4,53	90,92
	1/16	2,49	2,40	96,34

Table 1b

BEST-FIT VALUES	
Slope	1.04 ± 0.09
Y-intercept	0.03 ± 0.23
r^2	0,97

Table 2

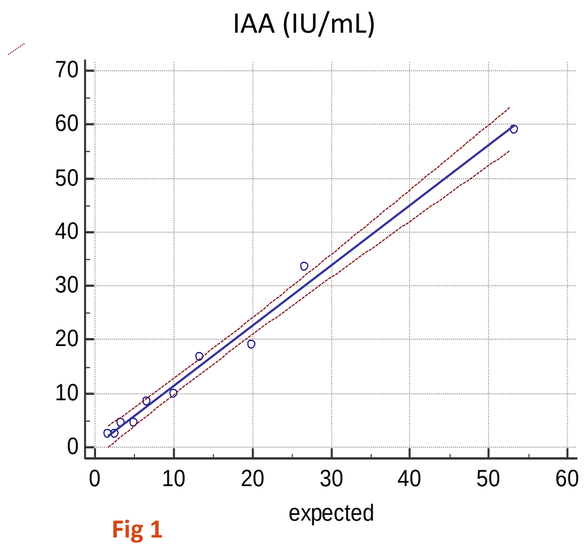

Fig 1

IAA REFERENCE INTERVAL	
95% RI, Double-sided	
Sample size	68
Lowest value	6,47
Highest value	17,30
Arithmetic mean	9,95
Median	9,29
Standard deviation	2,50
Coefficient of Skewness	1,1594 (P=0,0004)
Coefficient of Kurtosis	0,8687 (P=0,1510)
D'Agostino-Pearson test	reject Normality (P=0,0007)

Table 3

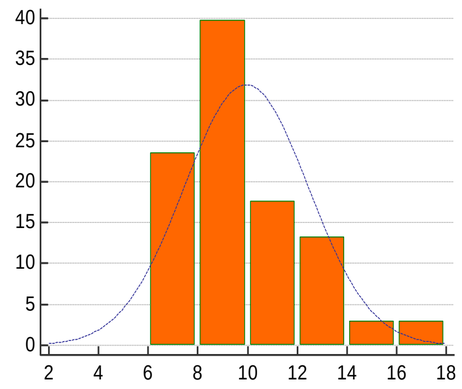

Fig 2a

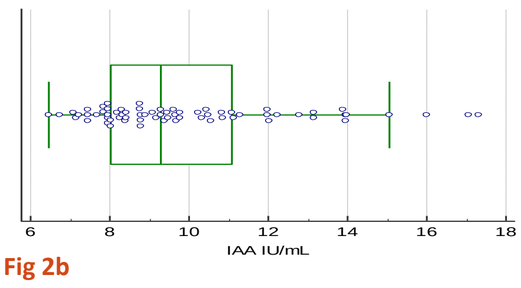

Fig 2b

IAA	
Sample size	68
Minimum (IU/mL)	6,47
Maximum (IU/mL)	17,30
Mean (IU/mL)	9,95
Median (IU/mL)	9,29
anti-GAD 65	
Sample size	68
Minimum (IU/mL)	1,20
Maximum (IU/mL)	12,01
Mean (IU/mL)	5,02
Median (IU/mL)	5,25

Table 4

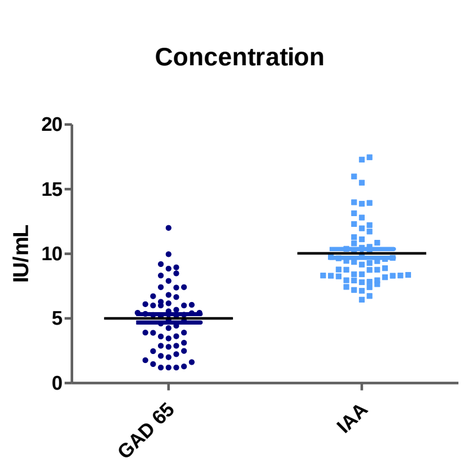

Fig 3

6 References

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3. Matti A, Westerlund A, Blomberg K. et al. Time-Resolved Immunofluorometric Dual-Label Assay for Simultaneous Detection of Autoantibodies to GAD65 and IA-2 in Children with Type 1 Diabetes. Clinical Chemistry. 2007;53: 472-479.