



Evaluation of precision, comparability and linearity of MAGLUMI™ 2000 Plus GAD65 antibody assay

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Contributions: (I) Conception and design: M Plebani; (II) Administrative support: C Cosma; (III) Provision of study materials or patients: C Cosma; (IV) Collection and assembly of data: A Padoan, C Cosma; (V) Data analysis and interpretation: A Padoan; (VI) Manuscript writing: All Authors; (VII) Final approval of manuscript: All authors.

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Background: Type 1a diabetes (TD1a) is an autoimmune chronic disease and more than 85% of newly diagnosed patients presented elevated levels of glutamate decarboxylase 65-kDa isoform (GAD65) antibodies in serum. Aim of this study was to evaluate the MAGLUMI™ 2000 Plus GAD65 antibody chemiluminescence immunoassay (CLIA) precision, linearity and comparability with EUROIMMUN Anti-GAD enzyme-linked immunosorbent assay (ELISA).

Methods: MAGLUMI™ 2000 Plus (Snibe, Keyuan Road, Nanshan District, Shenzhen, China) precision was estimated adopting the Clinical and Laboratory Standards Institute (CLSI) EP15-A3 protocol and compared against the manufacturer's claimed values. A series of 135 specimens were used for comparing GAD65 antibodies between MAGLUMI™ 2000 Plus and EUROIMMUN Anti-GAD ELISA (EUROIMMUN Medizinische Labordiagnostika AG, Lubeck, Germany). Linearity of dilution was assessed by mixtures of high-level pools and a low-level pool of samples.

Results: MAGLUMI™ 2000 Plus repeatability and intermediate precision were measured at 22.2, 58.4 and 101.2 IU/mL levels of GAD65 antibody. At those levels, results of repeatability were 2.96%, 1.02% and 1.95%, and of intermediate imprecisions were 4.62%, 3.34% and 2.17%. Comparability showed that a detectable proportional bias was present between the two assays, being GAD65 antibody levels underestimated by MAGLUMI™ 2000 Plus CLIA assay with respect to EUROIMMUN Anti-GAD ELISA. However, using the cut-off suggested by the manufacturer for dichotomizing results, a good agreement was found between the two methods (90.4%). Linearity was optimal within the range 60–100 IU/mL, poor in the lower range.

Conclusions: MAGLUMI™ 2000 Plus GAD65 antibody CLIA assay showed a satisfactory imprecision, supporting the utilization of this assay in clinical practice for diagnosing TD1a. However, our data confirm the need for better standardization/harmonization between GAD65 antibody assays.

Keywords: MAGLUMI™ 2000 Plus; GAD65 antibody; precision; linearity; comparability

Received: 16 August 2019; Accepted: 03 September 2019; Published: 17 September 2019.

doi: 10.21037/jlpm.2019.09.03

View this article at: <http://dx.doi.org/10.21037/jlpm.2019.09.03>

Introduction

Type 1 diabetes (T1D) is a chronic disease with a strong genetic component, characterized by deficiency of insulin release and resultant increased hyperglycaemia (1).

Epidemiology of T1D is known to be heterogeneous regarding geography and ethnicity, but recent data show that T1D incidence among young is globally increasing of about 1.4% per year, and it was estimated that

approximately 17,900 new cases per 100,000 people were recorded in the United States in the 2011–2012 period (2). Hence, T1D represent an important health issue and is associated with considerable clinical, social and economic consequences, especially because diabetic complications continue to be a major cause of morbidity and mortality in patients with T1D (3).

Unlike type 2 diabetes (T2D), T1D can occur at any age, but it tends to develop in childhood (3). Two subtypes of T1D, namely type 1a and type 1b, have been defined, of which only one (type 1a diabetes; T1Da) is characterized by autoimmune-mediated progressive destruction of pancreatic β -cells and presence of autoantibodies in serum. In fact, autoimmune mechanisms are not involved in type 1b diabetes. The presence of autoantibodies against of isles cells autoantigen (ICA), insulin (IAA), glutamate decarboxylase (GAD), tyrosine phosphatase (IA-2) or zinc transporter 8 (ZnT8) have been described in patients and with percentages varying from 60% to 90% of T1Da new cases (4). Among these, GAD has been extensively studies with regard to its pathogenic role in development of T1Da and is described to be present up to 85% of newly diagnosed patients (4,5).

GAD catalyses the α -decarboxylation of L-glutamic acid to synthesize gamma-amino butyric acid (GABA). Two distinct form of GAD, namely GAD67 (67 kDa) and GAD65 (65 kDa), were identified. These forms are encoded by different genes, showing 65% homologies of sequences. Both GAD67 and GAD65 isoforms are synthesised inside the cytoplasm, whilst only GAD65 is anchored to the membrane (6). There is a strong variation of the expression of the two isoforms, and GAD65 is predominantly expressed in pancreatic islets, thus GAD67 does not offer any clinical utility for diagnosing T1Da. In humans the major antigenic region of GAD65 has been identified in the middle and the carboxyterminal region (5).

For assessment of GAD65 antibody, isotopic labelled assays were extensively used in the past decades as they provided robustness and good analytical performances. Currently, non-isotopic enzyme-linked immunosorbent assay (ELISA) and chemiluminescent immunoassay (CLIA) methods are widely available. In this study we aimed to evaluate the precision, comparability and linearity of the GAD65 antibody CLIA on MAGLUMI™ 2000 Plus. To our knowledge, this is the first study investigating the performance of GAD65 antibody CLIA on the MAGLUMI™ 2000 Plus platform.

Methods

The MAGLUMI™ 2000 Plus [Shenzhen New Industries Biomedical Engineering Co., Ltd (Snibe), Keyuan Road, Nanshan District, Shenzhen, China] is a chemiluminescent analytical system (CLIA), featured by high throughput (up to 180 tests/hours), supporting stat modality, flexible connections for lab automation, large operational capacity (up to 144 samples tubes) and continuous loading. It is also equipped by an intuitive and easy software interface. MAGLUMI™ 2000 Plus utilises magnetic microbeads particles platform with flash chemiluminescence, ABEI label, reagents. GAD65 assay (ref 130205005M) is a sandwich immunoassay. According to manufacturer, the measuring range is 1.0–280.0 IU/mL, with a limit of blank (LoB) of 1 IU/mL. The Manufacturer declares no interferences with GAD67 antigen. Calibration curve is generated by a 2-point calibration master curve procedure. MAGLUMI™ 2000 Plus CLIA presents a traceable calibrator for GAD65 (WHO 1st Reference Reagent 97/550).

Precision evaluation

Precision was evaluated by utilizing three human serum pools of samples at different concentrations. Precision estimations were obtained by using quintuplicate measurements of aliquots of the same pool, performed for a total of five consecutive days, following the Clinical and Laboratory Standards Institute (CLSI) EP15-A3 protocol. Analysis of variance (ANOVA) was used to estimate precision (7). The manufacturer's claimed precision data were referred to human serum pools and internal control material, containing different concentrations of analytes, measured in duplicate in two separate runs per day for a total of 20 days, following the CLSI EP5-A2 protocol. Obtained results for precision were compared to that claimed by manufacturer's by using the procedure recommendation by EP15-A3 (7). Calculated repeatability and intermediate precision are in accordance with the conditions specified by the international vocabulary of metrology (VIM, JCGM 100:2012) for precision estimation in a 5-day period (8).

Methods comparability evaluation

A total of 135 serum specimens, covering the most clinically-relevant range of GAD65 antibody assay were

Table 1 Precision results for GAD65 antibody assay expressed as coefficient of variation (CV) in percentage (%), obtained by using pools of samples

Measurand	Level	Design	Measured repeatability, CV%	Measured intermediate precision, CV%	Manufacturer's claims for repeatability at that level [#] , CV%	Manufacturer's claims for intermediate precision at that level [#] , CV%
Anti-GAD	22.2 (IU/mL)	5x5 CLSI EP15-A3	2.96	3.43	4.62	6.55
	58.4 (IU/mL)		1.02	1.46	3.34	3.00
	101.2 (IU/mL)		1.95	1.96	2.17	2.50

[#], obtained from the MAGLUMI™ GAD65 (CLIA) insert, N 069 GAD65-en-EU, V9.4, 2018-08; *, Precision results were interpolated by using a linear function. GAD65, glutamate decarboxylase 65-kDa isoform; CLIA, chemiluminescence immunoassay; CLSI, Clinical and Laboratory Standards Institute.

collected from routine testing at the Department of Laboratory Medicine, University-Hospital of Padova. The EUROIMMUN Anti-GAD ELISA (IgG) (EUROIMMUN Medizinische Labordiagnostika AG, Lubeck, Germany) (ref. EA 1022-9601G) assay used in clinical practice was compared with respect to MAGLUMI™ 2000 Plus CLIA on residual specimens, previously anonymized.

Linearity assessment

Linearity was assessed using a series of mixtures of samples pools, prepared with different GAD65 antibody concentration, as specified in the CLSI EP06 A:2003 guideline (paragraph 4.3.1) (9). In brief, three serum pools with measured GAD65 antibody level of 109.5, 59.2 and 23.7 IU/mL (high-level pools) were diluted with a low GAD65 antibody level serum pool (3.5 IU/mL). Different dilutions were performed maintaining final volume of 500 µL. This approach allowed to explore assay linearity in the range from 5.47 to 98.9 IU/mL. All dilutions were tested in triplicate and values averaged. Assigned GAD65 values were calculated by considering the dilution factor.

Statistical analyses

For precision evaluation, ANOVA was used to estimate repeatability and intermediate precision (7). An in house developed R script (R Foundation for Statistical Computing, Vienna, Austria), implementing the CLSI EP15-A3 protocol, was used to perform ANOVA and to calculate the upper verification limit. For method comparison, proportional and/or constant bias were estimated by Passing-Bablok regression and Bland-Altman analysis. Deviation from linearity was detected by Cumsum test

during method comparison. MedCalc Statistical Software version 18.5 (MedCalc Software, Ostend, Belgium) was used for Passing-Bablok and Bland-Altman analyses. P values <0.05 were considered statistically significant.

Results

Precision evaluation

Repeatability and intermediate precision, calculated by the 5-day analysis, were reported on *Table 1*. Because the levels used by manufacturer to estimate precision were different from those used in this study, MAGLUMI™ 2000 Plus claimed precision results were re-estimated by linear interpolation.

Methods comparability evaluation

Method comparisons were performed for a total of 135 serum specimens, collected in a wide dynamic range, from <0.5 to >280.0 IU/mL.

Considering only GAD65 antibody results within the measurable range of both assays, Passing-Bablok and Bland-Altman analyses were performed. Results are shown in *Figure 1A,B*. The slope and intercept results, and their corresponding 95% confidence intervals (95% CIs), reported in *Figure 1*, underlined proportional bias between assays. Cumsum tests does not underlined significant deviations from linearity (P=0.200). Bland-Altman analysis confirmed a bias of -69%.

A further evaluation was performed, considering GAD65 antibody results as positive or negative, using the manufacturers' declared cut-offs to dichotomize results, that were: for MAGLUMI™ 2000 Plus CLIA 17 IU/mL,

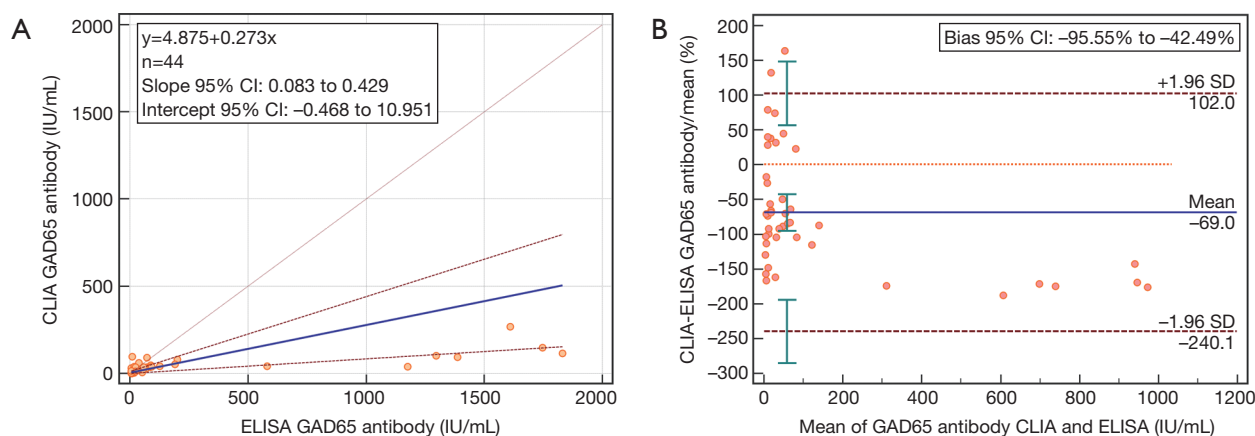


Figure 1 Method comparison results for GAD65 antibody (IU/mL). (A) Passing-Bablok regression and (B) Bland-Altman analyses of MAGLUMI™ 2000 Plus (CLIA) *vs.* EUROIMMUN Anti-GAD (IgG) (ELISA) analytical systems. GAD65, glutamate decarboxylase 65-kDa isoform; CLIA, chemiluminescence immunoassay; ELISA, enzyme-linked immunosorbent assay; CI, confidence interval; SD, standard deviation.

for EUROIMMUN Anti-GAD ELISA 10 IU/mL. This analysis showed that assays results, once considered as positive or negative, presented a good agreement (90.4%), with a Cohen's kappa of 0.761 (standard error = 0.085, $P < 0.001$).

Linearity assessment

Linearity results for the GAD65 antibody MAGLUMI™ 2000 Plus CLIA are reported in *Figure 2*. All tested mixtures of samples-pools deviated from linearity, according to CLSI EP06 A:2003 (9).

Discussion

In this study, the precision characteristics of GAD65 antibody MAGLUMI™ 2000 Plus CLIA have been evaluated by using samples pools. The used protocol from EP15-A3 allowed to demonstrate the precision characteristics of the assay at conditions specified for repeatability and intermediate precision (7). The results showed that MAGLUMI™ 2000 Plus presents very good precision characteristics at each level evaluated and at each condition. In fact, overall imprecision was lower than 4%. All precision results were inferior to those reported by the manufacturer using serum as matrix. Comparability evaluation was performed against the EUROIMMUN Anti-GAD ELISA assay. Of the 135 samples collected from the laboratory routine analyses, several results were

below or above the measurable range of one or both of the two assays. Therefore, to avoid misinterpretation of results, in the first analysis only the GAD65 antibody values in the measurable range of both assays were considered ($n=44$) and, thus, values below the lower, or above the upper measurable limits were excluded. Passing-Bablok and Bland-Altman analyses demonstrated that a detectable proportional bias was present between the two assays and shown that GAD65 antibody levels were underestimated by MAGLUMI™ 2000 Plus CLIA assay with respect to EUROIMMUN Anti-GAD ELISA. Notably, both assays are claimed to be traceable to the same calibrator for GAD65 (WHO 1st Reference Reagent 97/550), thus this systematic component of bias was not expected. A second analysis was hence performed, by dichotomising results considering manufacturers' recommended cut-offs. Overall, the agreement was elevated, with a Cohen's kappa demonstrating a good reliability between assays. The GAD65 antibody cut-offs declared by manufacturers are quite different (17 IU/mL for MAGLUMI™ 2000 Plus CLIA *vs.* 10 IU/mL for EUROIMMUN Anti-GAD ELISA) and after applying the transferability calculation using the equation derived from Passing-Bablok analysis, they are not overlapping.

Linearity of dilutions was also assessed to evaluate the ability of the method to provide results that are directly proportional to the concentration of GAD65 antibody in tested samples. Despite dilutions should be theoretically performed by spiked quantities, the approach performed in

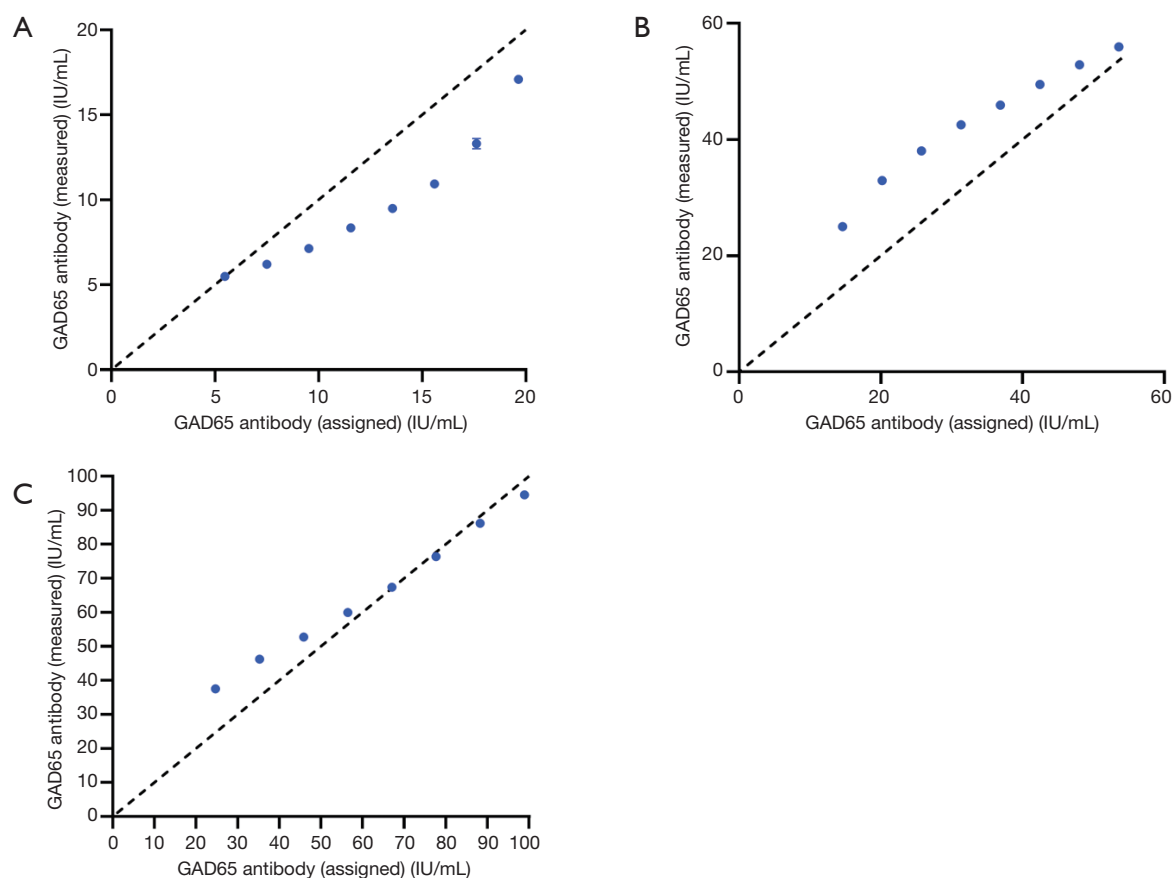


Figure 2 GAD65 antibody linearity results plotted for: (A) a high-level pool at 23.7 IU/mL, diluted with a low level pool at 3.45 IU/mL; (B) a high-level pool at 59.2 IU/mL, diluted with a low-level pool at 3.45 IU/mL; (C) a high-level pool at 109.5 IU/mL, diluted with a low-level pool at 3.45 IU/mL. GAD65, glutamate decarboxylase 65-kDa isoform.

this study was different and high-level pools were diluted in a low-level pool. The results clearly demonstrated that within the range 60–100 IU/mL results are linear, whilst linearity is poor in the lower range. These results may be attributable to the MAGLUMI™ 2000 Plus CLIA GAD65 assay calibration curve which is very flat for concentration below 60 IU/mL.

Some possible limitations of this study should be considered as well. For example, being the measured between-methods bias relevant, the measurement of trueness by the traceable calibrator for GAD65 (WHO 1st Reference Reagent 97/550) could have been performed. Further, limit of blank as well as limit of detection were not calculated in MAGLUMI™ 2000 Plus CLIA GAD65 assay.

Conclusions

To our knowledge, this is the first study performing an

analytical evaluation of the GAD65 antibody MAGLUMI™ 2000 Plus CLIA assay. Our data overall support utilization of this assay in clinical practice for diagnosing TD1a. However, additional efforts would be needed for improving harmonization/standardization of results between different assays.

Acknowledgments

Funding: None.

Footnote

Provenance and Peer Review: This article was commissioned by the editorial office, *Journal of Laboratory and Precision Medicine* for the series “2019 International Conference on Laboratory Medicine: From Bench to Diagnostic -

Therapeutic Pathways”. The article has undergone external peer review.

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at <http://dx.doi.org/10.21037/jlpm.2019.09.03>). The series “2019 International Conference on Laboratory Medicine: From Bench to Diagnostic - Therapeutic Pathways” was commissioned by the editorial office without any funding or sponsorship. Mario Plebani served as an unpaid Guest Editor of the series. The authors have no other conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This study was performed in accordance with the Declaration of Helsinki (as revised in 2013) and in accordance with the respective local Institutional Ethical Committee guidelines. Individual informed consent was waived.

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doi: 10.21037/jlpm.2019.09.03

Cite this article as: Cosma C, Padoan A, Plebani M. Evaluation of precision, comparability and linearity of MAGLUMI™ 2000 Plus GAD65 antibody assay. J Lab Precis Med 2019;4:31.

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