Original Article

COMPARISON OF TURNAROUND TIME (TAT) PERFORMANCE BETWEEN ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) VS. AUTOMATED CHEMILUMINESCENT IMMUNOASSAY (CLIA) IN MEASURING DIABETES AUTOANTIBODIES

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ABSTRACT

Autoantibodies to glutamate decarboxylase (GAD), islet antigen-2 (IA2) and islet cell antigen (ICA) are characteristic markers for type 1 diabetes among paediatric population and adults with latent autoimmune diabetes. We aim to compare the analysis performance of the enzyme-linked immunosorbent assay (ELISA) and the chemiluminescence immunoassay (CLIA) methods in the detection of diabetes autoantibodies. A cross-sectional study was conducted on 1,425 serum samples sent for diabetes autoantibodies measurement at Institute for Medical Research, Kuala Lumpur, Malaysia from April 2021 to July 2022. A total of 695 samples were measured using the Medizym® ELISA kit 96 wells and 730 samples were measured using Maglumi® CLIA kit. Both methods were able to produce timely results at more than 90% of the allowable laboratory turnaround time (LTAT). Cohen's kappa showed good and satisfactory agreement between the two methods: 0.734 (GAD), 0.413 (ICA), 0.514 (IA2). Additionally, CLIA method was significantly less time-consuming and less labour-intensive. CLIA method has a significant advantage over ELISA in offering better laboratory workflow with shorter time testing and TAT in laboratory practice.

KEYWORDS: method comparison, ELISA, CLIA, diabetes autoantibodies

INTRODUCTION

Autoantibodies to glutamate decarboxylase (GAD), Islet antigen 2(IA2) and Islet cell antigen (ICA) are characteristic markers for type 1 diabetes among paediatric population (1). They are also detectable in the subgroup of adults with latent autoimmune diabetes (2). Autoantibody assays are increasingly available to clinicians and are in great demand due to the high prevalence of diabetes worldwide (3-4). Early detection of diabetes type 1 is crucial to prevent life-threatening ketoacidosis by immediate insulin initiation (5). Diagnosis of diabetes type 1 or other types of autoimmune diabetes is mainly based on the detection of anti-GAD, anti-IA2 and anti-ICA antibodies via screening method such as the enzyme-linked immunosorbent assay technique (ELISA) (6).

ELISA is the well-known method in autoantibody detection using immunoassay technique that uses labelled immunoassay to detect and quantify substances, including antibodies, antigens, proteins, glycoproteins, and hormones (7). Basically, is a biochemical technique that detects antigens in liquid samples using an enzyme immunoassay. Antigens bind to a surface, followed by an enzyme-linked antibody, producing a colour change. The detection of these products is through measuring the antibodies complexes that can be read by a specific analyser (8). Despite its usefulness in the laboratory setting for years, the technique has been slowly replaced by a more advanced, sensitive and rapid automated analyser using the chemiluminescence immunoassay (CLIA) platform.

CLIA is an immunoassay technique that uses labels as indicator for the analytic reaction, in which the reaction forms a luminescent molecule (9). In general, luminescence is the emission of a visible or near-visible radiation which is generated when an electron transitions from an excited state to ground state. The resultant potential energy in the atom gets released in the form of light and can be detected and measured (10).

However, both methods have its own advantages and disadvantages in the laboratory setting and clinical diagnostics. However, ELISA has limitations in detecting ultra-low concentrations of biomarkers (11). CLIA provides higher sensitivity, faster processing, and automation, making it a superior alternative in clinical diagnostics (12). CLIA is particularly useful for detecting low-concentration substances like hormones, as its detection range is significantly lower than that of ELISA. (13). It is important to have a diagnostic testing tool that can produce accurate, reliable, and rapid results with faster turnaround time (TAT). Therefore, this study aims to compare the analysis performance of both ELISA and CLIA platform methods in diabetes autoantibodies detection.

MATERIALS AND METHODS

Setting and design

This cross-sectional study was performed from April 2021 to July 2022 at the Endocrine Unit of Specialised Diagnostic Centre of the Institute for Medical Research, Kuala Lumpur, Malaysia (NMRR No: NMRR-21-1283-60298). As of date, our laboratory is the main primary government centre for analysis diabetes autoantibodies in Malaysia.

Procedure

The serum samples received for diabetes autoantibodies detection from all Ministry of Health hospitals in Malaysia were stored at -30℃ for a maximum duration of 12 weeks. Upon analysis, samples were thawed at room temperature and mixed with vortex. Each sample will be tested on anti-GAD, anti-IA2 and anti-ICA resulting in three separate reactions per sample. For the purpose of the study, the Medizym® ELISA kit assay was compared with respect to the MAGLUMI™ 2000.

Medizym® ELISA kit contains 96 wells coated with indicated antibodies (GAD, ICA and IA2). Each plate consists of a calibrator and quality control (QC) to run along with the tested samples. The immunoassay reaction took place in the presence of indicated antibodies and measured using spectrophotometry at 450nm wavelength. Multiskan Go Plate reader from Thermo Scientific was use to read the wavelength of colour changes in testing using Medizym® ELISA kit. While for CLIA method, Maglumi® by Shenzhen New Industries Biomedical Engineering (SNIBE) analyser was used to measure wavelengths emit by fluorescent dye.

Maglumi® CLIA kit used special magnetic microbeads that are mixed with patients' samples. N-(aminobutyl)-N-(ethylisoluminol) (ABEI) was used as indicator to label the antigen-antibodies complexes. All reactions took place in the reaction cuvette. The detector will measure the emitted light in relative light unit (RLU) and will convert it to specific readable value.

Data collection

Comparison in performance was assessed in terms of the feasibility between the two methods, ease of operation, laboratory turnaround time (LTAT) performance and agreement of the measured results. Feasibility and ease of operation was assessed through handling experience among three medical lab technologists, while the agreement between the two methods were analysed from a total of 37 samples that were randomly selected to be concurrently analysed. Results were validated by two science officers and authorised by a pathologist.

Statistical analysis

Statistical analysis was performed using Microsoft Excel 2016 software. Descriptive nominal data were described using percentages and frequency. Statistical agreement between analysers were evaluated using Cohen's Kappa.

Ethical clearance

The study was registered with the National Medical Research Register (NMRR) of the Ministry of Health Malaysia (NMRR ID-22-00431-SWM) and received ethical clearance from the Medical Research and Ethics Committee, Ministry of Health Malaysia.

RESULTS

A total 1,425 of samples from all government hospitals in Malaysia were analysed using both ELISA and CLIA methods. Total of 695 samples were measured using the Medizym® ELISA kit of 96 wells and 730 samples were measured using Maglumi® CLIA kit. Data collected in three different months during the usage of ELISA and CLIA method can be referred in Table 1. All samples were tested for anti-GAD, anti-IA2 and anti-ICA. Both methods were able to produce timely results at more than 90% of the allowable LTAT. (Table 1).

Table 1. Laboratory turnaround time comparison between ELISA and CLIA

Method	ELISA (%)	CLIA (%)
Month 1	90.27	98.56
Month 2	98.48	99.30
Month 3	94.09	97.50
Mean	94.28	98.45

Cohen's Kappa analysis was conducted to assess the agreement between results obtained from 37 randomly selected samples. In Cohen's Kappa analysis values ≤ 0 as indicating no agreement and 0.01–0.20 as none to slight, 0.21–0.40 as fair, 0.41– 0.60 as moderate, 0.61–0.80 as substantial, and 0.81–1.00 as almost perfect agreement (16). The analysis demonstrated substantial agreement for anti-GAD (κ = 0.734) and moderate agreement for anti-ICA (κ = 0.413) and anti-IA2 (κ = 0.514) between CLIA and ELISA. However, the CLIA method produced results more quickly, delivering outcomes within one hour and completing a full batch ran within a day, whereas ELISA required 17 hours for individual results and nearly two days for a complete batch run.

DISCUSSION

In this study, we assessed the comparability of ELISA and CLIA methods in diabetes autoantibodies detection. Despite the advantages of CLIA, our study found good and moderate Cohen's kappa agreement between the two methods, indicating some degree of variability. Pre-analytical factors, including improper sample collection, storage, freeze-thaw cycles, handling, and processing, can interfere with test results, potentially leading to false positives or negatives (14-15). Poor sample quality can significantly impact the accuracy of immunoassays such as ELISA and CLIA (16). Furthermore, our laboratory receives samples from all peninsular Malaysia, including Sabah and Sarawak. The risks associated with poor handling, excessive agitation, and temperature fluctuations during transportation cannot be entirely ruled out. However, pre-analytical procedures are carried out in accordance with our laboratory's standards. Strict adherence to these protocols is crucial to ensuring the reliability and accuracy of diagnostic tests. Additionally, this study utilized a limited sample size to evaluate the comparability of both methods. To enhance reliability, Lapić, et al emphasised on further studies with larger sample sizes and diverse population groups were recommended to validate the findings and strengthen confidence in CLIA's diagnostic accuracy (13). Additionally, standardisation of assay protocols and calibration strategies should be considered to enhance agreement levels between different detection methods. Laboratories implementing CLIA should also conduct periodic quality assessments such as good internal quality control (IQC) monitoring and participate in external quality assurance (EQA) program to ensure consistency and reliability in clinical practice. Our findings align

with Cosma et al. (17), who found the Maglumi 2000 Plus CLIA method to be highly reliable for GAD65 antibody testing, comparable to Anti-GAD ELISA in accuracy and clinical applicability. However, this study specifically targeted only for anti-GAD antibodies. Given that IA-2 and ICA are also key autoantibodies in Type 1 diabetes, similar reliability may be expected for their detection, warranting further investigation. This reinforces the effectiveness of both CLIA and ELISA for diabetes antibody detection.

Similar to ELISA, CLIA method was able to produce fast results and achieved more than 90% of the targeted LTAT within 14 working days. Moreover, CLIA have more advantage compared to ELISA in which CLIA capable to run more sample at any time because of random access analyser. Unlike ELISA, which need to be run manually and limited number of samples can be run due to usage of 96 wells. Therefore, more samples can be run simultaneously in a batch and in turn shorten the time of TAT.

CLIA technique proved to be technically superior as it is less laborious to perform and able to provide results within a shorter time frame as compared to ELISA technique that requires skilled pipetting. Since CLIA offer less laborious, making it less prone to human error. Although ELISA method can give high predictive value, there are few issues that need to be considered (14). ELISA method needs to be run by batches, therefore there is possibility of keeping the sample until one batch full, eventually will lead in longer TAT. Meanwhile, CLIA has proved to have greater advantage of being less laborious and able to provide reliable results with faster TAT in comparison to ELISA.

The CLIA method has also demonstrated significant improvements in laboratory workflow procedures and can achieve faster TAT (within one working day) in laboratory practice. Unlike the ELISA method, which requires manual extraction of results from the analyser's plate reader, the CLIA method allows results to be directly retrieved from the software in a format Excel. This reduces transcription errors and minimises human error. Therefore, analysing samples for diabetes autoantibody detection using the CLIA method not only provides reliable results but is also quicker and simpler to perform, making it ideal for laboratories with a high workload.

Despite the advantages of CLIA in terms of automation and turnaround time, ELISA remains a preferred choice in many laboratories due to its cost-effectiveness. ELISA assays generally require lower initial equipment investment and operational costs, making them more accessible for laboratories with budget constraints, particularly in resource-limited settings (18). Additionally, ELISA kits are widely available and offer reliable sensitivity and specificity for detecting diabetes autoantibodies, further supporting their continued use in diagnostic workflows

CONCLUSION

This study highlights the comparability and efficiency of ELISA and CLIA methods in detecting diabetes autoantibodies. While ELISA remains widely utilised due to its high predictive value, it is labour-intensive, requiring batch processing, manual washing, and extended incubation periods. In contrast, CLIA provides distinct advantages, including automation, random-access capabilities, and enhanced capacity for high sample throughput. Its shorter TAT improves laboratory workflow and facilitates timely clinical decision-making. Given its superior efficiency, reduced manual workload, and reliable performance, CLIA emerges as a more practical and effective alternative for high-throughput diabetes autoantibody testing in modern laboratory settings. Early identification of autoantibodies allows clinicians to monitor at-risk individuals more closely, initiate timely interventions, and provide appropriate patient education. This can help delay disease progression, optimise glycaemic control,

and reduce the risk of complications. Additionally, early detection facilitates timely initiation of insulin therapy and personalised treatment plans, improving long-term outcomes and overall patient care. The implementation of CLIA in diagnostic laboratories could enhance disease surveillance and guiding proactive clinical decisions in Type 1 diabetes management.

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