

RESEARCH ARTICLE

CONCORDANCE OF THE ANALYTICAL PERFORMANCE OF AUTOIMMUNE ANTIBODIES ON THE HOB BIOCLIA® 1200 AUTOMATED IMMUNOASSAY ANALYZER TO THE PHADIA 250® SYSTEM

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Received: 2 December, 2019/ Revision: 9 December, 2019/ Accepted: 24 December, 2019

ABSTRACT: With more than 80 ADs (autoimmune diseases), e.g. Lupus or Rheumatoid arthritis, they are the third most common diseases worldwide. The diagnosis is difficult, because the generated autoantibodies are often not specific for a single disease. In fact, there is a need to increase the clinical efficiency in autoimmune diagnosis. Therefore, we tested and compared the CLIA-based HOB BioCLIA 1200® to the FEIA-based Phadia 250® system in both, handling and performance. 23 selected autoimmune parameters (e.g. in ANA, celiac disease or anti-phospholipids syndrome) and altogether 5982 measurements are done in our high-throughput lab. For the performance, the non-compliance and the κ -values are calculated to describe the effect of discrepant results. For 17 of 21 calculated parameters we found a good compliance, just four parameters, e.g. the Rheumatoid arthritis parameter anti-RF-M and the celiac parameter anti-DGP-A, just substantial κ -values are shown. A reason for the anti-DGP-A could be that celiac disease is not a relevant but rare disease in China. Thus the assay is far too sensitive or needs a higher reference range for a Caucasian patient pool as discussed with the manufacturers. The handling showed a stable running, random access system with an overall performance that makes it well usable in a medium sized laboratory.

KEY WORDS: Chemiluminescent immuno assay (CLIA); Automated autoimmune analyser; Autoimmune diseases (AD); HOB BioCLIA 1200®; Cohen's Kappa (κ) test

INTRODUCTION:

Introduction

ADs are the result of mismanagement in the immune system, leading to a chronic inflammatory process that damages specific organs or tissues. Epidemiological data show evidence of a steady state rise in ADs in the last decade's^{2,3} and a hygiene hypothesis for the Western societies was created.⁴

With more than 80 ADs, e.g. Lupus or Rheumatoid arthritis, they are the third most common diseases worldwide after cardiovascular disease and cancer^{5,6}. The difficulties in diagnosis are that the generated auto antibodies are often not specific for a single disease but closely related to clinical manifestations.⁷

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For the detection of such auto antibodies IFM is the gold standard. ⁸However, the method requires intensive personal operation, evaluation and lacks reproducibility.⁹ With regard to this background; there is a need for specific and sensitive tests to increase the clinical efficacy of antibody tests and to create easy running systems for healthcare labs. Alternative methods for the detection of antibodies in the autoimmune field are EIAs.¹ Actual established systems on the European market using the EIA method are Phadia (ThermoFisher Scientific, Uppsala, Sweden), Bio-Plex (bio-rad, California, USA) and Bio-Flash (Inova Diagnostics, San Diego, USA). The aim of the present work was the evaluation of the practical application of the HOB BioCLIA 1200® and the comparison to the Phadia 250® system.

The Phadia 250® system(ThermoFisher Scientific) is a fully automated FEIA system for allergy and autoimmunity testing, designed as a sandwich immunoassay.^{10,11} The Bio-Flash and HOB BioCLIA are both CLIAs for autoimmune disease.^{12,13} In contrast, the Bio-Plex® system(Bio-Rad)uses a bead-based immunoassay for the simultaneous detection of multiple analytes in a multiplex system.^{14,15}

Table 1: Comparison of actual used systems.

	Phadia 250®	Bio-Plex®	Bio-Flash®	HOB BioCLIA® 1200
Sensitivity	> 10 ⁻¹⁵ mol/L	10 ⁻¹³ -10 ⁻¹⁵ mol/L	10 ⁻¹³ -10 ⁻¹⁵ mol/L	10 ⁻¹⁸ mol/L
Dyn. range	< 10 ⁵	10 ² -10 ⁵	10 ² -10 ⁵	10 ⁷
Method	FEIA	ELISA	CLIA	BioCLIA
Through put	60 T/h	100 Samples/h	40 T/h	60 T/h
Sample loading	Random accessed	Batching	Random accessed	Random accessed
Flexibility	Flexible selection	Fixed panel	Flexible selection	Flexible selection
Reagents	Stored on board	Stored on board	Stored on board	Stored on board
Analytesavailable	22	18	21	51

MATERIALS AND METHODS:

HOB BioCLIA 1200®

The HOB BioCLIA 1200®chemiluminescent immunoassay system utilizes streptavidin-coated magnetic nano particles (beads). The homo-tetramers of the protein have an extraordinarily high affinity for biotin ($K_d \approx 10^{-14}$ mol/L)¹⁶, this non-covalent interaction is one of the strongest in nature.[¹⁷] After the beads are incubated with the diluted serum and washed, antihuman IgG conjugate antibody as a tracer is added. The generated complex is enzymatically oxidized with analkaline phosphatase solution (indirect CLIA) as trigger, and chemiluminescent light is produced.¹⁸ This reaction is measured in relative light units (RLUs), that are proportional to the amount of the complex (Figure. 1 below). The use of magnetic beads has made it possible to eliminate a number of time- and labour-intensive steps and reduce non-specific bindings.

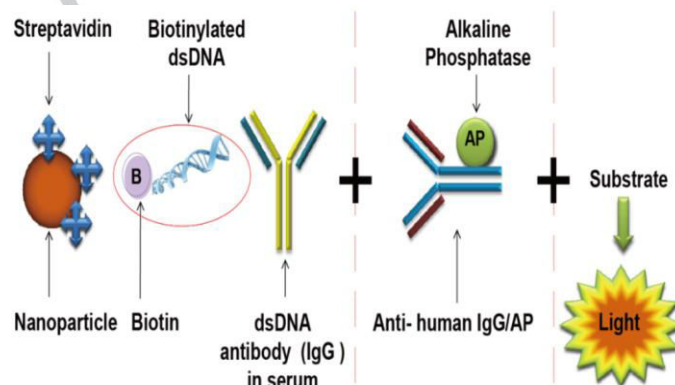


Figure 1: The enzyme-enhanced assay format of BioCLIA® 4G for e.g. anti-dsDNAIgG in the diagnostic testing of autoantibodies. ¹⁹ Suchlike immunoassays with enzymes (EIAs) for component labeling use combined immunologic and enzymatic tools generating high specificity results.²⁰

FEIAs are basically similar, but a fluorescent reaction product is used as substrate for the quantification. The inactive fluorochrome is colourless but induced by enzymatic dissociation; the activated form can be detected by uv-light irradiation.²¹

The HOB BioCLIA 1200® system is already established on the Asian, but not on the European

market. The analytical performances including LOD, precision (including intra-assay and inter-assay), linearity and the effect of interfering substances were evaluated in accordance with relevant *Clinical & Laboratory Standards Institute* (CLSI) guidelines.²⁴

For our study we used a selection of 23 parameters including antibodies for vasculitis, thyroid, anti-Phospholipids syndrome, celiac disease, rheumatoid arthritis and ANA.

Phadia 250®

The Phadia 250® system (ThermoFisher Scientific, Uppsala, Sweden) was used for the quantitative screening of auto antibodies. The used method in this system is an ELiA (FEIA), designed as a sandwich immunoassay. Quantitative detection of the antibodies in both, sera or plasma is done according to manufacturer's protocol. Calibrations are done with commercial standards in double determination every 28 days or after lot change. The evaluation of the results of the single control probe measurements is done according to the declared manufacturer areas for the quality management. In addition to the internal controls, periodic external quality controls via quality club (Phadia, monthly) and inter laboratory tests (RfB, two times per year) are done.

Statistical analysis

For the statistical analysis Microsoft Excel 2010 for Windows was used. The "non-compliance" is calculated as the quotient of discrepant results to the total number of measurements per parameter. The degree of agreement of the antibody concentration determinations, and therefore for the positive-positive-, negative-negative- and the discrepancy-rate in the Phadia 250® and HOB BioCLIA 1200® system, the Cohen's kappa (κ) is calculated.^[11] The statistical analysis is done for parameters with sample numbers >30. Thus, for the parameters anti-GBM (n = 7 sera), anti-CENP-B (n= 4 sera), κ was not calculated.

Materials:

Sera from patients with and without autoimmune disease were used for the study. In most cases clinical information (e.g. pregnancy) was not available. Hence, we can't say which of the two systems the preferable gold standard is for an automated autoimmune analyser in relation to the medical conditions.

Patients were included with an age range of all ages, minimum a few weeks (0) and maximum 93 years. The main proportions are the age group 51-65 years followed by the 36-50 years old patients, as expected.

Overall we had specimens from 1309 men (36%) and 2362 women (64%) respectively 575 from hospitals (16%) and 3095 from outpatient clinics (84%).

Calibrator and control reagents are supplied by the HOB Biotech Group and stored cold (4-8 °C). For calibration, high- and low- calibrators are used for a master curve principle, but not for anti-TPO and anti-TG. The master curve for the QC consists of six calibrators. High and low positive controls for each parameter are used. The patient panel was first tested at the Phadia 250® system, then at the BioCLIA 1200®.

Routine Procedure

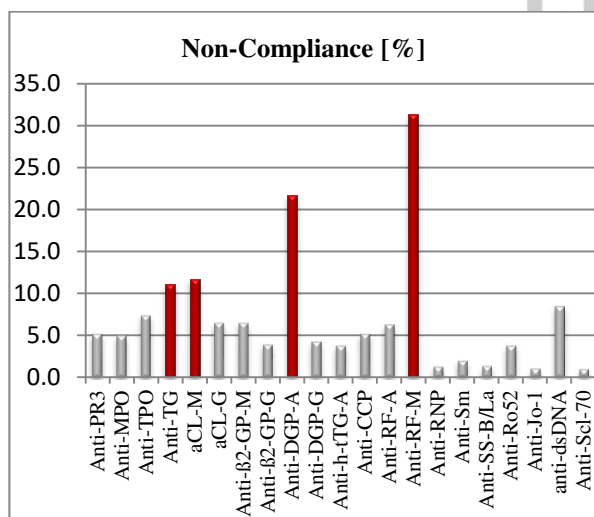
The cooled control and/or calibration materials are equilibrated to room temperature on the roll mixer for 20-25 min. filling levels of sample tubes, wash buffer, waste etc., and have to be checked. The prepared materials are inserted into the sample carousel and the barcode is scanned automatically. After pipetting, the controls and calibrators are unloaded from the sample carousel, and stored cold directly. After the run the sera are started.

RESULTS :

Comparison of the concordance: BioCLIA 1200® versus Phadia 250®

This study was conducted in the serological department at the LADR GmbH MVZ Nord-West in Schüttorf, Germany, a private lab for laboratory medicine. A comparative study of the HOB BioCLIA 1200® (HOB Biotech Group, China) and the established Phadia 250® system (ThermoFisher Scientific, Sweden) was performed. Between the beginning of January and the beginning of June 2018, a total of 5982 measurements of 23 parameters are done (see Table 02) together with 600 control measurements (RU/mL). The measurements are performed on a same-day basis on both systems. Besides the comparison of the analyses, the operability and the daily handling is another point of the evaluation. The comparison resp. concordance of the parameters concerning discrepant results and the κ -values of the Cohen's kappa test are shown in graph 01 and graph 02.

In terms of agreement between the different methods the ANA-parameters anti-Scl-70 (p= 1.0 %), anti-Jo-1 (p= 1.1 %), anti-RNP (p= 1.3 %) and anti-SS-B/La (p= 1.4 %) achieved the best agreements with a compliance >98.5% for the detection of the antibodies in the sera.



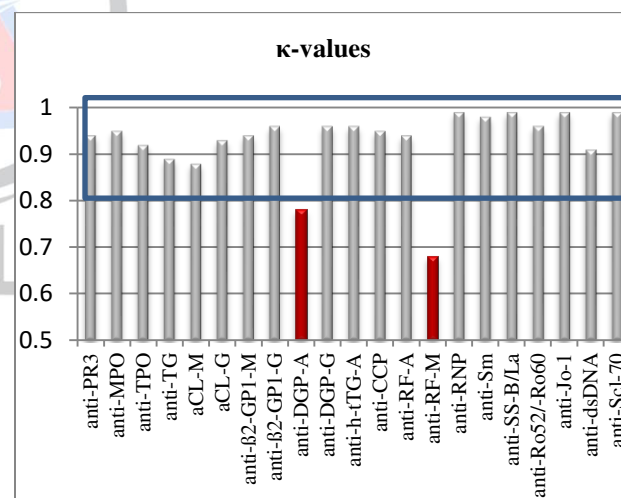
Graph 01: Overview of the parameters and their non-compliance (positive-negative /negative-positive)

measurements) between the Phadia 250® and HOB BioCLIA 1200® system. Values of < 10 % are acceptable and grey coloured. Red coloured bars have a non-compliance of ≥ 10 % and show a bold difference. Minimum one positive-positive-result is measured expecting anti-Jo-1, aCL-M and aCL-G.

Furthermore, the vasculitis parameters anti-PR3 and -MPO have also good concordance with only 5.2 % and 5.0 % discrepant results. Anti-GBM isn't mentioned due to the low number of sera.

Bad compliances (red bars, graph 01; ≥ 10 %) are found in four cases, for anti-TG (11.1 % of 208 measurements), aCL-M (11.7 % of 206 measurements), anti-DGP-A (21.7 % of 253 measurements) and anti-RF-M (31.3 % of 131 measurements).

Overall, the HOB BioCLIA 1200® system showed a higher positive rate compared to the Phadia 250® (see Table 02). Just in three cases, anti-TG, dsDNA and anti-Scl-70, the Phadia 250® system measured more positive results.



Graph 02: Calculated κ-values in the Cohen's kappa test. Strength of agreement: 1.00-0.81: almost perfect (blue box); 0.80-0.61: substantial; 0.60-0.41: moderate²³

The statistical agreement between the two systems is in 19 of 21 cases almost perfect. The two worst non-compliance results anti-DGP-A (21.7 %) and -RF-M (31.3 %) are calculated with a substantial (anti-DGP-A: 0.78; anti-RF-M: 0.68) agreement.

DISCUSSION:

Different immobilization techniques have led to an improvement of immunoassays with a regard to specificity and sensitivity, but the aim of the diagnostic tests to distinguish between patients with and without an autoimmune disease is the same. ELISA tests are moderately fast with assay times between 1.5 to 3 hours. The focus shifted towards a decrease in assay time and fully automated technologies. To reduce time to result and minimize hands-on time in the laboratory, new systems combining random access and CLIA technology have been developed and offer single patient testing together with assay times under 50 minutes. CLIAs are significantly different from ELISA techniques, as the antigen is covalently attached to the surface of the bead particles unlike the passive adsorption used for most ELISAs.²⁴

The group of patients shows a typical distribution pattern for autoimmunity for this laboratory, e.g. more samples of female than male and an age group focus on older adults. Thus, they are well suited for the evaluation.

As seen in Table 02, we observed in 13 of 21 parameters higher positive rates for the HOB BioCLIA 1200® system. Moreover, the celiac parameter anti-DGP-A and the rheumatic factor anti-RF-M have the poorest compliance and κ -values. In the ANA-group, anti-dsDNA showed a non-compliance of 8.5 % and a higher positive rate for the Phadia 250® system. This could be explained by the CLIA method's wide dynamic range and therefore a higher analytical sensitivity. This has to be clarified in a study with known diagnosis. The κ -value for anti-dsDNA is calculated with almost perfect (0.91). Whereas, the rheumatoid factor anti-IgM is calculated just with a substantial κ -value (0.68).

The third group of parameter with a higher positive rate are the celiac parameters anti-DGP-IgA and anti-DGP-IgG. The higher positive rate seems to be not plausible. A reason for this is obviously that celiac disease is not a relevant but rare disease in China.

Table 2: Overview of the non-compliance, the higher positive rates and statistical data in detail. Red coloured data show a bad non-compliance ($\geq 10\%$), in contrast the green coloured ones show the best accordance between the systems ($\leq 1.5\%$).

Parameter	Sera [n]	Non Compliance [%]	κ -values	Higher Positive Rate
Anti-PR3	135	5.2	0.94	HOB BioCLIA
Anti-MPO	80	5	0.95	HOB BioCLIA
Anti-TPO	866	7.4	0.92	HOB BioCLIA
Anti-TG	208	11.1	0.89	Phadia
aCL-M	206	11.7	0.88	HOB BioCLIA
aCL-G	199	6.5	0.93	HOB BioCLIA
Anti- β 2-GP-M	31	6.5	0.94	-
Anti- β 2-GP-G	26	3.9	0.96	-
Anti-DGP-A	253	21.7	0.78	HOB BioCLIA
Anti-DGP-G	235	4.3	0.96	HOB BioCLIA
Anti-h-tTG-A	714	3.8	0.96	HOB BioCLIA
Anti-CCP	1010	5.2	0.95	HOB BioCLIA
Anti-RF-A	127	6.3	0.94	HOB BioCLIA
Anti-RF-M	131	31.3	0.68	HOB BioCLIA
Anti-RNP	231	1.3	0.99	HOB BioCLIA
Anti-Sm	205	2	0.98	HOB BioCLIA
Anti-SS-B/La	211	1.4	0.99	-
Anti-Ro52	413	3.8	0.96	-
Anti-Jo-1	187	1.1	0.99	-
dsDNA	305	8.5	0.91	Phadia
Anti-Scl-70	198	1	0.99	Phadia
Anti-GBM	7	-	-	
Anti-CENP-B	4	-	-	

Thus, the assay is far too sensitive for a Caucasian patient pool.²⁵ Discussions with the manufacturers confirm the suspicion and leads to revalidation of the assays. An option could be to adapt the cut-off. In the current design this assay is not useable for a European market. Nevertheless, the most important serologic parameter for the celiac diagnostic is anti-h-tTG-A (tissue transglutaminase IgA) which exhibits a good concordance (non-compliance 3.8 %).

As expected from the literature very good correlations between CLIA and ELIA are shown

For anti-PR3, we found a very good agreement as in earlier studies, where a high percentage of agreement (95 %) could be shown between the CLIA and ELIA.²⁶ We observed the same result in our study (non-compliance 5.2 %; see Graph 01).

Also good results in terms of agreement between the different methods anti-Scl-70 (p= 1.0 %), anti-Jo-1 (p= 1.1 %), anti-RNP (p= 1.3 %) and anti-SS-B/La (p= 1.4 %) achieved the best agreements, in accordance to the manufacturers own studies.²⁷ The specificity of these four parameters were observed with e.g. 97.9 % (anti-RNP) to 100 % (anti-Jo-1).²⁸

One of the tasks of this study was to evaluate the daily routine in our high throughput lab. This is mainly based on subjective impressions of the technical staff being in charged for this study and well experienced in using fully automated instruments. The performance of the weekly and monthly service is very easy and not time-consuming. The containers for the wash buffer have a good size and are convenient. The software is neatly arranged, easy to handle and has an intuitive user interface/desktop. The system runs very stable, no technical problems were observed in the used period. The dead volume for a single test is very high (400 μ L). Controls and calibrators have no bar code. The tests have been added to the respective rack position manually. The (un-)load of the racks is uncomfortable. Each rack or sample position in the carousel has to be reselected via the software, manually.

CONCLUSION:

We evaluated the CLIA-based HOB BioCLIA 1200® in both, handling and performance in comparison. We tested 23 parameters overall but calculated the statistical parameters for 21 due to a too less value numbers for two parameters. In 17 of the examined 21 analytes we found a good compliance to the FEIA-based Phadia 250® system. In four cases (anti-TG, aCL-M, anti-DGP-A and -RF-M), we found a poor

compliance of over 10 %. The statistical agreement between the two systems is in 19 of 21 cases almost perfect with κ -values between 0.88-0.99. Overall, the HOB BioCLIA 1200® showed a higher positive rate due to a higher sensitivity or a lower reference range. In case of the celiac parameters it is obvious that celiac disease is not a relevant but rare disease in China. Thus, the assay is far too sensitive or needs a higher cut-off for a Caucasian patient pool. This is discussed with the manufacturers. The discrepant results for anti-RF-M are discussed. Currently, we are testing new kits for celiac disease and rheumatoid factors. The HOB BioCLIA 1200® is a stable running, random access system with an overall performance that makes it well usable in a medium sized laboratory.

ABBREVIATIONS

CLIA: chemiluminescent immunoassay; FEIA: fluorescent enzyme immune assay; EIA: enzyme immune assay; resp.: respectively; κ : Cohen's Kappa; AD: autoimmune disease; IgG/A/M: immunoglobuline G/A/M; uv: ultraviolet; LOD: limit of detection; IFM: immunofluorescence microscopy; RfB: Referenzinstitut für Bioanalytik; RU/ml: relative units per milliliter; k_d : dissociation constant; e.g.: exempli gratia or for example; ANA: antinuclear antibody.

Acknowledgements

The authors would like to thank Mrs Jutta Otten (LADR, Schüttorf; managing medical technical assistant) for organizing the lab work and supporting the threshold workers in this project and Prof. Dr. Arne Ring (medac, Wedel) for his advice for the statistical analysis.

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Cite of article: Knies C, Olivo M, Tönnies R, Von Landenberg P. Concordance of the analytical performance of autoimmune antibodies on the hob bioclia® 1200 automated immunoassay analyzer to the phadia 250® system. Int. J. Med. Lab. Res. 2019, 4(3): 21-28

CONFLICT OF INTEREST: Authors declared no conflict of interest

SOURCE OF FINANCIAL SUPPORT: Nil

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