Routinely use of BioCLIA® 6500 (HOB Biotech / Eurobio Scientific), a novel chemiluminescent immunoanalyzer in autoimmune diseases diagnosis

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Introduction

The detection of antibodies is often useful in diagnosing and/or classifying autoimmune diseases such as connective tissue diseases and antiphospholipid syndrome. Traditional dichotomous investigations were often time-consuming and did not allow prompt response to clinicians. Currently, the laboratory must perform safety diagnostic tests as soon as possible to reduce hospitalization time. BioCLIA® 6500 is a fully automated chemiluminescent immunoanalyzer described to perform safety diagnostic tests in a short time.

Objective

The aim of this study was to compare the predictive and discriminative performance of BioCLIA® Anticardiolipin (ACL), anti-B2GP1, anti-ENA (extractable nuclear antigen) and anti-dsDNA tests to conventional ELISAs or multiplex assays on well-defined clinical groups of patients and to daily evaluate, using multiparametric runs, the determination of these antibodies in a hospital laboratory.

Characteristics of coupled antigens in BioCLIA® reagents

Connective tissue diseases	dsDNA	Native, Calf Thymus
	Ro60	Native, Bovine
	SS-B/La	Native, Calf Thymus
	nRNP/Sm	Native, Bovine tissues
	Sm	Native, Calf Thymus
	Jo-1	Recombinant human histidyl-tRNA synthetase
	Scl-70	Native, Calf Thymus
	Ro52	Recombinant human Ro-52 (SSA) protein
	Rib-P	Recombinant Human ribosomal phosphoprotein P0
	PCNA	Recombinant Human PCNA
	PM-Scl	Recombinant Human PM-Scl protein
	His	Native, Calf Thymus
	CENP-B	Human recombinant centromere protein B
	Nuc	Native, Calf Thymus
	ENA Screen-I	Same antigens as individual assays (Sm, nRNP/Sm, SS-B/La, Ro52, Scl-70,Jo-1, Ro60 and CENP-B)
	CTD Screen	Same antigens as individual assays (Sm、dsDNA、Nucleosome、Histone、Rib-P、PCNA、Ro60、Ro52、SSB/La、Scl-70、Jo-1、PM/Scl、CENP-B、nRNP/Sm、AMA-M2)
Antiphospholipid syndrome	aCL-A	Synthetic cardiolipin & Native β2GP1
	aCL-G	Synthetic cardiolipin & Native β2GP1
	aCL-M	Synthetic cardiolipin & Native β2GP1
	aCL-A/M/G	Synthetic cardiolipin & Native β2GP1
	β2GP1-A	Native, Human β2GP1
	β2GP1-G	Native, Human β2GP1
	β2GP1-M	Native, Human β2GP1
	β2GP1-A/M/G	Native, Human β2GP1

BioCLIA Autoimmune Reagent kit CTD Screen
(Sm, nRNP/Sm, SS-B/La, Ro52, Scl-70, Jo-1, Ro60, CENP-B, dsDNA, nucleosome, histones, Rib-P, PCNA, PM/Scl, et AMA -M2)

BioCLIA Autoimmune Reagent kit ENA Screen-I
(Sm, nRNP/Sm, SS-B/La, Ro52, Scl-70, Jo-1, Ro60 et CENP-B)

Material and methods

Serum samples from 200 patients with well-defined diseases, according to the classification criteria approved by the American College of Rheumatology or defined by the revised Sapporo laboratory criteria (60 patients with systemic lupus erythematosus (SLE), 20 patients with Sjögren syndrome, 10 patients with systemic sclerosis, 10 patients with myositis and 100 patients with Anti-phospholipid syndrome), were included in the first part of the study. Simultaneously, consecutive serum samples (n = 150) sent for detection of anti-ENA, anti-dsDNA antibodies and 150 sent for anti-phospholipid antibodies were analyzed in comparison with conventional assays. The BioCLIA® assay is based on a two-step indirect chemiluminescence immunoassay (CLIA) using biotinylated antigen and magnetic streptavidin-coated microparticles as solid phase. In the first step, the specific antibodies present in the sample bound to the solid phase and after extensive washing, a second antibody labeled with alkaline phosphatase as detection marker was added. After washing, a light signal was generated by chemiluminescent reaction. The product is based on the indirect chemical luminescence method of AMPPD and alkaline phosphatase (ALP). The principle of the reaction is ALP-promoting chemical luminescence using magnetic particles as carriers. It uses the free energy released by chemical reaction to stimulate the intermediates to return from the excited state to the ground state. During this change of state, photons of equal energy are released. Photons are measured for quantitative analysis and the signal was proportional to the amount of antibodies bound to the solid phase. The results were then compared to those obtained with our routinely used tests.

Results

Each calibration curve is kept in the automate for 28 days, and daily controls were performed to valid the run. BioCLIA® reagents have shown good diagnostic performance, in terms of sensitivity and specificity. Global agreements between BioCLIA® and routinely used assays were from **90% to 98%** for anti-ENA and **70% to 97%** for antiphospholipid antibodies in screening assays as well as specific assays. Discrepancy analysis showed higher sensitivity for the detection of anti-Ro52, anti-RNP and anti-B2GP1 IgM on BioCLIA® and it appeared to be less sensitive for anti-dsDNA and ACL IgG determinations.

Anti -	Ro52 Luminex	SSA Luminex	SSB Luminex	SM Luminex	RNP Luminex	Jo1 Luminex	Scl-70 Luminex	dsDNA ELISA	aCL-G ELISA	aCL-M ELISA	B2GP1-G FEIA	B2GP1-M FEIA
Agreement %	94	91	89.5	94	94	98	97	92	70	77	87.5	83

Anti- Ro 52

LUMINEX	BioCLIA	
	N	P
N	71	7
P	1	54

Anti - SSA

LUMINEX	BioCLIA	
	N	P
N	65	5
P	7	55

Anti - SSB

LUMINEX	BioCLIA	
	N	P
N	88	6
P	8	31

Anti- Sm

LUMINEX	BioCLIA	
	N	P
N	108	3
P	5	17

Anti- RNP

LUMINEX	BioCLIA	
	N	P
N	87	7
P	1	37

Anti - Jo1

LUMINEX	BioCLIA	
	N	P
N	116	2
P	1	14

Anti- Scl70

LUMINEX	BioCLIA	
	N	P
N	126	3
P	1	3

Anti - dsDNA

ELISA	BioCLIA	
	N	P
N	52	2
P	7	54

aCL - G

ELISA	BioCLIA	
	N	P
N	40	1
P	34	42

aCL- M

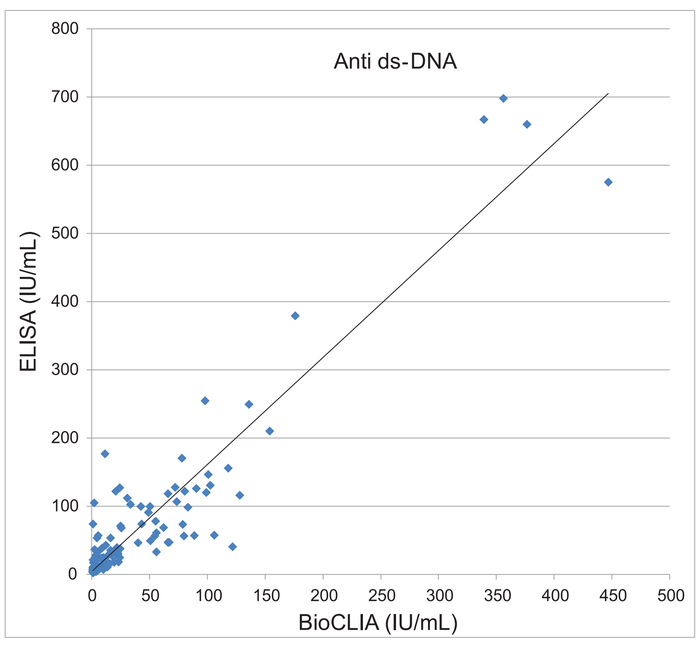
ELISA	BioCLIA	
	N	P
N	51	21
P	6	38

Anti- B2GP1- G

FEIA	BioCLIA	
	N	P
N	49	0
P	12	35

Anti- B2GP1- M

FEIA	BioCLIA	
	N	P
N	43	9
P	8	41



Good correlation was available between CLIA and ELISA for anti-dsDNA antibodies (r^2 = **0.88**), while titer correlations for ACL and anti-B2GP1 antibodies were not easy due to different standards and units in comparative tests.

Discussion

Recent advances in diagnostic technologies have enhanced the importance of antibody determination in autoimmune diseases, especially at early stages. The practical approach in an autoimmunity laboratory tended to perform multiparameter tests in a short time. Automation can improve the reproducibility and reduce interlaboratory variation, which remains a major problem in autoimmunity. The reagents available on the BioCLIA® analyzer exhibit good diagnostic performances, in terms of sensitivity, specificity, positive and negative predictive values. The discrepancies observed with our conventional routine tests were not higher than the discrepancies between other ELISA or other technologies, depending on the different nature of the antigens and the lack of standardization. Moreover, the majority of the discrepancies were close to the cutoff zones for either test. The main advantages of BioCLIA® were full automation and flexibility of work modalities. Quality and safety are ensured by barcode scanning for all reagents and samples, allowing full traceability of samples, reagents and operators. Different reagent cartridges can stay on board in a refrigerated area with stability for 28 days and each calibration is stable for 28 days. The software is user-friendly and shows good visibility of remaining reagents, lot numbers, expiration date and good tracking of quality controls. Then the analyzer is always ready for use after daily automatic wake-up. All types of tubes were accepted in the loading tray to limit decantation of serum samples and risk of error.

Conclusion

BioCLIA® 6500 analyzer is easy to rapidly detect the most common autoantibodies in autoimmune diseases. This system has the potential to provide clinically useful data in a short time. By means of the flexibility of its work modalities (random access and stat position), it is well adapted to determine antigenic specificities in daily practice, even in emergency.