Anti-double stranded deoxyribonucleic acid antibodies testing - comparison between immunofluorescence assay and automated enzyme immunoassay: A single center experience

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Background
Autoimmune rheumatic diseases are autoimmune disorders presented with joint and muscles manifestations. They are characterized by presence of antinuclear antibodies (ANA). ANA include autoantibodies to extractable nuclear antigens, autoantibodies to histones and deoxyribonucleic acid (DNA). Anti-double stranded DNA (dsDNA) antibodies are recognized as diagnostic markers of systemic lupus erythematosus (SLE) and as indicators of SLE disease activity, especially in lupus nephritis. The significance of anti-dsDNA in SLE diagnosis and in monitoring SLE disease activity has led to increase in this test laboratory requests as well as in the number of commercially available kits.

Aim of the work
This study aims to evaluate the performance of Alegria anti-dsDNA screen test method in the Laboratory of Clinical Immunology, Assiut University and to compare its results to the results obtained by immunofluorescence method.

Materials and methods
Evaluation of the performance of Alegria anti-dsDNA screen test as a qualitative test was evaluated through method comparison according to Clinical and Laboratory Standard Institute (CLSI) guidelines. Results obtained by Alegria anti-dsDNA screen kit were compared with that obtained by nDNA Fluoro-Kit indirect fluorescent antibody test. Manufacturer's recommended reference interval of Alegria anti-dsDNA screen was verified according to CLSI guideline.

Result
Alegria anti-dsDNA screen ELISA kit showed 94.4% positive agreement, 37% negative agreement and 65.7% overall agreement with nDNA Fluorokit.
In the verification study of manufacturer's reference interval (negative <25 U/ml), 5/40 specimens (12.5%) from healthy subjects were positive which exceeds the acceptance criteria of 10%. ROC curve methodology was used to analyze results of both methods and cut off value was adjusted. With cut off adjustment, method specificity increased but sensitivity was decreased. Optimal cut off was determined to be 74U/ml with acceptable level of both sensitivity and specificity (72% and 74%, respectively).

Conclusion
Alegria anti-dsDNA screen method demonstrated good sensitivity and low specificity. With manufacturer's cut off adjustment, specificity was improved. Manufacturer's reference interval of Alegria anti-dsDNA screen was not verified. A new cut off value was suggested, for further validation in an independent study.

Keywords:
anti-dsDNA antibodies, Crithidia Lucillaie immunofluorescence, enzyme-linked immunosorbent assay, systemic lupus erythematosus

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Introduction

Autoimmune rheumatic diseases (ARDs) are autoimmune disorders presented with joint and muscles manifestations. However, other organs may be involved at a varying degree in different conditions. They are also called connective tissue diseases (CTDs) or collagen diseases. They include systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), Sjogren's syndrome, systemic sclerosis, polymyositis and dermatomyositis and mixed connective tissue disease [1].

Autoimmune rheumatic diseases are characterized by presence of antinuclear antibodies (ANA). ANA include autoantibodies to extractable nuclear antigens, autoantibodies to histones and deoxyribonucleic acid (DNA). These antibodies are involved in disease

pathogenesis, and their presence in patients' sera constitute one of the criteria used (together with the clinical manifestations) for disease diagnosis [2].

Anti-DNA antibodies include those against single and double stranded DNA (ssDNA and dsDNA, respectively). Anti-dsDNA antibodies are recognized as diagnostic markers of SLE and as indicators of SLE disease activity, especially in lupus nephritis [3]. However, high anti-dsDNA levels are found only in 50–70% of SLE patients. So, negative anti-dsDNA test does not exclude SLE. Also, anti-dsDNA antibodies can be detected in other autoimmune diseases such as RA, as well as in healthy blood donors [3]. The significance of anti-dsDNA in SLE diagnosis and in monitoring SLE disease activity has led to increase in this test laboratory requests as well as in the number of commercially available kits [4].

Many different assays have been used for the detection of anti-ds DNA. The most widely used and documented methods are radioimmuno-assay (RIA) or Farr assay, Crithidia Lucillaie immunofluorescence (CLIFT), and enzyme-linked immunosorbent assay (ELISA) [5]. Farr assay was considered the gold standard procedure for measuring anti-dsDNA antibodies but, its use became extremely limited as the method is time consuming and due to the use of radioactive materials [6,7]. CLIFT has high specificity for anti-dsDNA antibodies and currently considered the gold standard method [8]. ELISA is becoming a prevalent method used in laboratory practice. It is relatively cheap and rapid assay, can be automated [9] and can detect different isotypes of anti-dsDNA [10].

This research was designed to compare anti ds-DNA test results performance obtained by an automated ELISA system to that obtained by CLIFT as a gold standard method.

Materials and methods

This study was approved by the Ethical Committee of the Faculty of Medicine, Assiut University (IRB no. 17100369) and it was done in the laboratory of clinical immunology, Clinical Pathology Department, Assiut University Hospital.

Evaluation of Alegria anti-dsDNA screen test method as a qualitative test was done through comparison method study according to Clinical and Laboratory Standard Institute (CLSI) guideline; CLSI EP12-A2. Fifty four positive and 54 negative serum samples by the nDNA Fluoro-kit indirect fluorescent antibody test (Cat. no. 1852, Diasorin, USA) were tested by Alegria anti-dsDNA screen test method (Cat. no. ORG204S, Orgentec Diagnostika GmbH, Germany). Alegria anti-dsDNA screen test was performed according to manufacturers' instructions using Alegria automated instrument (Orgentec, Germany). Also, manufacturer's recommended reference interval of alegria anti-dsDNA screen (negative <25 U/ml) was verified according to CLSI guideline; CLSI EP28-A3. Forty serum specimens from apparently healthy subjects with normal laboratory findings (negative ANA) were tested by Alegria anti-dsDNA screen method.

Statistical analysis

IBM SPSS statistical package version 26 was used in performing Receiver Operating Characteristics (ROC) curve analysis.

Results

Alegria anti-dsDNA screen testing of 54 positive specimens and 54 negative specimens revealed 51 true positive, 34 false positive, 20 true negative and 3 false negative (Table 1).

The positive percent agreement (PPA) was 94.4% (95% CI: 88.07–94.9%), negative percent agreement (NPA) was 37.03% (95% CI: 25.4–50.37%), and overall percent agreement was 65.7% (95% CI: 56.38–74.01%).

Out of the 40 serum specimens from healthy control subjects, 5 specimens (12.5%) were positive (≥25 U/ml) by alegria anti-dsDNA screen method. This percentage exceeds the acceptance criteria of 10%. These 5 positive specimens were retested with nDNA Fluoro immunofluorescence kit and gave negative results.

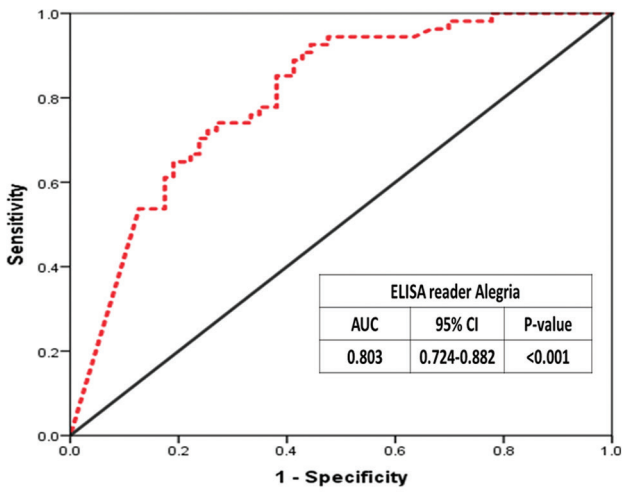
According to the results of reference interval verification together with the increased number of false positive results in method comparison, re-assessment of the cut off value (25 U/ml) and investigation of other values as cut off level were suggested.

Test results of 118 serum specimens by both alegria anti-dsDNA screen and nDNA Fluoro immunofluorescence methods were analyzed by the ROC curve methodology. ROC curve analysis results are shown in Fig. 1 and Table 2.

Table 1 Results of comparison method study

Candidate Method Alegria anti-dsDNA screen	Comparative Method nDNA Fluoro Kit (Immunofluorescence)		
	Positive	Negative	Total
Positive	51	34	85
Negative	3	20	23
Total	54	54	108

Figure 1



ROC curve for Alegria anti-dsDNA screen method.

Table 2 Examples of cut offs given by the ROC curve and their corresponding sensitivity and specificity

Parameter	Anti-ds DNA by Alegria					
AUC	0.803 (0.724–0.882)					
Cut-off	25	35	40	50	60	70
Accuracy	67%	72.5%	73.5%	70%	71.5%	72.5%
Sensitivity%	94%	94%	91%	78%	76%	72%
Specificity%	40%	51%	56%	62%	67%	73%

With the manufacturer's cut off value of 25 U/ml, sensitivity was good (94%), specificity was low (40%) and overall method accuracy was 67%. As the cut off value increases, method specificity was increased, but, with concomitant decrease in its sensitivity.

Determination of the optimal cut off which is the cut off with highest acceptable level of both sensitivity and specificity was done according to (Zhou *et al.*, 2002) [11]. The optimal cut off could be achieved by adjusting the cut off value from 25 U/ml up to 74 U/ml with acceptable level of both sensitivity and specificity (72% and 74%, respectively). As cut off value increases, false positive results would decrease.

Discussion

In this study, Alegria anti-dsDNA screen ELISA kit showed 94.4% positive agreement, 37% negative agreement and 65.7% overall agreement with nDNA Fluoro CLIFT kit. In the study of [4], positive agreement between different ELISA assays (six kits not including Orgentec kits) and CLIFT ranged from 57% to 76%, negative agreement ranged was 65–92%, and overall agreement was 70–80%. In another study [12], positive agreements between two ELISA assays (not including Orgentec kits) and CLIFT were 94.1% and 88.2%, and negative agreements were 93.4% and 72.6%. The variation among different ELISA assay in agreement with CLIFT method may be related to the source of antigen used. DNA antigen sources include calf thymus, salmon testes, bacteriophage X, plasmid DNA and recombinant DNA [13]. Alegria anti-dsDNA screen ELISA kit uses recombinant human DNA as antigen.

In our study, 34 out of 54 CLIFT negative serum specimens were positive by Alegria anti-dsDNA screen ELISA method; 34 false positive specimens. Anti-dsDNA ELISAs may give false-positive results due to binding of immune complexes to the pre-coat intermediates [3]. Another explanation is the presence of anti-ssDNA antibodies that may cross-react with the anti-dsDNA ELISA kits [12]. Also, ELISA can detect low-avidity anti-dsDNA antibodies which are not detected by CLIFT. The low avidity antibodies generally have little clinical importance and may be present in other CTDs, inflammatory and infectious diseases [14,15]. High avidity anti-dsDNA antibodies are more specific for SLE diagnosis and more closely associated with the pathogenesis of lupus nephritis. Low avidity antibodies may be associated with cerebral involvement [14,16].

It was reported that ELISA positive and CLIFT negative anti-dsDNA testing may have a clinical significance. Nearly 80% of ELISA positive and CLIFT negative patients met ≥3 of the SLE classification criteria (other than anti-dsDNA) [13]. Anti-dsDNA antibodies may be present in asymptomatic patients many years before a clinical diagnosis of SLE can be made [17].

In the current study, Alegria anti-dsDNA screen ELISA kit showed 94% sensitivity and 40% specificity at cut off value of 25 U/ml. When ROC curve was used to adjust the cut off value, method specificity increased as the cut off value was increased. Specificity reached 73% at cut off value of 70 U/ml. However, this specificity increment was accompanied with decrease in method sensitivity (sensitivity decreased to 72% at cut off value of 70 U/ml). Similarly, the study of [18] reported that the specificity of two anti-dsDNA ELISA kits (not including Orgentec kits) was increased after adjustment of the cut off using the ROC curve analysis.

Optimal cut off was determined to be 74 U/ml with acceptable level of both sensitivity and specificity (72% and 74%, respectively). This new cut off should be validated in an independent study.

Conclusion

Alegria anti-dsDNA screen method demonstrated good sensitivity and low specificity. With

manufacturer's cut off adjustment, specificity showed improvement. Manufacturer's reference interval of Alegria anti-dsDNA screen was not verified. A new cut off value was suggested, for further validation in an independent study.

Meanwhile, alegria anti-dsDNA screen method can be used with manufacturer's cut off value, as a screening test followed by confirmation of positive specimens by nDNA Fluoro kit.

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Nil.

Conflicts of interest
There are no conflicts of interest.

References

1 Brambilla C, Rice A, Nicholson AG. Histology of pulmonary and bronchiolar disorders in connective tissue diseases. Semin Respir Crit Care Med 2019; 40:147–158. doi: 10.1055/s-0039-1684029.

2 Emad Y, Gheita T, Darweesh H, Klooster P, Gamal R, Fathi H, *et al*. Antibodies to extractable nuclear antigens (ENAS) in systemic lupus erythematosus patients: correlations with clinical manifestations and disease activity. Reumatismo 2018;70:85-91. doi: 10.4081/reumatismo. 2018.1027.

3 Steiman AJ, Urowitz MB, Ibanez D, Li TT, Gladman DD, Wither J. Anti-dsDNA and Antichromatin antibody isotypes in serologically active clinically quiescent systemic Lupus Erythematosus. J Rheumatol 2015; 42:810–816. doi: 10.3899/jrheum. 140796.

4 Chiaro TR, Davis KW, Wilson A, Suh-Lailam B, Tebo AE. Significant differences in the analytic concordance between anti-dsDNA IgG antibody assays for the diagnosis of systemic lupus erythematosus—Implications for inter-laboratory testing. Clin Chim Acta 2011; 412:1076–1080. https://doi.org/10.1016/J.CCA.2011.02.025.

5 Martin J, Durant C, Rimbert M, Hemont C, Hamidou M, Audrain M. Evaluation of two antibodies against double-stranded DNA assays in discriminating between active and non-active systemic lupus erythematosus: Correlation between the cut-off and the specificity. Pathol Biol 2012; 60:387–391. https://doi.org/10.1016/J.PATBIO.2011.12.003.

6 Rouquette A, Desgruelles C. Detection of antibodies to dsDNA: An overview of laboratory assays. Lupus. 2016;15:403–407. http://dx.doi. org/10.1191/0961203306lu2324oa.https://doi.org/10.1191/0961203306LU2324OA.

7 Zhao J, Wang K, Wang X, Li T, Guo L, Gu L, *et al*. The performance of different anti-dsDNA autoantibodies assays in Chinese systemic lupus erythematosus patients. Clin Rheumatol 2018; 37:139–144. https://doi.org/10.1007/s10067-017-3771-x.

8 Enocsson H, Sjöwall C, Wirestam L, Dahle C, Kastbom A, Rönnelid J, *et al*. Four anti-dsDNA antibody assays in relation to systemic lupus erythematosus disease specificity and activity. J Rheumatol 2015; 42:817-25. https://doi.org/10.3899/jrheum.140677.

9 Yung S, Chan TM. Mechanisms of Kidney injury in Lupus Nephritis – the role of anti-dsDNA antibodies. Front Immunol 2015; 6:475. doi: 10.3389/fimmu.2015.00475.

10 Biesen R, Dähnrich C, Rosemann A, Barkhudarova F, Rose T, Jakob O, *et al*. Anti-dsDNA-NcX ELISA: dsDNA-loaded nucleosomes improve diagnosis and monitoring of disease activity in systemic lupus erythematosus. Arthritis Res Ther 2011; 13:26. 1–9. https://doi.org/10.1186/AR3250.

11 Zhou XH, Nancy A, Donna K. Statistical Methods in Diagnostic Medicine. Indiana University: Wiley; 2002. pp. 50–96.

12 Pavlovic M, Kats A, Cavallo M, Chen R, Hartmann JX, Shoenfeld Y. Pathogenic and Epiphenomenal anti-DNA antibodies in SLE. Autoimmune Dis 2010; 2010:1–18. doi: 10.4061/2010/462841.

13 Cavalcante EO, Suzuki LA, Rossi CL. A quantitative enzyme-linked immunosorbent assay (ELISA) for the detection of anti-double-stranded DNA IgG antibodies. Jornal Brasileiro de Patologia e Medicina Laboratorial 2019; 55:160–169. [Accessed 29 May 2022]. Available from: <https://doi.org/10.5935/1676-2444.20190018>. Epub 23 May 2019. ISSN 1678-4774. https://doi.org/10.5935/1676-2444.20190018.

14 Andrejevic S, Jeremic I, Sefik-Bukilica M, Nikolic M, Stojimirovic B, Bonaci-Nikolic B. Immunoserological parameters in SLE: High-avidity anti-dsDNA detected by ELISA are the most closely associated with the disease activity. Clin Rheumatol 2013; 32:1619-26. https://doi.org/10.1007/s10067-013-2330-3.

15 Antico A, Platzgummer S, Bassetti D, Bizzaro N, Tozzoli R, Villalta D, (SIMeL), S.G. on A.D. of the I.S. of L.M. Diagnosing systemic lupus erythematosus: new-generation immunoassays for measurement of anti-dsDNA antibodies are an effective alternative to the Farr technique and the Crithidia luciliae immunofluorescence test. Lupus. 2010; 19:906–912. http://dx.doi.org/10.1177/0961203310362995. https://doi.org/10.1177/0961203310362995.

16 Sohrabian A, Parodis I, Carlströmer-Berthén N, Frodlund M, Jönsen A, Zickert A, *et al*. Increased levels of anti-dsDNA antibodies in immune complexes before treatment with belimumab associate with clinical response in patients with systemic lupus erythematosus. Arthr Res Ther 2019; 21,259. https://doi.org/10.1186/s13075-019-2056-y.

17 Mohammed AGA, Alghamdi AA, ALjahlan MA, Al-Homood IA. Echocardiographic findings in asymptomatic systemic lupus erythematosus patients. Clin Rheumatol 2017; 36:563–568. doi: 10.1007/s10067-016-3486-4.

18 Zhao J, Wang K, Wang X, Li T, Guo L, Gu L, *et al*. The performance of different anti-dsDNA autoantibodies assays in Chinese systemic lupus erythematosus patients. Clin Rheumatol 2018; 37, 139-144. https://doi.org/10.1007/s10067-017-3771-x.