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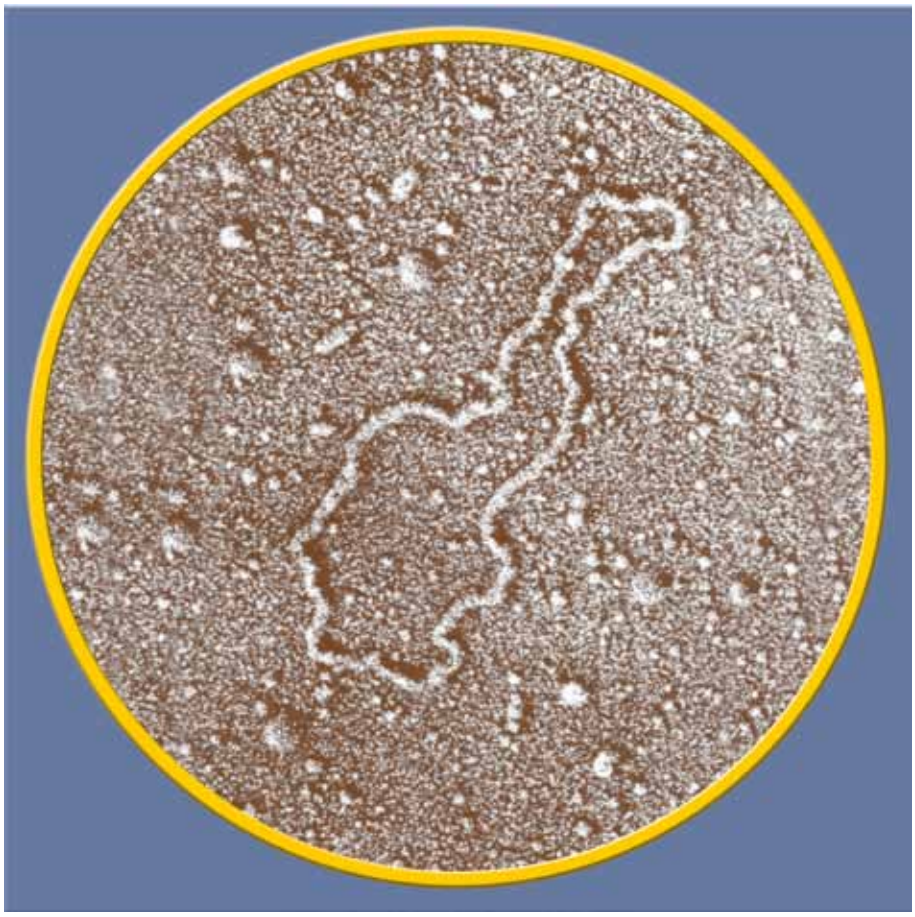

EliA™
Excellence in Autoimmunity

CONTENTS

Electron-microscopic picture of the plasmid DNA pSC101 (JD Watson et al. (eds), Recombinant DNA, 2nd Ed., 1992, WH Freeman & Company, New York, Oxford, Scientific American Books)

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N. Olschowka
G. Messer
P. White
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EDITORIAL

dsDNA Antibodies - the never-ending story in autoimmune diagnosis. What is the right method, are they really specific, which antibodies are the right ones? Every time the discussion is taken up again, it causes a stir and one gets the impression it was a question of faith.

Since the beginning of the Elias Journal in 1992, dsDNA antibodies have been a subject, and even just two Journals ago (2/2001) we reported on the new EliA dsDNA and the first studies with this new automated EIA.

Pharmacia Diagnostics produces enzyme-linked immunosorbent assays (ELISAs), so we always have been confronted with the typical problem of the ELISA in the dsDNA antibody detection: it is too sensitive. With the use of ELISAs for the measurement of dsDNA antibodies the low-avidity antibodies were detected - and dsDNA antibodies which were believed to be highly specific for SLE suddenly could be found in numerous clinical syndromes like uveitis, discoid lupus erythematosus, rheumatoid arthritis, juvenile rheumatoid arthritis and further in a wide variety of patients. For achieving an SLE disease specificity it was necessary to measure only high avidity antibodies. This is possible with the Farr RIA, which makes this method so specific for SLE. But nevertheless, the Farr RIA has never been the most common test in practical routine, because it is time-consuming and involves the use of radioactive material. With the development of the EliA™ dsDNA, it was our aim to offer a non-radioactive, fully automated test for IgG, more sensitive and easier to handle than IIF tests on Crithidia luciliae and as specific as the Farr RIA for SLE. First studies with the EliA™ dsDNA let us believe that we reached the goal! This should be reason enough to focus another Elias Journal on the topic dsDNA antibodies - the never-ending story in autoimmune diagnosis.

Prof. Rekvig from Norway is a specialist on dsDNA antibody detection and summarizes for us the up-to-date knowledge on this topic. Further on page 7, we give a short overview on the methods of dsDNA antibody detection with their pros and cons. This is followed by some interesting results comparing EliA™ with ELISA in the clinical laboratory, obtained by Dr. Messer from Munich. And last but not least, Peter White from the UK NEQAS reviews the dsDNA antibody data from 1999 to 2001, showing existing problems with the detection of dsDNA antibodies in practical routine.

Pleasant reading

The immunological basis for selecting anti-dsDNA antibody assays

Ole Petter Rekvig

Department of Biochemistry, Molecular Genetic research group, Institute of Medical Biology, Faculty of Medicine, University of Tromsø, N-9037 Tromsø, Norway

In systemic lupus erythematosus (SLE), as we understand the disease today, B and T cell autoimmunity to nucleosomes, and particularly to the individual components of nucleosomes, native (ds)DNA and histones, is important [1]. These autoimmune phenomena may be of diagnostic value for SLE. Furthermore, subpopulations of anti-dsDNA antibodies have the potential to induce nephritis, typical of SLE [2-7]. Although the insight into molecular and cellular processes responsible for the production of antibodies to dsDNA has increased significantly over the last decade, the exact mechanism(s) in SLE remains to be established. In this review, the diagnostic impact of anti-DNA antibodies will be discussed in relation to our insight into the processes responsible for their production. The main question is whether anti-DNA antibodies in general or only subpopulations binding dsDNA at high avidity should be taken into diagnostic considerations.

Experimental observations argue for the view that humoral immune responses to ssDNA and dsDNA arise by molecular and cellular processes common to classical immune responses [8-11]. Such immune responses are, with some exceptions (e.g. polyclonal B cell activation), antigen selective, T cell dependent, undergo immunoglobulin class switch, and the antibodies affinity maturate towards the driving antigen. This generalized conclusion is based on analysis of the variable region structure of monoclonal anti-DNA antibodies [8,11-16]. More direct evidence comes from experiments where immunologically normal mice were immunized with DNA-peptide complexes [17-22]. These studies demonstrated that low avidity antibodies to ssDNA and/or dsDNA converge towards dsDNA with increasing avidity as the immune response progresses in context of deliberate immunizations. Although such structural and genetic data point to DNA as the unique and central antigen driving the immune response, we do not yet understand why, or if, these immune responses depend on, or define, an SLE disposition. A central question is how the initial immune response to e.g. ssDNA that occurs in virtually every individual can result in high avidity anti-dsDNA immune responses that will define SLE.

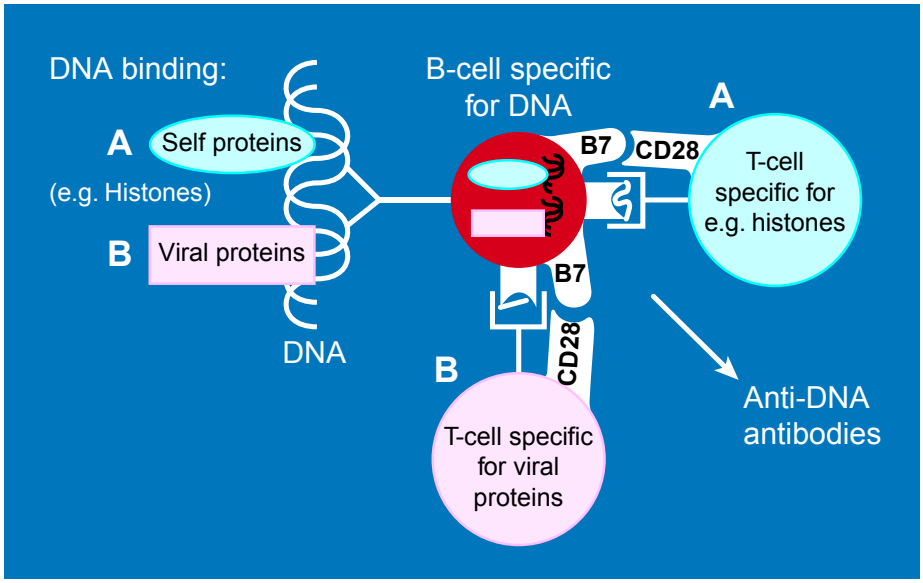

Figure 1. *A model based on experimental data for cognate B cell/T cell interaction for production of anti-DNA antibodies. Eventual stimulation of DNA-specific B cells by cross-reacting antigens are not considered*

The anti-DNA antibody response - a T cell dependent, antigen-selective process.

In the context of this problem, it is important to consider the elements involved in an anti-DNA antibody response. Antibodies to DNA may be generated providing that DNA is available to the immune system, and complexed with an immunogenic carrier protein, consistent with the traditional hapten-carrier model for initiation of anti-hapten antibodies. Such carrier proteins may derive from two sources; self or non-self. There is experimental evidence for both systems, as nucleosomes, and particularly histones, seem to be immunogenic for autoimmune T cells (Figure 1A) in SLE [23,24], but occasionally also in healthy individuals [24,25]. Viral, DNA-binding proteins may represent examples of non-self carrier proteins that may render DNA and nucleosomes immunogenic through their potential to stimulate T helper cells (Figure 1B) [22,25]. This general model, as outlined in Figure 1, describes how DNA may act as an immunogen with the potential to stimulate DNA-specific B cells.

Provided that DNA is complexed with immunogenic carrier proteins, an anti-DNA antibody response may be initiated. The early anti-DNA response is characterized by IgM antibodies with a dominant specificity for ssDNA (Figure 2A). Sustained stimulation results in immunoglobulin class switch to IgG, and accumulation of somatically mutated antibody variable regions. If these mutations introduce arginines at discrete positions (e.g. amino acid positions 99-100 of the heavy chain variable region [16,26]), specificity for dsDNA may develop. A B cell clone initially specific for ssDNA thus may gain specificity for dsDNA, and as the immune response progresses, affinity for dsDNA may increase resulting in high avidity anti-dsDNA antibodies. Alternatively, as outlined in

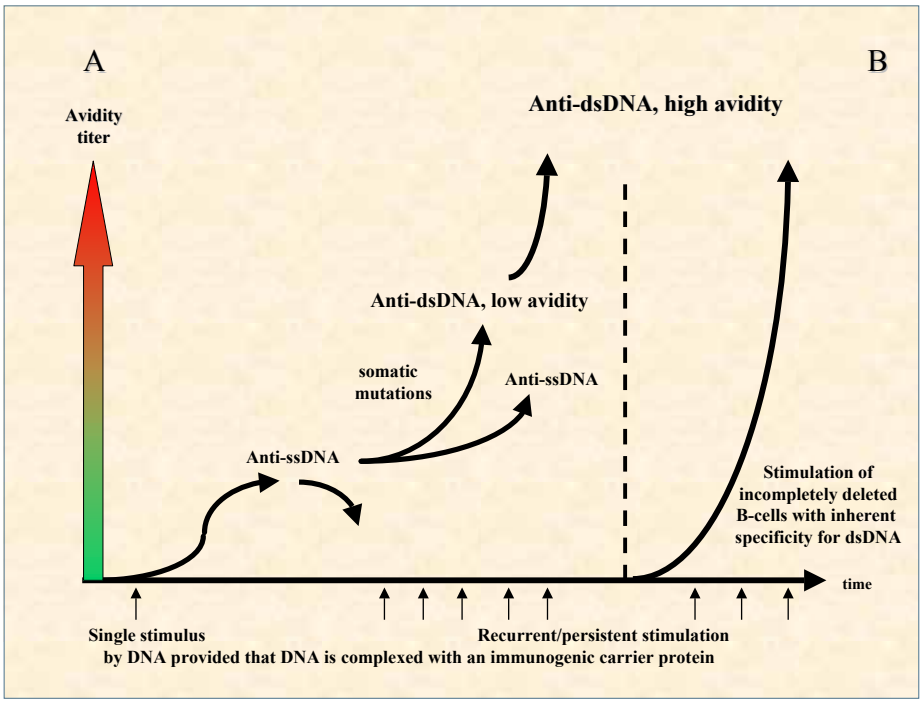

Figure 2. *Progressive development of anti-dsDNA antibodies as a consequence of sustained stimulation by DNA.*

Figure 2B, B cell clones with inherent specificity for dsDNA may escape deletion [27] and respond to immunogenic DNA. These may then clonally expand into high avidity anti-dsDNA antibody producing cells. Data consistent with this pathway for generation of anti-dsDNA antibodies have recently been provided [15]. B cells with specificity for dsDNA may also be functionally inactivated but not deleted. In this case, they may persist in the periphery where they may be subjected to activation that overcomes their anergic state.

A significant proportion of the total B cell population has the potential to bind, and eventually respond to, immunogenic DNA [28,29]. Such B cells may be stimulated by several different mechanisms, involving e.g. non-selective stimulation by Epstein Barr virus [30], or by polyclonal B cell activators like LPS [31,32], or antigen-selective processes as outlined in Figure 1 [17,23,25,33]. Also drugs may correlate to the development of anti-DNA antibodies, but this process is not yet fully understood. Nevertheless, whether the stimulation of these B cells are caused by antigen-selective or non-selective processes, antibodies to ssDNA may be produced. Since DNA-specific B cells and ligands with the potential to stimulate these are abundant, one should expect the presence of anti-ssDNA antibodies among individuals irrespective of their clinical conditions, and also amongst healthy individuals as well.

Consistent with this view, and not unexpectedly, anti-ssDNA antibodies are frequently observed among healthy individuals, among individuals suffering from chronic viral, bacterial or parasitic infections, or after ingestion of certain drugs. Anti-ssDNA, and occasionally anti-dsDNA antibodies, are also observed in context of malignancies and in individuals with no apparent or known cause for this immune response (reviewed in reference [1]). Thus, presence of antibodies to ssDNA does not distinguish healthy individuals from individuals suffering from SLE. Antibodies to ssDNA should therefore not be considered clinically important (see below). This may be somewhat of a paradox as antibodies to ssDNA may develop into antibodies to dsDNA as the immune response progresses [9,11,20,34,35] (Figure 2A).

This fact revitalizes the old problem whether specific activation of autoimmune dsDNA specific B cells is regulated differently in normal individuals and SLE, or whether appropriate immunogenic autoantigen availability is increased in SLE. Currently, there is an ongoing discussion whether levels of apoptotic nucleosomes are increased in SLE compared to normal individuals [36,37]. Increased levels of nucleosomes may represent a reservoir of antigens that may stimulate DNA-specific B cells, but concrete evidence for this is still unavailable.

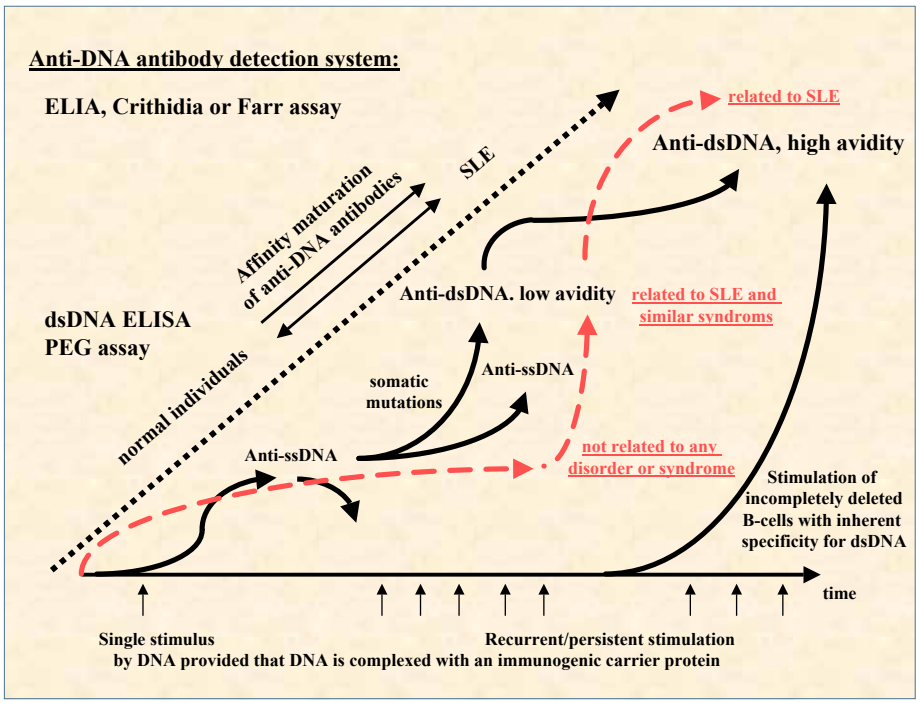

Figure 3. *Progressive development of anti-dsDNA antibodies related to clinical conditions and to different analytical assays.*

Are anti-ssDNA antibodies clinically important?

The processes described in Figure 2 may point at two possibilities when we consider the clinical impact of anti-ssDNA antibodies. Either, antibodies to ssDNA may be regarded clinically insignificant as they may be produced by virtually all individuals irrespective of their clinical condition (Figure 3). In this context, they do not distinguish an autoimmune disease from a healthy situation. Alternatively, anti-ssDNA antibodies may be regarded as precursor antibodies for anti-dsDNA antibodies. That is, anti-ssDNA antibodies may in certain situations indicate a potential for development of anti-dsDNA antibodies. However, we have today no known parameter to distinguish those individuals who produce anti-ssDNA antibodies that will progressively develop into high avidity anti-dsDNA antibodies from those who will not. From this rational, to test for antibodies to ssDNA should definitively be discouraged.

Diagnostically valuable anti-dsDNA antibodies: what is the correct method for their detection?

While the progressive development of high avidity antibodies specific for dsDNA relates to SLE (graphically presented in Figure 3), the potential to produce anti-ssDNA antibodies and possibly also low avidity anti-dsDNA antibodies may be inherent properties of the normal immune system. The latter antibodies may therefore be of less diagnostic value for SLE [38,39]. Nevertheless, for routine diagnostic work, an assay repertoire should be selected that allows detection also of low avidity anti-dsDNA antibodies, leaving anti-ssDNA undetected. ELISA is suitable for assaying this anti-dsDNA antibody sub-population; is quantitative, relatively sensitive, and detects anti-dsDNA antibodies at a higher frequency than Crithidia luciliae or Farr assays do [38,40]. The same is true for Pharmacia's newly developed EliA™ assay for anti-dsDNA antibodies (unpublished observations). It may therefore be advisable to implement anti-dsDNA ELISA as support for other less sensitive, but diagnostically more precise, anti-dsDNA antibody assays. The drawback of the ELISA assay is that it does not clearly discriminate between SLE and other disorders (see above and Figure 3).

In summary, this presentation describes the principal processes that result in anti-DNA antibodies. It is, from contemporary knowledge, evident that the ability to produce anti-DNA antibodies belongs to all of us. Therefore, it is important to consider this knowledge when selecting tests for anti-dsDNA antibodies, claimed to be marker antibodies for SLE. Tests for high avidity anti-dsDNA antibodies virtually diagnostic for SLE have low sensitivity. This links such tests to a conflict between clinical sensitivity and specificity. As analytically sensitive tests are not exact diagnostic instruments, those that are more disease-specific have a lower detection rate, also in definitive SLE. Only the latter may be regarded as relatively specific for SLE, while the dsDNA ELISA test may be valuable to perform, but isolated results from this assay should be interpreted with caution.

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Methods for the detection of anti-dsDNA autoantibodies

Nina Olschowka
Pharmacia Deutschland, Diagnostics Division, Global Marketing, Munzinger Str. 7, D-79111 Freiburg

Systemic lupus erythematosus (SLE) is an inflammatory multi-systemic disease. Clinical course and laboratory manifestations vary widely, making diagnosis difficult. Among the immunological abnormalities, the production of anti-dsDNA and anti-Sm antibodies is specific for the disease and, therefore, included in the diagnostic criteria for SLE.

In addition, dsDNA antibodies reflect disease activity, serve as a predictor of disease exacerbation and are suitable to monitor the response to therapy. Dependent on disease activity and detection method, DNA antibodies are found in 10-96% (!) of SLE patients (see table). Their accurate detection is of great clinical interest.

Among the multitude of anti-DNA antibody assays the most commonly used are IIF on Crithidia luciliae, radiobinding assays (mainly Farr assay) and enzyme-linked immunosorbent assays (ELISAs).

Anti-dsDNA antibodies, however, are not homogeneous but differ with respect to avidity, Ig-class, antigenic specificity, complement fixing ability and cross-reactivity. Depending on the methods used this can often lead to discrepant results. The pros and cons of the different methods shall be discussed in the following.

Crithidia luciliae immunofluorescence test (CLIFT)

The CLIFT is technically easy to handle and combines acceptable sensitivity with high disease specificity. In most studies, the sensitivity is lower than in ELISA or RIA (see table) but, nevertheless, it is considered to be sufficient for routine diagnosis.

The antibody concentration in a patient's serum is evaluated by titration. Therefore, quantitation in the CLIF test is limited. Thus, although the CLIFT is most widely used in routine practice, it is only useful for diagnosis, not for monitoring [1].

To obtain good reproducibility, the quality of the substrate is criticial. According to Smeenk et al. (1982) it is important to never let the slides dry out during the IIF procedure, since local increases in salt concentration will lead to dissociation of low avidity DNA-antibody interactions [2].

CLIFT measures antibodies of all avidities. The selection of the conjugate antibody enables differentiation of the autoantibody class.

Radioimmunological assay (RIA)

For the detection of anti-dsDNA the most widely used RIA is the Farr assay, which has been advocated as the most reliable method with the highest disease specific-

	ELISA	CLIFT	RIA
Aarden et al. 1975, Ann NY Acad Sci 254:505		30%	
Blazek et al. 1991, Lab Med 15:490	94%		
Clarke et al. 1986, Diagn Immunol 4:288	60%	68%	
Eaton et al. 1983, Arthritis Rheum 26:52	80%	58%	
Emlen et al. 1990, J Immunol Meth 132:91	52%		
Geißler et al. 1986, Immun Infekt 3:119	87%	47%	
Halbert et al. 1981, J Lab Clin Med 97:97	85%		
Isenberg et al. 1987, Ann Rheum Dis 46:448	33%	13%	
Kadlubowski et al. 1991, J Clin Pathol 44:246	46%		86%
McMillan et al. 1988, J Clin Pathol 41: 1223	91%	53%	96%
Miller et al. 1981, Arthritis Rheum 24:602	91%		
Rubin et al. 1983, Immunol Meth 63:359	22%		
Smeenk et al. 1992, Mol Biol Rep 17:71	100%	96%	98%
Tipping et al. 1991, Pathology 23:21	42%	35%	53%
Tzioufas et al. 1987, Clin Exp Immunol 5:247	73%		
Tzioufas et al. 1990, Clin Rheum 9:186	80%	33%	
Wigand et al. 1995, Lab Med 19:219	61%	58%	
	70%		
	84%		
Wigand et al. 1996, J Lab Med 20:283	93%	59%	62%
	82%		
Wigand et al. 1997 [4], Z Rheumatol 56:53	83%	56%	
	81%		
	73%		
Wong et al. 1998, Pathology 30:57	32%	17%	
	68%		

Table: *Percentage of positive dsDNA antibodies measured in SLE patients. Citated from R.Wigand et al. 1997; highlighted data was added by the author of this article*

ity. The high ionic strength of the ammonium sulfate solution used to precipitate the immune complexes specifically causes the dissociation of radiolabeled DNA and antibodies of low avidity. Therefore, the Farr assay is very selective for antibodies of high avidity, which have been reported to be most specific for SLE. The WHO standard is based on Farr-RIA, which positioned it as „golden standard" for anti-dsDNA measurement.

On the other hand this assay is time-consuming, technically more difficult to handle and involves the use of radioactive material. Another big disadvantage is, that a differentiation of the antibody-class is not possible. Different studies show that anti-dsDNA antibodies in patients without known autoimmune diseases most often belonged to the IgM class. Bootsma et al. concluded in their study, that rises of IgM class anti-dsDNA, in contrast with rises in IgG class anti-dsDNA, are not a sensitive tool for predicting a relapse and are not associated with specific clinical manifestations of SLE [3]. Comparative pre-launch studies with EliA™ dsDNA supported these findings.

Enzyme linked immunosorbent assay (ELISA)

The ELISA technique is easy to handle and can be used for automation, which is more and more important for routine laboratories. The specificity of the ELISA for SLE is often lower than that of CLIFT or Farr. Probably, this is due to the sensitive detection of low avidity antibodies, which also may occur in rheumatoid disorders other than SLE. The disease specificity can be increased by changes of the assay conditions. For example, EliA™ dsDNA does not detect low avidity antibodies and thus was shown to be highly specific for SLE.

Quantitation and excellent sensitivity make the ELISA the monitoring tool of choice. The immunoglobulin class can be determined by variation of the specificity of the labeled antibody.

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Clinical aspects of testing with the EliA dsDNA autoantibody detection system in a patient with SLE and cerebral involvement

Gerald Messer

Klinik und Poliklinik für Dermatologie und Allergologie, Ludwig-Maximilians-Universität München, Frauenlobstr. 9-11, D-80337 München, Germany

Lupus erythematosus (LE) can be divided into systemic LE (SLE) and the different subforms of cutaneous LE (CLE). CLE is further subdivided by clinical and immunological characteristics. From the dermatological view, discoid LE (DLE), subacute cutaneous LE (SCLE), lupus erythematosus tumidus (LET), profound LE (LEP) and bullous LE (BLE) are described. For the diagnosis of SLE, 4 of 11 criteria of the American College of Rheumatism (ACR) are mandatory; two of those are based on the typing of autoantibodies: antinuclear autoantibodies (ANA) and specificities like anti-dsDNA and anti-Sm. Depending on the selection of the patient collectives, dsDNA antibodies are reported in 60-90% in SLE patients. The positive finding of ANA plus anti-dsDNA antibodies provides already two of 4 ACR criteria for SLE. Thus, the precise definition of the autoantibody pattern is of great importance in every patient.

Table 1: SLE-patient with cerebral involvement. ELISA vs. EliA™

Sampling date	Varelisa [IU/ml]	EliA™ [IU/ml]
08/99	370	12.1
12/99	330	14.7
02/2000	266	**(above)**
04/2000 syst.therapy	352	**83**
07/2000	336	**25.7**
11/2000	334	10.1
05/01	212	n.d.
07/01	236	n.d.

Since our laboratory started the new method EliA™ in 2001, data on the first 150 serum samples were generated and compared to the data received with the ELISA method (Varelisa®). The EliA™ method was easy to establish. Anti-dsDNA measurements of 150 sera from 115 patients - most often with SLE - were analysed. Other diagnoses were systemic scleroderma, Sjögren´s syndrome, Sharp´s syndrome, polymyositis, lichen planus, temporal arteriitis, chronic urticaria, pemphigus foliaceus and further patients with hemiatrophy of the face (Parry Romberg syndrome), granuloma anulare and chronic graft versus host disease, all of which showed positive or equivocal results in the Varelisa® before. Some dsDNA-negative sera were included as negative controls.

Results: Of those patients formerly showing high dsDNA values in the Varelisa®, the EliA™ results were either less pronounced (equivocal) (systemic scleroderma, Sharp´s syndrome, lichen planus, graft versus host disease) or negative (facial hemiatrophy, granuloma anulare). Certain LE-patients were followed for a longer time peroid to correlate dsDNA-results with clinical changes.

One 54-year-old woman with SLE suffered from severe central nervous system involvement and epilepsy. The dsDNA values obtained by the ELISA method did not indicate major changes from August of 1999 to July 2001 (Table 1). In April 2000, a rebound of SLE with worsening of clinical symptoms and inflammatory blood values required a systemic treatment with higher doses of oral corticosteroids and azathioprine. Despite the immunosuppressive therapy and clinical improvement of the SLE, no changes in the anti-dsDNA values were detected by ELISA (Varelisa®) (Table 1). With the EliA™ system, the same serum samples were analysed. In parallel to the clinical course, a downregulation of dsDNA-antibodies after beginning of the systemic therapy was noted (right panel, 83 IU/ml 25,7 IU/ml). Thus, the results obtained by EliA were in concordance to the clinical course of SLE.

Regarding the cut-off values (pos. / equiv. / neg.) of Varelisa® and EliA™ as proposed by the manufacturer, the test values of the 150 samples showed a 90% correlation for both tests. Overall, fewer positive sera were detected with the EliA™.

Taken together with the clinical observations, our preliminary results on the EliA™ for detection of dsDNA antibodies showed that this method is a fast, sensitive and specific system for the diagnostics of sera of autoimmune patients in dermatology.

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UK NEQAS:

In vitro diagnostic kits in comparison

Peter White
UK NEQAS, PO Box 894, Sheffield S5 7YT, UK

INTRODUCTION

European standards are being prepared on the use of external quality assessment (EQA) schemes in the assessment of ‚in vitro' diagnostic procedures. It is acknowledged that EQA is an essential element of mechanisms which help to maintain and improve analytical quality. It can also serve a valuable function in raising standards in diagnostic laboratory medicine and has a general educational role in informing participants about the current state of the art including the relationship of clinical laboratory data to medical needs. It allows comparisons to be made between alternative analytical procedures in the post market surveillance of commercial assays and associated analytical equipment. This can be to the benefit of both manufacturers and users. EQA may also allow accuracy to be assessed if survey samples have reference measurement values.

The United Kingdom National External Quality Assessment Service (UK NEQAS) for Immunology was created in 1982 as the centre for EQA of autoimmune serology and is based at the Northern General Hospital in Sheffield. The centre is also contracted by the Medical Devices Agency to perform critical evaluations of in vitro diagnostic kits and associated analytical systems on behalf of the Department of Health. These evaluations are published by the Agency as a guide to purchasing within the NHS.

SCHEME DESIGN

UK NEQAS for Immunology offers a variety of programmes for the survey of laboratory performance in autoimmune serology. The scheme which encompasses antinuclear and dsDNA antibodies comprises a liquid sample which is dispatched to each participant laboratory approximately every six weeks. Materials used in the programme may be either pathological single donor human serum or normal human serum. Each sample is validated prior to dispatch and is stable for the three weeks in which participants are allowed to return data.

Laboratories are required to submit qualitative responses for each analyte (positive, negative or equivocal) which are assessed in relation to a designated response (DR) as defined by a pre-selected group of reference laboratories. Individual laboratory performance is based on the qualitative result submitted by participants (or the interpretation of a quantitative result) using the misclassification index score (MIS) system. This is an indication of the number of times that a laboratory has returned a result which is not the same as DR. The MIS is the number of times that a laboratory has given a wrong result in a six distribution window. The ideal score is therefore zero! Reports also give a summary of the quantitative results and show kit or method related statistics including the range of numerical data returned for each of the different assays.

dsDNA ANTIBODY - a review of data January 1999 to September 2001

The number of laboratories submitting data for ANA and dsDNA antibodies has increased significantly; 44% of participants in the programme are now from outside the UK.

	ANA	dsDNA
Distribution 991	279	227 (81%)
Distribution 016	336	322 (96%)

A much higher proportion of laboratories currently submit data for both ANA and dsDNA antibodies than was the case in early 1999.

The number of assays which are commercially available for the detection and quantitation of dsDNA antibody has also increased markedly. In addition to the traditional Farr radioimmunoassay and indirect immunofluorescence using Crithidia luciliae substrate, which are regarded as being the gold standard methods, 28 different commercial enzyme immunoassays (EIAs) now feature in the programme. Some laboratories use a combination of methods.

There have been a total of 22 distributions of single donor sera during the period of review; 15 having been clearly positive, 5 having produced results close to the reference interval of most methods and 2 were normal human sera. The series of distributions produced a total of 274 misclassifications, an overall 4.7% misclassification rate.

Misclassifications by method/manufacturer Distribution window 991 to 016

Method	Misclassification rate
Farr assays	1.2%
IIF Crithidia	1.3%
CLS Melisa	2.4%
Pharmacia Varelisa	2.5%
Hycor Cogent	2.7%
Binding Site	2.8%
Axis-Shield	4.7%
Inova	5.9%
CLS Autozyme	6.2%
Sigma	8.6%
ORGenTec	10.9%

Assays are only shown which featured in the programme for the entire review period. Only 4 EIAs compared favourably with Farr and IIF.

The range of values submitted by users of the various commercial EIAs is usually extremely wide even when the manufacturer has indicated that calibration of the kit is in relation to the Wo/80 International Standard.

Range of EIA mean values in relation to IIF titre

Distribution	DR	Crithidia IIF mean titre	Range of EIA mean values
994	Neg	12	3 - 140
015	Equiv	16	5 - 456
996	Pos	90	11 - 280
007	Pos	140	20 - 640
011	Pos	360	40 - 1280
001	Pos	860	19 - 2170
016	Pos	1480	35 - 10000
002	Pos	2940	90 - 18500

The reference intervals specified by manufacturers also varies considerably.

dsDNA Antibody- lower reference intervals specified by manufacturer

Bio Diagnostics	40 IU/ml
CLS Autozyme	73.3
Cogent Autostat II	40 (40-60 Equivocal)
IBL	40 (40-60 Equivocal)
Inova Quanta Lite	68.7 (68.7-103 Borderline positive)
ORGenTec	20
Pharmacia Varelisa	35 (35-55 Equivocal)
Sera Quest	27 (27-33 Equivocal)
Axis Shield	30 (30-50 Borderline positive)
Zeus Scientific	150 (150-180 Equivocal)

dsDNA ANTIBODY - summary of situation 2001

Calibration and the reference intervals quoted by manufacturers are responsible for some of the variation observed in quantitation values submitted to the programme and the relatively high proportion of misclassifications. It is becoming increasingly clear that these are not the only components in a very complex scenario.

Since the methods generally in use differ considerably in their physicochemistry, it is probably not surprising that reported comparisons have described them as giving very different results. Discrepancies between the various anti-dsDNA assays are mainly attributable to the difference in avidity of the antibodies. At present the EIA has been shown to be the most sensitive method whilst Farr and Crithidia assays are considered to be the most specific for SLE.

For a large laboratory with a heavy service commitment, cost effectiveness and efficiency usually dictate the use of only one available method for routine diagnosis and manufacturers are always seeking to develop and enhance their repertoire. There are so many products available that the diagnostic laboratory is facing an ever increasing dilemma in deciding which option is likely to be the most appropriate system for its needs and which produces reliable results.

The MDA evaluation programme aims to give assistance in the decision making process as new systems become available. An evaluation to assess the performance of the Pharmacia EliA™ anti-dsDNA IgG assay was performed by the MDA Evaluation Unit in Sheffield during August 2001.

The Pharmacia EliA™ kit detects IgG class autoantibodies directed against dsDNA. The assay is based on the principles of coating a microplate well with double stranded plasmid DNA and has been formulated to produce results which are equivalent to the Farr assay.

The wells are automatically dispensed and processed in the UniCAP® 100 instrument.

The following table summarises results obtained by Crithidia IIF and Pharmacia EliA™ dsDNA antibody assays with sera from SLE patients and normal blood donors. Relative sensitivity and specificity were calculated from the data.

EliA™ dsDNA vs Crithidia IFA (n=190)

Crithidia IIF	EliA™ dsDNA Pos.	EliA™ dsDNA Neg.
Pos.	26	5
Neg.	5	154

Relative sensitivity and specificity

Sensitivity (%)	84
Specificity (%)	97
Overall agreement (%)	95

There were excellent indices of within assay variation (3.9% to 5.4%) and between assay variation (3.7% to 4.5%). A mean value of 174 IU/ml was recorded for the IRP Wo/80 against an assigned value of 200 IU/ml. In summary, the Pharmacia EliA™ IgG dsDNA assay produced results which compared favourably with the traditional assays.

Copies of the report (MDA Number 01130) which was published in September 2001 can be obtained from the MDA - http://www.medical-devices.gov.uk

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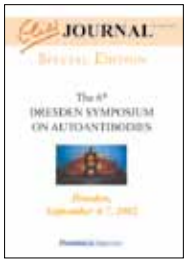

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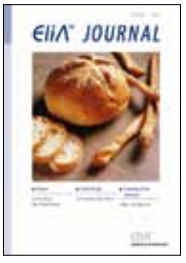

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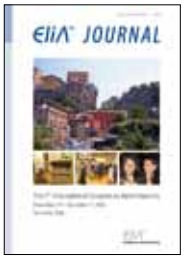

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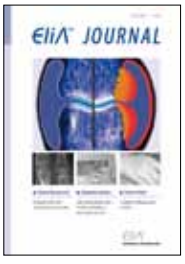

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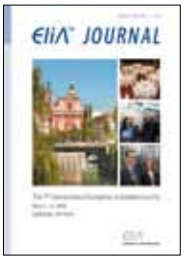

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Phadia GmbH, *Munzinger Str. 7, D-79111 Freiburg Germany, Tel: +49 761 47-805-0, Fax: +49 761 47-805-120, autoimmunity@phadia.com, www.phadia.com*

Head office Sweden *+46 18 16 50 00* ***Austria*** *+43 1 270 20 20* ***Belgium*** *+32 2 749 55 15* ***Brazil*** *+55 11 3345 5050* ***Czech Republic*** *+420 220 518 743* ***Denmark*** *+45 70 23 33 06* ***Finland*** *+358 9 8520 2560*
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