Detection of antibodies to the extractable nuclear antigens by enzyme linked immunosorbent assay.

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Anti-extractable nuclear antigen (ENA) antibodies are a group of autoantibodies that are directed against various components of the cell nucleus. Six different ENA have been well characterized and these include Sjogren's syndrome-A antigen (SS-A also known as Ro), Sjogren's syndrome-B antigen (SS-B also known as La), Smith (Sm) antigen, Scleroderma-70 (Scl-70) antigen, ribonuclear protein (RNP) and topoisomerase-1 (Jo-1).[1] Antibodies to these antigens are closely associated with connective tissue diseases (CTD) with varying prevalence (**Table 1**).[2]

Connective tissue diseases are a group of systemic autoimmune inflammatory diseases comprising of systemic lupus erythematosus (SLE), Sjogren's syndrome (SS), systemic sclerosis (Scl), polymyositis/dermatomyositis (PM/DM) and mixed connective tissue disease (MCTD).

Patients with CTD can present with clinical manifestations related to any organ-system of the body and often without the signs and symptoms that are classically associated with these diseases.[1] Consequently, early diagnosis of these diseases, based on clinical examination, can prove very difficult and therefore, clinicians rely heavily on the use of anti-ENA antibody testing for the exclusion, or early diagnosis prognosis and monitoring of CTD (**Table 1**). Due to the importance of anti-ENA antibodies in the diagnosis and management of CTD, assays used for testing should, therefore, be sensitive, specific and have a quick turn around time. Testing for anti-ENA antibodies has traditionally been carried out using classical gel-assays including the simple immunodiffusion and the counter current immunoelectrophoresis (CCIE) assays. However, these methods are time consuming, require great skills and have rather low sensitivities for the detection of anti-ENA antibodies, particularly those directed against the SS-A and Scl-70 antigens.[3] For these reasons, increasing number of clinical immunology laboratories are switching to testing for anti-ENA antibodies by enzyme linked immunosorbent assay (ELISA). The latter assays are more sensitive, require little skills, have a quick turn around time and are amenable to automation.

In the present study, we have investigated a number of different ELISA preparations with a view of changing our anti-ENA-antibody testing from the CCIE-method to an ELISA. The study was conducted at the regional department of Immunology, Birmingham Heartlands Hospital, during the period of 2003. We tested a number of ENA-positive and negative samples using 3

Table 1 - Association of anti-ENA-antibodies with CTD and their prognostic significance.

Antibodies	Associated CTD	Prevalence %	Monitoring suggested	Prognostic indicator
SS-A/SS-B	SS	65/60	Yes	Associated with development of extraglandular manifestations (arthralgia, vasculitis, nephritis, lymphoadenopathy and leucopenia).
	SLE	35/15	Yes	Associated with subacute cutaneous lupus and neonatal lupus syndrome
Sm	SLE	30-40	No	Renal disease and poor prognosis
RNP	MCTD	90	No	
	SLE	30-40	No	
Scl-70	Scl	20-40	No	Poor prognosis with cardiopulmonary disease and severe skin disease. Predictor for the development of systemic sclerosis in patients with Raynaud's phenomena
Jo-1	PM/DM	20-40	Yes	Predict an aggressive form of the disease with arthritis and interstitial lung disease (require close monitoring of pulmonary function for the early detection of lung involvement and aggressive treatment).

* Both Ro and La antibodies in pregnant patients can cross the placenta and cause fetal complete heart block and neonatal lupus.
SLE - systemic lupus erythematosus, SS - Sjogren's syndrome, Scl - scleroderma, MCTD - mixed connective tissue disease, PM/DM - polymyositis/dermatomyositis, ENA - extractable nuclear antigens, CTD - connective tissue disease, RNP - ribonuclear protein. SS-A/B - Sjogren's syndrome-A/B, Sm - Smith antigen, Scl-70 -Scleroderma-70, Jo-1 - topoisomerase-1.

different commercial ELISA preparations and then compared the results generated with that obtained by the traditional CCIE-assay. The results obtained are illustrated in **Table 2**. As can be seen from this table, the 3 ELISA preparations confirmed the positive results obtained by the CCI-assay for SS-A, SS-A/SS-B and Scl-70 antibodies. Moreover, all 3 ELISA preparations detected, additionally, SS-A and Jo-1 antibodies from samples previously shown to contain unidentified anti-ENA antibodies by the CCIE assay. The latter results can not be attributed to false positive results since, firstly, all 3 ELISA preparations produced the same strong positive results, and, secondly, such results correlated well with the clinical picture. In addition, 2 out of 3 ELISA detected 2 more additional antibodies to Sm and RNP. However, the significance of the latter results is questionable, since the results obtained were either equivocal or weak positive and they were not reproduced by the third ELISA preparation, or the CCIE-method. Therefore, these latter results would seem to be truly false positives and this would fit with the previous studies showing that ELISA have reduced specificity for some of the anti-ENA antibodies.[3] In contrast to previous studies, the present study has revealed that some ELISA preparations can be more sensitive than, and as specific as, the CCIE method. There are a number of possible explanations for the generation of false positive results by some of the ELISA preparations observed in the present and previous studies.[4] These include, firstly, the use of impure substrates to coat ELISA-plates, and, secondly, as a result of detection of low affinity antibodies by some of the ELISA preparations which would not be detected by the CCIE-assay. The possibility of contamination would be supported by the fact that, firstly, not all ELISA preparations produced false positive results (as shown in the present study) and, secondly, false positive results were only obtained by ELISA preparations coated with proteins purified from human materials (Sm and RNP), but not by recombinant proteins (Ro, La, Scl-70 and Jo-1). Refining the purification procedures for ENA, or the use of recombinant proteins should increase the specificity of ELISA for anti-ENA antibodies. Existence of low affinity anti-ENA antibodies would be the other explanation for the generation of 'false positive' results by ELISA and this suggestions would be supported by previous investigations into other autoantibodies (dsDNA and Scl-70 antibodies). Anti-dsDNA antibodies measured by the Farr, or the Crithidia Luciliae assays were regarded as highly specific for SLE. However, when ELISAs were introduced, it became apparent that such antibodies were not restricted to SLE, but associated with many other conditions. Further work revealed that antibodies associated with SLE were of high affinity, whereas those associated with other conditions were of low affinity.[5] Similarly, anti-Scl-70 antibodies obtained by the CCIE-assay were regarded as highly specific for scleroderma.[3] However, when the latter method was replaced with ELISA, positive results for anti-Scl-70 antibodies were found associated with SLE,in addition to Sclerodermal, and such results were initially labeled as false positives. However, these results were shown subsequently to be truly positive-results and corresponded to low affinity antibodies. These antibodies are now taken as a marker of a subgroup of SLE patients who are at an increasing risk of developing pulmonary hypertension and renal disease.[3] It is important therefore, to audit anti-ENA antibody results,

Table 2 - Anti-ENA antibody results obtained by the CCIE and ELISA.

Methods	**Anti-ENA-antibody results**										
	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11
CCIE	N	wp-uENA	wp-uENA	Wp-uENA	Ro	Ro La	Ro La	Ro La	Ro La	Ro La	Scl-70
ELISA-1	N	N	Jo-1	Ro	Ro	Ro La	Ro La	Ro La	Ro La	Ro La	Scl-70
ELISA-2	N	N	Jo-1	Ro	Ro	Ro La Sm	Ro La	Ro La	Ro La	Ro La	Scl-70
ELISA-3	N	Sm	Jo-1	Ro RNP	Ro	Ro La	Ro La	Ro La	Ro La	Ro La	Scl-70

Serum samples from 11 patients (P1-11) were assesed for antibodies to total and specific ENA using counter current immunoelectrophoresis (CCEI) and different preparations of enzyme linked immunosorbent assay (ELISA).
Wp-uENA - weak positive-ENA antibodies, N - negative, ENA - extractable nuclear antigen,
Sm - Smith antigen, Jo-1 - topoisomerase-1, Ro - Sjogren's syndrome-A antigens, La - Sjogren's syndrome-B antigens,
RNP - ribonuclear protein, Scl-70 - Scleroderma-70.

obtained by ELISAs, in order to determine their true significance.

In conclusion, the present study has revealed that some ELISA preparations can be more sensitive, and as specific as the CCIE method for the detection of anti-ENA antibodies. Laboratories that are still using the later method should consider switching to ELISA. However, it is important that laboratories evaluate a range of different ELISA preparations before selecting the most optimal one. In addition, it is recommended that laboratories then audit results in order to determine the true significance of such results. Finally, until the true significance of ELISA-generated results is known, positive ENA-results should be interpreted in conjunction with the clinical picture and this would require close liaison between the clinical Immunology Laboratory and clinicians.

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Antiphospholipid syndrome among Bahraini patients

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Antiphospholipid syndrome (APS) is defined as the presence of antiphospholipid (APL) antibodies, arterial or venous thrombosis, recurrent spontaneous abortions, and thrombocytopenia. However, not all patients develop such complications. The risk of thrombotic event in patients with APS is 0.5-30%. The syndrome can occur within the context of several diseases, mainly autoimmune, or it may be present without any recognizable disease, the so-called primary APS.[1]

Systemic manifestations of APS are multisymptomatic and can affect most of the systems. The symptoms are secondary to thrombosis that can be located in the vessels of each caliber. Most commonly, APS is associated with systemic lupus erythematosus (SLE). Approximately 35% of SLE patients have elevated levels of APL antibodies.[2] The diagnosis of APS is based on the presence of any clinical manifestation associated with the syndrome in addition to the presence of anticardiolipin (ACL) antibodies or lupus anticoagulant (LAC).[1]

A retrospective study was performed on 22 patients with APS who where treated in Salmaniya Medical Complex (Ministry of Health), largest hospital in Bahrain (1000 bed), over 16 year period from 1988 - 2002. Anticardiolipin immunoglobulin G (IgG) and immunoglobulin (IgM) were tested by enzyme link immunoabsorbent assay (ELISA) technique.

Primary APS was 45.8% while the secondary 54.5%. History of thrombosis was present in 50% of primary APS, while 33.3% of the secondary APS patients. The female to male ratio was 10:1. Among our female patients, 90.5% were married and percentage of pregnancies was 71.4% and number of miscarriages was 57.1%. In the primary APS, history of miscarriage was 80% while 50% in secondary APS group. All secondary APS were SLE patients. Percentage of ACL IgG was higher in primary APS (80%) compared to secondary APS 66.7%. On the contrary ACL, IgM was higher in secondary (75%) compared to primary APS (60%). Venereal Disease Research Laboratory (VDRL) was reactive in 90% of the primary group while 50% in secondary group. Antinuclear antibodies were much common in secondary APS (100%) than in primary APS as expected (20%). Regarding anti double stranded DNA it was positive in 83.3% of the secondary APS while absent in the primary form. Prolonged partial thromboplastin time (PTT) was present in 90% of the primary APS versus 66.7% of the secondary form. Treatment was given to 95.2% after diagnosis: 33.3% received aspirin, 76.2% received steroid, 28.6% received heparin and 28.6% received warfarin.

The present study on Bahraini patients showed that Hughes syndrome is not a common problem among hospitalized patients in Bahrain. Neurological, ophthalmological and cardiac manifestations known to be among the manifestations of APS were uncommon in the present study.