CHAPTER I

INTRODUCTION

Background of the problem

SLE patients sera appears contain antibodies against a variety of cellular macromolecules. These include antibodies against DNA, nuclear protein, histones and various RNA protein. In addition, SLE patients frequently produce antibodies against nuclear nonnucleic acids and other nuclear and cytoplasmic macromolecules. Attention has been directed in particular to antibodies reactive with deoxyribonucleic acid (DNA) (1, 2, 3), because anti-DNA antibodies appear to be almost specific for SLE. Detection of anti-DNA antibodies is not only of value in the diagnosis and clinical management of SLE but their levels correlate with clinical activity and DNA-anti DNA complexes may play a role in the pathogenesis of nephritis complicating SLE (4, 5).

Since the discovery that sera of patients with SLE contain antibodies to nuclear components (1), more refined methods have been developed to define the antigen involved in the reaction and to measure the amount of antibody. The solid phase radio-immune assay was recently developed (6) and this technic has a

number of advantages. In the first place, the simplicity of the precedure and the sensitivity, it gives a direct measure of antibody binding to the DNA but the disadvantages were the use of expensive equipments, the enviromental pollution, and the instability of reagents. Enzyme-antibody conjugated was then substituted for the radiolabelled (7).

The aim of this study is to develop the enzyme-linked antiimmunoglobulin to determine serum anti-DNA antibody for the use in routine serological laboratory.

Objective

The objective of this study is to develop the microtiter solid phase enzyme-linked immunoassay for determining anti-DNA antibodies in serum of patients with SLE.

Literature review

Preliminary criteria for the classification of SLE

The proposed criteria for the classification of SLE patients in clinical trials, population surveys and other such studies, a person shall be said to have systemic lupus erythomatosus (SLE) if any four or more of the following 14 manifestations are present, serially or simultaneously, during any interval of observation (8):

1. Facial erythema (butterfly rash): Diffuse erythema, flat or raised, over the malar eminence (s) and for bridge of the nose; may be unilateral.
2. Discoid lupus erythematosus (DLE): Erythematous raised patches with adherent keratotic cealing and follicular plugging, strophic scarring may occur in older lesions, may be present anywhere on the body.
3. Raynaud's phenomenon: Required a two-phase color reaction, by patient's history or physician's observation.
4. Alopecia: Rapid loss of large amount of the scalp hair, by patient's history or physician's observation.
5. Photosensitivity: Unusual skin reaction from exposure to sunlight, patient's history or physician's observation.
6. Oral or nasopharyngeal ulceration.
7. Arthritis without deformity: One or more peripheral joints involved with any of the following in the absence of deformity:
   (a) pain on motion; (b) tenderness;
   (c) effusion or periarticular soft tissue

swelling. (Peripheral joints are defined for this purpose as feet, ankles, knees, hips, shoulders, elbows, wrists, meta-carphophalangeal, proximal interphalangeal, terminal interphalangeal, and temporomandibular joints)

8. LE cells: Few or more classical LE cells seen on one occasion of one cell seen on two or more occasions, using an accepted published method.

9. Chronic false-positive serologic test for syphilis: Known to be present for at least six months and confirmed by Treponema pallidum immobilizing or Reiter's tests.

10. Profuse proteinuria: Greater than 3.5 g/day

11. Cellular casts: May be red cell, homoglobin, granular, tubular, or mixed.

12. One or both of the following: (a) Pleuritis, convincing history of pleuritic pain; or rub heard by a physician; or reentgenography evidence of both pleural thickening and fluid; (b) pericarditis, documented by ECG or rub.

13. One or both of the following: (a) Psychosis; (b) convulsion by patient's history or

physician's observation in the absence of uremia and offending drugs.

14.. One or more of the following: (a) Hemolytic anemia; (b) leukopenia: white blood cell count less than 4,000/cumm. on two or more occasions; (c) thrombocytopenia, platelet count less than 100,000/cumm.

Unusual cutaneous manifestations (70)

- Erythromelalgia with red discoloration of the extremities

- Soft tissue calcification and multiple dermatofibroma

- Clubbing finger

- Erythema annulare centrifugum

- Lupus panniculitis cutaneous nodules occur that resolve leaving depressed areas. Sites of predilection include the cheeks, back, buttocks and upper arms. The histology is that of a lymphocytic vasculitis and panniculitis. Antimalarial drugs and systemic corticosteroids may be useful.

- Occasionally patients are seen in whom one cannot differentiate LE from lichen planus clinically, histologically or by immunopathology. The term "mixed lichen planus-lupus erythematosus disease" is proposed.

- Hypocomplementemic vasculitis is a lupus variant in which the patients have chronic urticaria-like lesions that

histologically show necrotizing vasculitis. One or more of the complement components usually are decreased. These patients are usually resistant to therapy.

Common histocompatibility phenotypes, with the association of HLA-B8, HLA-B5, HLA-BW15 and HLA-BW35 have been reported in SLE.

An increased incidence of HLA-B7 and HLA-B8 has been reported in discoid lupus. In discoid lupus, patient with HLA-B8 appeared to have an increased risk of conversion of disease to SLE.

LE may be seen in the newborn. The cutaneous LE disappear by age 1 year. Antibodies that can cross the placenta are present in the newborn but become negative within a few months. The disease may be self-limited but may persist.

Many patients with complement deficiencies, particularly C_2 have LE. Use of CH_{50}, not C_3 determination, is necessary to diagnose genetic complement deficiency.

Immunology of Systemic lupus Erythematosus

Systemic lupus erythematosus was previously manifested in many forms. At present SLE is known as a chronic systemic inflammatory disease with multiple organs involvement. An erythematosus rash occurs on the face and other exposed sunlight

areas. No cause and no cure are yet known for SLE. The age at onset ranges from 15-30 years and it is more prevalent on females to males (4:1) of childbearing age (9).

The study of SLE started with the discovery of LE cell phenomenon. Then lead to the finding of antinuclear factors and antibodies to DNA in the sera of patients with SLE. The studies of renal eluates from these patients found that DNA containing immune complexes were the cause of lupus glomerulonephritis (71).

Several autoantibodies were found in the sera of SLE patients. Physical or chemical stimuli may initiate a process releasing cellular antigens from normal cellular breakdown products. This intrinsic immunological hyperactivity may cause the formation of multiple antibodies to antologous constituents. Lymphocytotoxic antibodies occur in many patients with SLE, are capable of killing T lymphocytes in the presence of complement. This lead to depressing T cell activity and enhancing B cell activity. Delayed hypersensitivity responses may be impaired. Excessive and high titered antibody are found. Autoantibody formation is normally regulated by suppressor T cells, decreasing of these cells may lead to the production of large amount of autoantibodies (9).

Serum complement levels are usually decreased which may be due to utilization by immune complex, reduction of synthesis or a combination of both factors. Antinuclear factors detected by immunofluorescence technic are usually found in patients with active SLE. High titered anti-DNA antibodies and low serum complement levels are often observed in active renal disease (9).

Clinical features:

Many clinical patterns may be presented in SLE. Several organs may be involved and there are peroids of active disease and remission. The frequent manifestation of SLE involving skin, joints and muscles, kidneys, heart, lungs, nervous system, eyes, gastrointestinal system, hematopoietic system are frequently seen. Certain drugs such as apresoline, procainamide may also provoke a lupus-like picture (70).

Immunopathology (70):

In diagnostic problems, without skin lesions, the biopsy specimen is taken from a sun exposed area for diagnosis. Non-sun-exposed area are biopsied for prognosis. Deposition of IgG, IgM, IgA, C_3' proactivator can be demonstrated.

If immune complexes are present in non-involved non-sun exposed area prognosis is probable poor.

Deposition of IgA and complement is also present in the mesangium and the loops of renal glemeruli.

Pathogenesis (70):

Viruses: The tubuloreticular structures resembling paramyxoviruses have been found in involved and uninvolved DLE and SLE skin. They are also present in neonatal DLE and SLE skin. Type C RNA Virus expression has been reported.

Drugs: Precainamide and hydralazine, thiouracil, chlordiazepoxide chlorpromazine, penicillamine, methydopa, oral contraceptive and etc.

Ultraviolet light: Cutaneous LE can be induced with monochromatic radiation of 250-313 mm.

Endocrine: SLE occurs in women and is related to hormonal activity. Oral contraceptives may induces a flare up of LE. Pregnancy often affravates SLE.

Immune responsiveness: There is generalized dimunition of cell mediated immunity. Decrease CMI seems to correlate with disease activity. Circulation T lymphocytes are decreased. Antilymphocyto-toxins are found and are directed against T lymphocytes. The B lymphocyte system exhibits signs of increased activity. They appeared to be increased immunity to RNA. The increased B cell activity may be caused by

depressed suppressor T cell function. The B lymphocytes could block Fc-receptor function.

Immune complexes: Circulation free DNA and Anti-DNA antibodies are present. Immune complexes can be demonstrated in the serum and skin. A defect in F_c-receptor function of splenic macrophage in SLE might have defective clearance of soluble immune complexes from the circulation.

Laboratory studies (79, 80)

1. Chemical findings:

- Total protein; Often an increase in the globulin is compensated for by a decrease in serum albumin, yielding a normal total protein figure.

- Serum albumin; The value is consistently low in active disease.

- Serum creatinine values are frequently in the normal range.

- Blood Urea Nitrogen (BUN)

- Bilirubin is seldom elevated in SLE in the absence of complicating viral hepatitis or pharmacologic hepatic injury.

Cholesterol levels; Prednisone is roughly linear and approximates a 3 mg/100 ml rise in cholesterol for every mg/day of prednisone given. It is possible that, this side effect may be a significant component in arterial pathology.

2. Hematological findings in SLE patients:

- Anemia (80% of eases)
- Leukopenia usually not below 2,000 cells/cumm.
- Thrombocytopenia
- increased Erythrocyte sedimatation rate (ESR)

3. Urinalysis will demonstrate:

- hematuria
- proteinuria
- red and white cell casts

4. Serological findings

4.1 LE cell phenomenon: The presence of 7 S IgG antibody reacted with deoxy-ribonucleoprotein in the nucleus of damaged leukocytes leads to the destruction of normal chromatin pattern. The LE cell is the leukocyte (PMN or monocyte) that has engulfed one or more of homogenous mass composing DNA, immunoglobulin and complement. The LE cell phenomenon is deserved in 75-80% patients with SLE, 15% of patients with rheumatoid arthritis, scleroderma or polydermatomyositis.

4.2 Fluorescent antinuclear antibodies

Method: Cells of rat liver incubated with FITC anti-human IgG

Results: Morphologic patterns of nuclear fluorescence.

Non particulate

a) peripheral pattern reflects antibodies to DNA, found in severe SLE nephritis.

b) homogenous pattern reflects antibodies to nucleoprotein, found in connective tissue disease.

Particulate

c) speckled pattern reflects antibodies to saline soluble protein, then test for anti RNA-ase resistant Extractable nuclear antigen (ENA or Sm antigen) antibodies, if positive, suggest SLE.

d) nucleolar pattern reflects antibodies to RNA, found in scleroderma

e) threads pattern, found in SLE and connective tissue disease.

Negative

Virtually excludes active SLE if the patient is not on an immunosuppressant.

4.3 Anti-DNA antibodies: Three major types of anti-DNA antibodies can be found

a) anti-single stranded or denatured DNA (ss-DNA)

b) anti-double stranded or native DNA (ds-DNA or nDNA)

c) antibodies to both DNAs.

Anti-ds-DNA antibodies are found in one-third to two-thirds of SLE serums are highly specific while anti-ss-DNA antibodies are found in other autoimmune disease and in drug-induced lupus-liked syndrome. The amount of antibodies correlate well with disease activity and the antibody titer decreases when patients enter remission.

4.4 Anti-RNA antibodies: 70% of patients with SLE are found to have anti-double stranded RNA but it is not specific for SLE. They are also found in patients with viral infection.

4.5 Serum complement components: They are reduced in nephritis, extensive skin and/or central nervous system involvement.

4.6 False-positive serologic test for syphilis: About 10-20% of patients with SLE give false-positive VDRL test.

4.7 Rheumatoid factors: Almost 30% of patien s with SLE have a positive latex fixation test for Rheumatoid factors.

4.8 Antierythrocyte antibodies: Detected by direct Coombs test About 10-65% of SLE patients have been found.

4.9 Anticytoplasmic antibodies: Numerous anti-cytoplasmic antibodies have been found in patients with SLE.

4.10 Circulating anticoagulants and antiplateletes antibodies: The IgG antibodies can decrease the prothrombin time. Antiplatelets antibodies are found in 75-80% of SLE.

4.11 Organ-specific antibodies: Antibodies to thyroglobulin are found in 20% patients with SLE. Antibodies against liver, kidney and joint tissue have been found.

5. Other laboratory findings:

5.1 X-ray finding: May reveal cardiomegaly, pleural effusion, platelike atelectasis or interstitial fibrosis.

5.2 Tissue studies: Kidney may show accumulation immunoglobulins and complement deposition along the glomerular basement mambrane. 90% of patients with SLE have immunoglobulin and complement deposition in the dermal epidermal junction of skin.

Management

DLE

1. avoid sun light and photosensitive drugs

2. topical sunscreen agent, derivatives of para-aminobenzoic acid and titanium dioxide.

3. topical and intra lesional corticosteroids

4. antimalarial drugs using chloroquin 250 mg. 1 tab bid (drugs should not be used during pregnancy) or

5. clofazimine (Lamprene) 100 mg. bid (65% of the cases showed improvement)

SLE

1. same as in DLE

2. Antimalarial drugs in patients with discoid lesions

3. combination of systemic corticosteroid and cytotoxic drugs for example azathioprine, cyclophosphamide or chlorambucil in lupus nephritis.

Table showing line of treatment by using combination of prednisone and cyclophosphamide (the Institute of Demotology, Bangkok, Thailand)

Month	prednisone mg/day	+ Cyclophosphamide mg/day in lupus nephritis
1	60	100 (2^{nd} week)
2	45	100
3-9	30	50
10-13	30-15 (single dose)	50
13	30-15 (single dose) every other day	50 every other day

Result of the latest treatment (72).

Many reports attest the unfavourable course of patients with moderate and severe glomerulonephritis when steroids are used alone, especially in patient with reduced renal function and/or the nephrotic syndrome. Combination of corticosteroids and cyclophosphamide is not better than corticosteroids alone in many cases of diffuse proliferative glomerulonephritis.

Treatment with azathioprine in combination with corticosteroid was not better than those in patients treated by corticosteroid alone.

Corticosteroid - cyclophosphamide is definitely more effective in suppressing lupus nephritis than corticosteroid alone.

Excellent therapeutic results and be clearly attributed to the use of chlorambucil (10 mg/day) with steroids. Chlorambucil not only induced a resolution of the lesions of diffuse proliferative lupus glomerulonephritis but also had the minimal side effects.

An average of maintanance dose of prednisolone is reduced when combined drugs are used (15 mg/day is reduced to 7.5 mg/day)

4. Other supportive treatments

antacid

calcium

antihypertensive and diuretic

antibiotic for urinary tract infection

antidiabetic

5. Plasmapharesis: Removal of 5-8 litres of plasma weekly was lifesaving in patients with high levels of circulating immune complexes. It was ineffective in patients without evidence of increased circulating immune complexes.

In 1969 Hargraves et al (10) discovered that serum from patients with systemic lupus erythematosus when incubating with human leukocytes would induce alternation of nuclei leading to phagocytosis of nuclear remnants. This phenomenon was called LE. cells phenomenon. Many experiments were used to find out the property and activity of LE to be serum factor. It was found to be γ-globulin and was proved to be autoantibody to nuclear protein or DNA (1).

Various methods were then developed for studying anti-DNA antibody. Nowadays antibodies to DNA are known to have a high degree of diagnostic specificity for active systemic lupus erythematosus.

In 1957 Seligmann studied the property of anti-DNA antibodies using immunodiffusion method. In 1966 Tan et al (3) developed double diffusion in agarose. Calf thymus DNA was used as standard. This method lack of sensitivity and was difficult to quantitate.

Robbins et al in 1957 (2) also studied anti-DNA antibodies employing complement fixation test. The result was

positive in 22 of 30 patients with SLE. It was found to relate LE cell phenomenon but this method was not specific for SLE. Patients with hyperglobulinemia would have positive result. Quantitative micro complement fixation method was developed in 1961 by Wasserman and Levine (11) which could detect for milli-micro gram of antibody.

In 1958 Farr described the ammonium sulfate precipitation method (12). The method based on the insolubility of antigen-antibody complex in 50% ammonium sulfate solution white free antigen was still soluble. In 1968 Wold et al (13) developed the Farr method for anti-DNA antibody, by adding radio-labelled DNA to serum and then add ammonium sulfate to 50% saturation, to precipitate the radioactive DNA complexed with anti-DNA in serum. The supernatant were counted and calculated for DNA binding activity. The sensitivity of this method was about 0.1 ug of antibody. The positive results were found in 33 of 44 patients with SLE.

Latex agglutination for anti-DNA antibody in patients with SLE first used by Christions et al in 1958 (14). Polystyrene latex containing anti-DNA antibody. The results were paralleled to LE cell but it gave rosetts with rheumatoid arthritis sera.

In 1965 an indirect hemagglutination test was described by Lawlis, using formalinized human erythrocytes sensitized with DNA. Jokinen and Julkunen in 1965 (15) used this method to study 63 patients with SLE and the test was positive in 19 cases. The test was negative in collagen diseases and miscellaneous diseases. Koffler et al in 1969 (5) also employed this method to SLE and found that about 60% of patients had antibodies to nDNA and 92% had antibodies to single stranded DNA. Anti-DNA antibodies were also found in patients with rheumatoid arthritis, infectious mononucleosis, chronic active hepatitis and in patients chosen at random as controls.

In 1966 Jones and Berg (16) reported the usefulness of nitrocellulose membrane filters in detecting DNA-protein complexes. They found that white RNA polymerase and native DNA were each filtrable through such a membrane, a complex of the two was retained. Kredich et al (17). This method for direct measurement of anti-DNA antibody, depending on the ability of radio-labelled DNA to pass through a nitrocellulose membrane filter while radio-labelled DNA-antiDNA complexes were retained. The radioactivity in both supernatant and precipitate were then counted and calculated for DNA binding activity. Anti-DNA antibodies were found in 19 of 21 patients with active SLE, 10 of 12 patients with inactive SLE and 3 of 217 controls.

The solid phase radio immunoassay was first described by Catt et al in 1966 (18). After the radio-labelled antigen was fixed to specific antibody, a "solid phase antibody" was added to seperate free radio-labelled antigen from antibody from antibody bound radio-labelled antigen. Tan and Epstein in 1973 (19) used a solid phase immunoassay for antibody to DNA, using polynucleotides adsorbed to plastic cups incubated with tested serum. Anti-DNA antibodies were detected by this method in 60.8% of patients with SLE. It was positive approximately 19% of rheumatoid arthritis. In 1976 lange et al (6) incubated patient's serum to a DNA-coated plastic cup. Anti-DNA antibody in serum was estimated by adding I^{125}-labelled purified anti-human immunoglobulin. This method has the advantage of being able to define immunoglobulin class of anti-DNA antibody. The antibodies were found in 69% of patients with SLE. It was found in 3% of rheumatoid arthritis and 35% of other collagen disease. In 1974, Pesce et al (7) described a solid phase adsorbent enzyme linked immunoassay for measuring the amount of anti-DNA antibodies in serum. The anti-DNA serum reacted with DNA coated to polystyrene tube. Antihuman γ -globulin peroxidase enzyme conjugate was added to react with anti-DNA. The enzyme activity was proportional to the amount of anti-DNA antibodies. This method was developed to substitute radioimmonoassay. The positive results were found in 18 of 42 patients with SLE, and 2

of 8 sera from patients with rheumatoid arthritis.

In 1976, Schuller et al (20) determined anti-DNA antibodies in sera using counter immunoelectrophoresis method. DNA and serially diluted sera were applied on agarose gel running in an electric current using Michaelis's buffer, pH 7.0. This method depended on optimal condition. The results were found to be more sensitive than the Farr assay, precipitin lines were found in 21 of 24 sera of patients with SLE, 6 of 71 control patients, 11 of 35 patients with other immunological diseases.

Slater et al (21) describer an immunofluorescence test for antibodies to native DNA, using the kinetoplast of Crithidia luciliae as substrate. The immunofluorescence test appears to have great specificity as a diagnostic test for SLE, though lacking the sensitivity of the Farr technic.

In 1978 Zasshi determined anti-DNA antibodies by electrosyneresis and complement fixation technic in the serum of systemic lupus erythematosus (73).

Enzyme-Linked Immunosorbent Assay

The assay involving the use of antigen or antibody labelled with an enzyme have recently been applied to the measurment of substances in biological fluids. These are known

under a variety of names such as enzyme immunoassay (EIA) (13), enzyme labelled assay (EIA) (74), competitive enzyme linked immunoassay (CELIA) (75), and enzyme-linked immunosorbent assay (ELISA) (22). The latter is perhaps the most useful term since it identifies the heterogenous enzyme assay yet at the same time clearly differentiates it from the tests which employ antibody-peroxidase conjugates for staining reactions for microscopy. The introduction of enzyme immunoassay was described by Engvall and Perlmann (22).

General principles of ELISA:

1. The indirect method of ELISA for assay of antibody (Fig. 1):

The indirect method of ELISA has been used on infections diseases, where the need is often for measurment of antibody. In the indirect method the antigen is immobilised by passive adsorption on to the solid phase antibody. Test sera are then incubated with the solid phase and any antibody in the test sera becomes attached to the antigen on the solid phase. After washing to remove unreacted serum components, an anti-globulin enzyme conjugate is added and incubated. This will become attached to any antibody already fixed to the antigen. Washing again removes unreacted material and finally the enzyme substrate is added. Its colour change will be a measure of the

amount of the conjugate fixed, which is itself proportional to the antibody level in the test sample.

2. Double antibody sandwich method of ELISA for assay of antigen (Fig. 2):

An alternative method for antigen measurement is the double antibody sandwich technique. In this modification a solid phase is coated with specific antibody. This is then reacted with the test sample containing antigen, then enzyme labelled apecific antibody is added, followed by the enzyme substrate. The antigen in the test sample is captured (step 2) and immobilised on to the sensitised solid phase where it can itself then fix the enzyme labelled antibody (step 3). This technique is analogous to the immunoradiometric assays of Miles and Hales (76), and has the same sensitivity and specificity for high molecular weight antigens.

3. Competitive ELISA for assay of antigen (Fig. 3)

ELISA tests can be competitive for the assay of antigen. Enzyme labelled antigen is mixed with the test sample containing antigen, which competes for a limited amount of antibody. The reacted (bound) antigen is then separated from the free material, and its enzyme activity is estimated by addition of substrate.

4. The double antibody sandwich antiglobulin ELISA for assay of antigen (Fig. 4):

Various other modifications of ELISA have been used. For example, Fig. 4 shows a system were the second antibody used in the double antibody sandwich method is from a different species, and this is then reacted with an anti-immunoglobulin enzyme conjugate. The advantage of this is that it avoids the labelling of the specific antibody, which may be in short supply and of low potency. This same method can be used to assay antibody (step 3) where only an impure antigen is available; the specific reactive antigens are selected by the antibody immobilised on the solid phase.

5. Completitive antigen modification of the indirect ELISA for assay of antigen (Fig. 5):

Plates are coated with the specific antigen and these are then incubated with a mixture of reference antibody and the test sample. If there is no antigen in the test sample the reference antibody becomes fixed to the antigen sensitised surface. If there is antigen in the test solution this combines with the reference antibody, which cannot then react with the sensitised solid phase. The amount of antibody attached is then indicated by an enzyme labelled antiglobulin conjugate and enzyme substrate. The amount of inhibition of substrate

degradation in the test sample (as compared with the reference system) is proportional to the amount of antigen in the test system.

In all these method passive adsorption to the solid phase can be used in the first step. Absorption of other reagents can be prevented by inclusion of wetting agents in all the subsequent washing and incubation steps. The washing must be sufficient to prevent carry-over of reagents from one step to the next.

Uses of ELISA:

Today ELISA have extensive application in many fields including the followings.-

Endocrinology: Van Weemen and Schuurs set up ELISA for measuring human chorionic gonadotropin (13). Detailed evaluations of the HCG, Oestrogen and luteinising hormone assays have been made by Van Weemen (13) and Schuurs (23). Parrallel studies with ELISA on human placental lactogen (24).only resulted in assays which were much less sensitive than RIA. Insulin is another large hormone which has been measured succeessfully by ELISA (25). Thyroid stimulating hormone has also been assayed with accuracy at very low levels (26). Smaller molecular wight hormones have also been measured, with a high sensitivity, including progesterone (27, 28) aestradiol

(23, 24, 29) and cortisol (30, 31).

Oncofetal proteins: Double antibody sandwich and competitive sandwich ELISA methods for alpha-fetal protein (AFP) have been described by several groups of workers. (32, 33, 34, 35, 36, 37, 38). ELISA for carcinoembryonic antigen have been described by Engvall and collaborators (39, 40, 41, 42).

Serum proteins: IgG was first measured using competitive ELISA methods by Engvall and Perlmann.(22, 43, 44). The ELISA which had been developed for IgE by Hoffmann (45) and by Weltmen et al (46) could be of considerable practical value.

Antigens of infectious agents: Carlsson et al (47) assayed antigens from Brueetta, Yersinia, and Salmonellae and more recently by Yolken et al (48) who detected Escherichia coli toxins by ELISA. Halbert and Anken (49) described a rather similar double antibody sandwich assay for hepatitis B surface (HBs) antigen Warren et al, showed that candida antigen could be proved of use in indicating invasive candidiasis (50).

Antibodies: In 1975 Voller and Bidwell showed that rubella antibody (51) could be measured by a microplate ELISA and the results found to correlate with those measured by hemagglutination inhibition test, this was confirmed by Gravell et al (52). Very early detection of antibodies to rabies by a sensitive ELISA was also reported by Atanasiu et al (53) and by

Thraenhart and Kuwert (54), and this could lead to a useful test for assessing vaccine responses.

Antibodies to various bacterial antigens were successfully measured by this technic. For example, Holmaren and Svennerholm found ELISA satisfactory for assaying antibody to Vibrio cholerrae (55). Recently Halle et al and Herrman et al introduced ELISA for detection of antibodies anainst Rickettsiae and they found it suitable for both clinical and research laboratories (56, 57).

ELISA has also been used for a variety of other human parasitic infections such as amoebiasis (61), hydatid disease (62), onchocerciasis and hookworm (63), leishmaniasis (64), and trichinosis (65). Malaria was studies by Voller et al (58, 59, 60) in a number of geographical locations using ELISA, and the authors showed that ELISA values reflected the endemicity.

Diagnosis of mycotic infections is also heavily dependent on serological tests. Hornmel et al (66) showed that antibody to Aspergillus and Candida could be measured by ELISA, but it is perhaps of more diagnostic relevance that circulating candida antigen can also be assayed (50).

Further more, ELISA has been used with success for detecting immune complexes which can be of dignostic

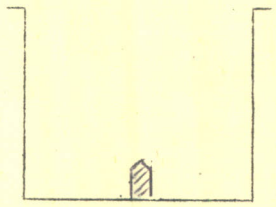
importance (66, 67).

Many substances in human biological fluids are sucessfully studied using enzyme immunoassay.

Fig. 1 The indirect method of ELISA for assay of antibody

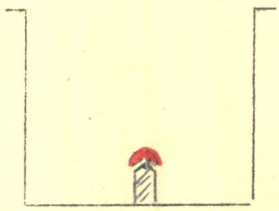
1. Antigen absorbed to plate

   wash



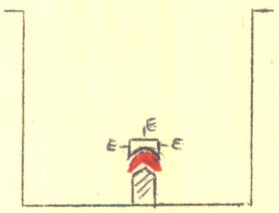
2. Add serum any specific antibody attaches to antigen

   wash



3. Add enzymed labelled antiglobulin which attaches to antibody

   wash



4. Add substrate

Amount hydrolysed = Amount antibody present

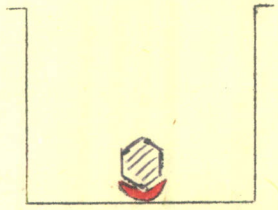
Fig. 2 Double antibody sandwich method of ELISA for assay of antigen

1. Antibody adsorbed to plate

   wash

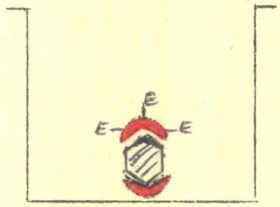
2. Test solution containing antigen added

   wash



3. Add enzyme labelled specific antibody

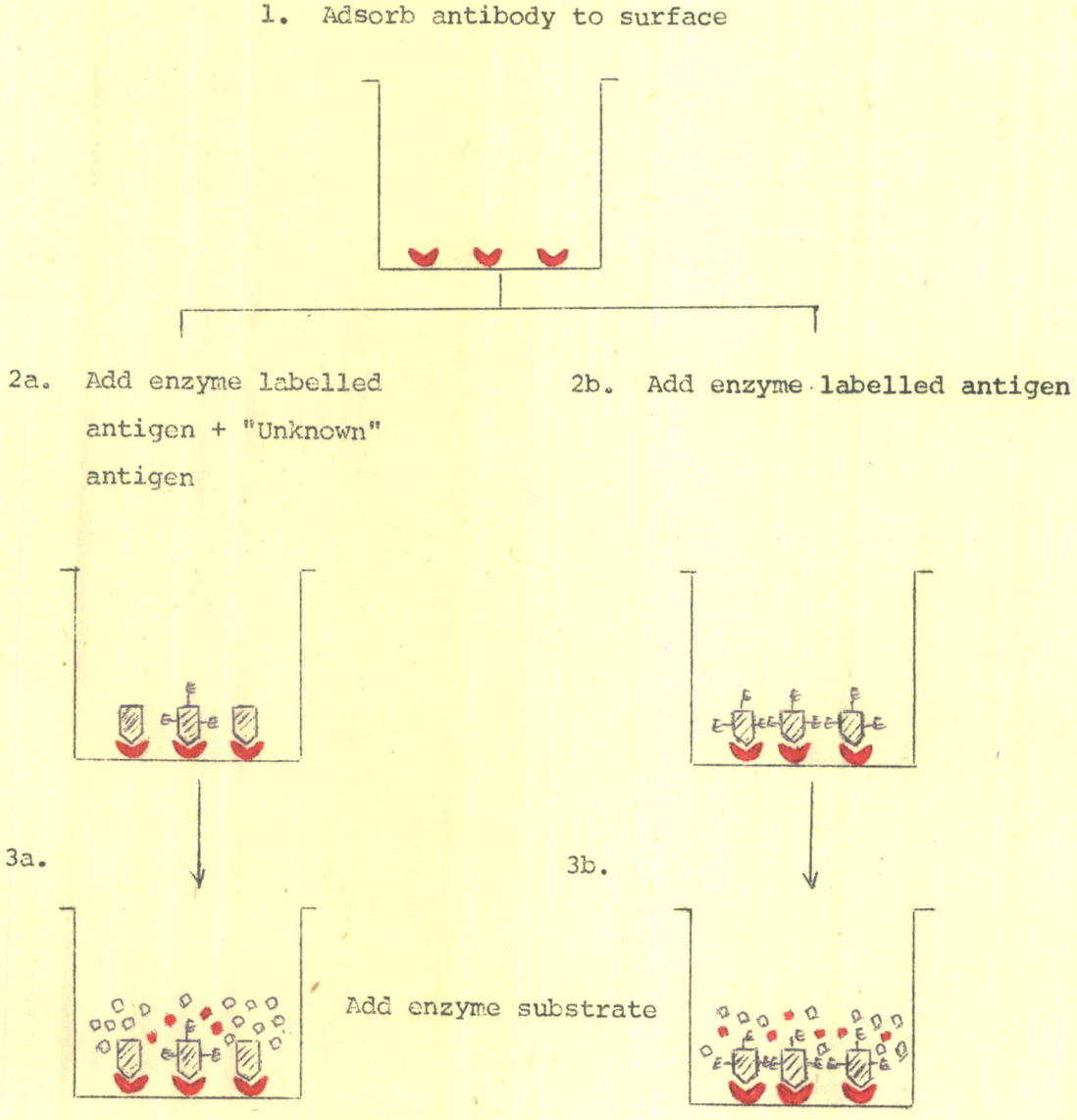
   wash



4. Add enzyme substrate

Amount hydrolysis = Amount antigen present

Fig. 3 Competitive ELISA for assay of antigen



Substrate hydrolysis = Labelled (Antigen)

Difference between 3a. & 3b. = "Unknown" (Antigen)

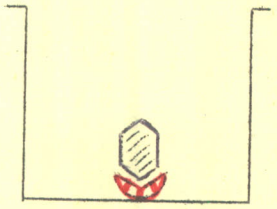
Fig. 4 The double antibody sandwich-antiglobulin ELISA for assay of antigen

1. Plate coated with specific antibody A (e.g. rabbit)

   plate washed

2. Test sample containing antigen reacted

   plate washed

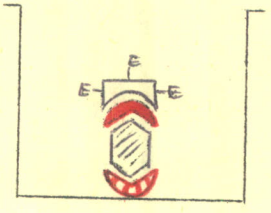


3. Specific antibody B added

   (of different species e.g. goat)

4. Enzyme labelled anti B globulin added e.g. anti goat Ig

   plate washed



5. Enzyme substrate added

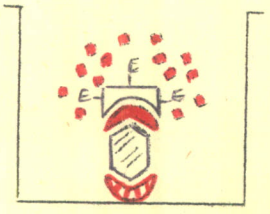


Fig. 5 Competitive antigen modification of the indirect ELISA for assay of antigen.