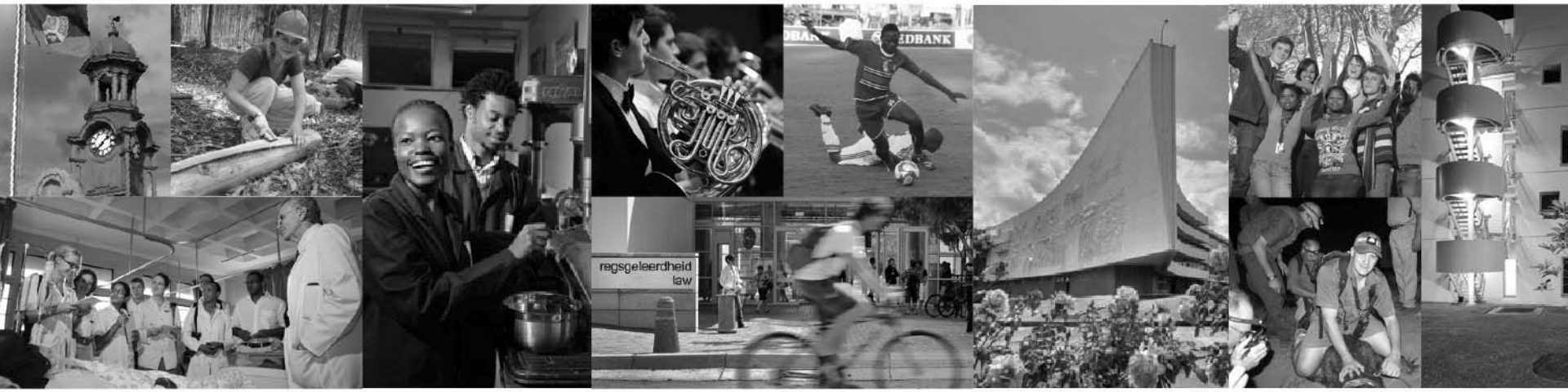
Serodiagnostic Procedures Performed in the Department of Immunology



UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA
Denkleiers • Leading Minds • Dikgopolo tša Dihlalefi

Dr Pieter WA Meyer

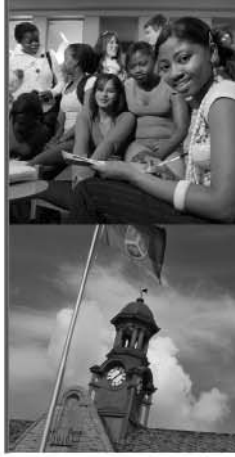
1.Autoimmune Diseases

Automated

- Anti-nuclear antibodies
- Anti-gliadin/ tissue transglutaminase (Coeliac)
- ANCA (MPO/PR3/GBM)
- Anti-CCP
- RF
- Cardiolipin (incl. B_2 Glucoprotein 1)
- Thyroid TPO/TG
- Parietal/intrinsic Factor
- Mannan (ASCA – Crohn's)

ELISA/IF

- Anti-nuclear antibodies
- Smooth muscle/LKM
- GAD/IA2
- Acetylcholine receptor
- IFBA
- Skin, cardiac, etc

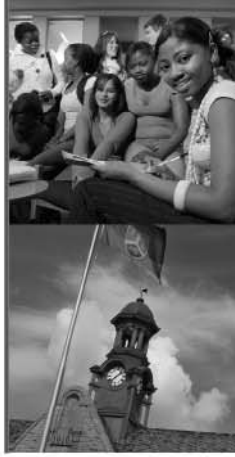


Diagnostic algorithm for CTD

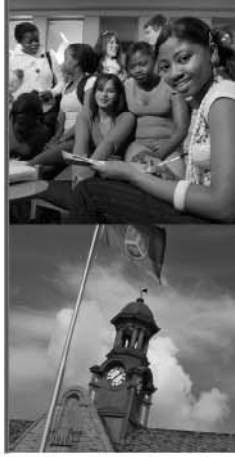
SLE and other connective diseases

- ANF (IIF/Hep-2 cells)
  - Negative
    - Titre < 1:160
  - Positive
    - ≥ 1:160
    - Report Titre and pattern
      - Homogenous, Peripheral, Speckled, Nucleolar, Centromere or combinations thereof.
- CTD Screen (ENA)
  - Cocktail of 18 associated antigens
  - Negative
    - <0.7 ratio
  - Positive
    - Breakdown
    - Quantitative determination of 18 antigens
      - dsDNA, Ro(SSA), La(SSB), Sm^{DP}, *U1RNP, RNP70, Scl-70, CENP, Jo-1, Rib-P, Fibrillarin, PM-Scl, Mi-2, PCNA*

Extractable Nuclear Antigens and Associated Diseases



Antigen	Disease Association
dsDNA	>85% SLE **Diagnostic**
SS-A (Ro 52/Ro60)	30-40% SLE; 50-80% Primary SJS; neonatal lupus, 5% RA
SS-B (La)	10-30% SLE; 50-80% Primary SJS, neonatal lupus
Sm^{DP}	About 30% of patients with SLE: **Diagnostic**
U1-snRNP (70kD, A an	Diagnostic for MCTD (overlap SLE/Scl/PM); 45% SLE
RNP70	Accompanies U1RNP, but not diagnostic; 45% SLE
Jo-1	30% of patients with polymyositis (PM): **Diagnostic**
Scl-70 (topoisomerase I)	70% progressive systemic sclerosis: **Diagnostic**
Centromere P	90% CREST Syndrome: **Diagnostic**
Ribosomal P	Neuropsychiatric SLE: **Diagnostic**
CENP	Scleroderma. >90% CREST **Diagnostic**
Fibrillarin	Scleroderma. Exclusively identifies systemic sclerosis (SSc)
Mi-2	Identifies idiopathic inflammatory myopathies (IIM), polymyositis dermatomyositis
PCNA	SLE, highly sensitive if highly elevated
PM-Scl	70% polymyositis/scleroderma (PM/SSc) overlap syndrome



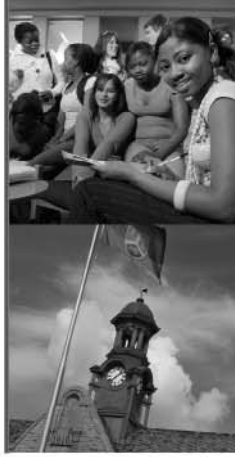
Patient 1: Female, 37 years (suspected SLE)

Tests requested: Measurement of ANA, CTD screen andantibodies to: dsDNA, nucleosomes, RF, CCP, and levels of the complement components C3 and C4.

Test	Result	Normal range
ANA	Positive 1/160(Homogeneous)	Negative
CTD Screen	32	<0.7 Ratio
dsDNA	381 U/ml	10 U/ml
RF	10	<15 IU/ml
CCP	0.2	<10 IU/ml
C3	0.3	0.9-1.8 g/l
C4	0.05	0.2-1.0 g/l
CIC	30	<10 U/ml

*Strongly positive for antibodies to SS-A, SS-B, Sm and U1RNP

Interpretation: SLE



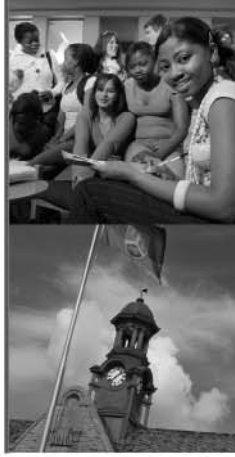
Patient 2: Female, 33 years, Limited Scleroderma (CREST Variant)

Tests requested: Measurement of ANA, CTD screen andantibodies to dsDNA and nucleosomes.

Test	Result	Normal Range
ANA	Positive 1:160(Centromere)	Negative
CTD Screen	12.3	<0.7 Ratio
dsDNA	1.0	<10 U/ml
Nucleosome	2	<20 U/ml

*Strongly positive for anti-centromere antibodies

Interpretation: Scleroderma



Patient 3: Female, 10 years (suspected polymyositis)

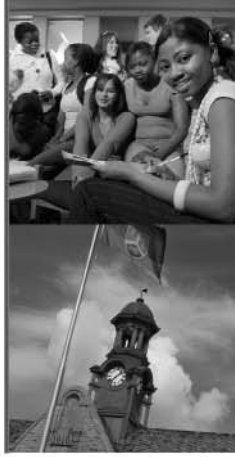
Tests requested: Measurement of ANA, CTD Screen and antibodies to dsDNA and nucleosomes.

Test	Results	Normal Range
ANA	Positive <1:160(Speckled)	Negative
CTD Screen	5.3	<0.7 Ratio
dsDNA	3.0	<10 U/ml
Nucleosome	0.9	<20 U/ml

Strongly positive for anti-Jo1 antibodies

Interpretation: Polymyositis

Serodiagnosis of Rheumatoid Arthritis



- Rheumatoid factor (about 80% of patients are seropositive)
  - Highly elevated RF results >400 IU/ml, strongly associated with more severe RA
- Anti-CCP antibodies (anti-cyclic citrullinated peptide antibodies, about 80% of patients are seropositive)
- Anti-CCP is present years before clinical onset of RA

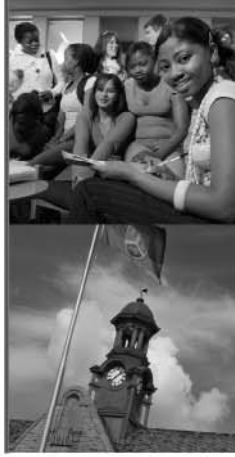
Recent studies in predominantly Black South African population (Gauteng Tertiary Institutes)

- RF and anti-CCP was very significantly correlated with HAD[1]
- RF and especially anti-CCP have high risk factors when associated with the "Shared Epitope" (a known genetic predisposition for RA)[2]

1. Meyer PWA et. al. Mediators of Inflammation, vol. 2010.doi:10.1155/2010/158514.

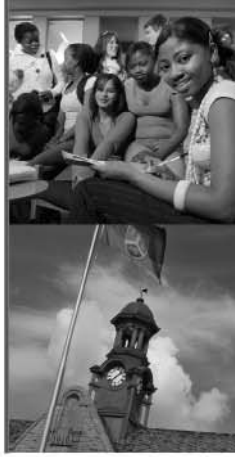
2. Meyer PWA et. al. Arthritis Research & Therapy 2011, 13:R160, doi:10.1186/ar3479

Laboratory Diagnosis of Allergic Sensitivity



- Measurement of total IgE in serum.
- Measurement of allergen-specific IgE in serum using the Immunocap / RAST system
  - Single allergens or groups of allergens
  - Screen using allergen mixes followed by testing for specific sensitivities.

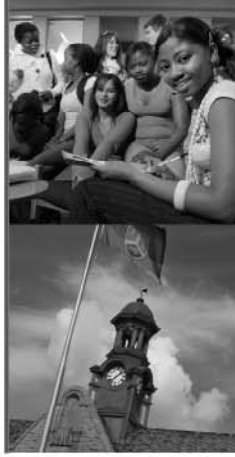
- Allergen microarrays containing 103 different allergens
- CAST-ELISA based on release of the cysteinyl leukotrienes C4 and D4 from allergen treated blood.



Unicap Allergen Mixes for Screening of Patients' Serum

- Aeroallergen mix (7 allergens):
  - House dust mite (x2) (*d1, d2)*
  - Cat dander *(e1)*
  - Dog dander *(e5)*
  - Cockroach*(i6)*
  - Grass *(g6)*
  - *Aspergillus fumigatus (m3)*
  - *Alternaria alternata (m6)*
- Food mix (6 allergens):
  - Cow's milk protein *(f2)*
  - Fish *(f3)*
  - Wheat *(f4)*
  - Peanut *(f13)*
  - Soya bean *(f14)*
  - Egg white *(f75)*

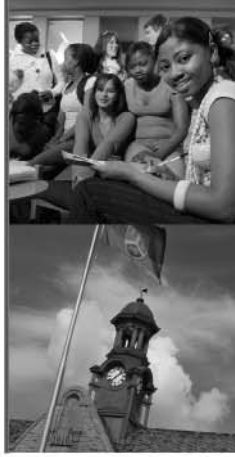
If positive, then test for reactivity to specific allergens



Laboratory Investigation of Patients with Suspected Primary Immunodeficiency Syndromes (PIDs)

Screening procedures: measurement of:

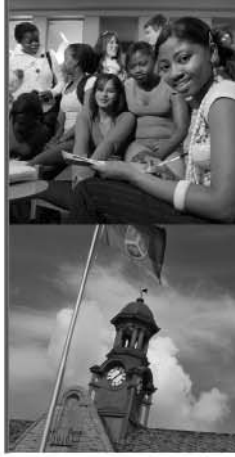
- Full blood count and differential.
- Flowcytometric analysis of:
  - circulating B cells (CD19).
  - T cells/subsets (CD3, CD4, CD8),
  - NK cells (CD56).
- Total serum immunoglobulins
  - IgG, IgA, IgM & Total IgE
  - IgG subclasses(if necessary)
- Total haemolytic complement
  - C3 and C4



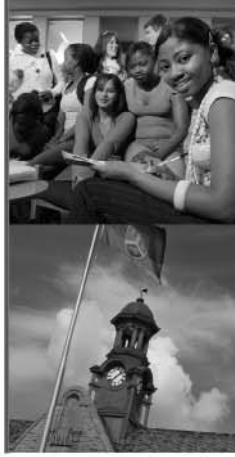
Follow-up Procedures: PIDs

- Neutrophil functions (antimicrobial activity, adhesion molecule expression).
- Monocyte/macrophage functions (production of cytokines e.g. IL-12 and expression the IFN-γ receptor).
- T-lymphocyte functions (proliferation, cytokine production e.g. IL-2 and IFN-γ, expression of the IL-2 receptor and CD154).
- Molecular/genetic analysis (if available)

Anti-Phospholipid Syndrome

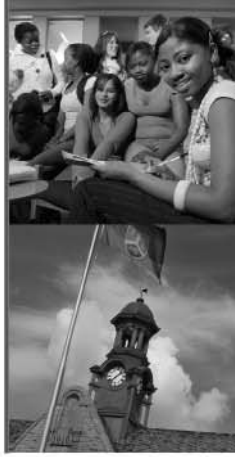


- Characterised by;
  - Arterial / venous thrombosis
  - Recurrent foetal loss / intra-uterine growth restriction
  - Thrombocytopenia
  - Female: Male ratio 2:1
- Several potential Risk Factors
  - Pre-existing inflammatory disorders
  - Cigarette smoking
  - Hyperlipidemia
  - Hypertension
- Associated with production of autoantibodies



Serodiagnostic Detection of Anti-Cardiolipin Antibodies

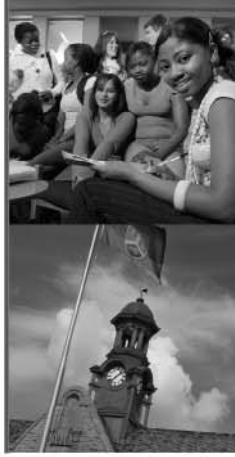
- ACLA IgG
- ACLA IgA
  - Predominant in Africanpopulation
- ACLA IgM
- Antibodies to β_2 glycoprotein-1
  - Assothciated with thrombosis and fetal loss.



Anti-Cardiolipin Antibodies

- Function as a pro-coagulant
- Target cardiolipin-β2 GP-1 complex, inhibiting thrombin / thrombomodulin activation of protein C.
- Inhibit degradation of factor V by protein S
- Bind to vascular endothelium causing activation of complement and generation of thromboxanes
- Bind to decidual tissue causing complement activation and generation of TNF

Anti-Phospholipid Antibody – Induced Pregnancy Loss



Binding of ACL to decidual tissue

↓

Complement Activation

↓

C5a

↓

TNF

↓

Activation of endothelial cells and leucocytes

↓

Activation of endothelial cells and leucocytes