Recent Diagnostic Tests In Pathology

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Metropolis Healthcare Services Ltd.
Categories

1. Immunochemistry – Cardiac markers, Allergy testing, Autoimmune disorders- Neurology, Liver, Renal, GIT
2. Heamatology- CD34 stem cell counts
3. Infections- HCV, TB, HIV
4. Histopathology- Liquid based cytology, IHC
5. Genetics & Molecular Biology - karyotyping, FISH, DNA sequencing
Biomarkers in Heart Failure

- **Natriuretic peptides** comprise a family of 3 structurally related molecules: atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and C-type natriuretic peptide, CNP.

- These peptides possess potent **natriuretic**, **diuretic**, and **vasodilating** activities and are implicated in body fluid homeostasis and blood pressure control. Unlike ANP and BNP, CNP does not have direct natriuretic activity.

- **Brain natriuretic peptide** (BNP), is a 32 amino acid polypeptide secreted by the **ventricles** of the heart in response to excessive stretching of heart muscle cells.

- BNP is co-secreted along with a 76 amino acid N-terminal fragment (NT-proBNP) which is biologically inactive. BNP binds to and activates the atrial natriuretic factor receptors in a fashion similar to **atrial natriuretic peptide** (ANP) but with 10-fold lower affinity.

- The **biological half-life** of BNP (20 mins), however, is twice as long as that of ANP, and that of NT-proBNP (1-2 hrs) is even longer, making these peptides better targets than ANP for diagnostic blood testing.

- Both BNP and NT-proBNP levels in the blood are used for screening, diagnosis of acute **congestive heart failure** (CHF) and may be useful to establish prognosis in heart failure, as both markers are typically higher in patients with worse outcome.

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**Heart failure in ambulatory setting**

<table>
<thead>
<tr>
<th>NT-proBNP</th>
<th>&lt;125 pg/ml &lt;75 years</th>
<th>NT-proBNP &lt;300 pg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>BNP</td>
<td>&lt;70 pg/ml</td>
<td>BNP &lt;100 pg/ml</td>
</tr>
<tr>
<td>GFR</td>
<td>&lt;60 ml/min</td>
<td>BNP 200 pg/ml</td>
</tr>
</tbody>
</table>

**Rule-out heart failure**

<table>
<thead>
<tr>
<th>NT-proBNP</th>
<th>&lt;450 pg/ml 75 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>BNP</td>
<td>GFR &lt;60 ml/min</td>
</tr>
</tbody>
</table>

**Rule-in heart failure**

<table>
<thead>
<tr>
<th>NT-proBNP</th>
<th>Age &gt;75: NT-proBNP &gt;1800 pg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Age 50–75: NT-proBNP &gt;900 pg/ml</td>
</tr>
<tr>
<td></td>
<td>Age &lt;50: NT-proBNP &gt;450 pg/ml</td>
</tr>
<tr>
<td>BNP</td>
<td>&gt;500 pg/ml</td>
</tr>
</tbody>
</table>

The Allergic Disorders

- Food Allergy
- Atopic dermatitis (Eczema)
- Gastrointestinal symptoms
- Asthma (cough; wheeze)
- Nervous system: Headaches, Irritability
- Allergic rhinoconjunctivitis (hay fever)
- Anaphylaxis
## The Allergic Disorders

<table>
<thead>
<tr>
<th>Sr. no.</th>
<th>Screening Category</th>
<th>Name of Panel</th>
<th>Included Allergens</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Allergy screening</td>
<td>Phasedrop</td>
<td>&gt; 4 years</td>
</tr>
<tr>
<td>2</td>
<td>Allergy screening</td>
<td>Phasedrop</td>
<td>0 to 4 years</td>
</tr>
<tr>
<td>3</td>
<td>Allergy screening</td>
<td>Phasedrop &amp; Total IgE</td>
<td></td>
</tr>
</tbody>
</table>

### Adult Panel - Comprehensive

<table>
<thead>
<tr>
<th>Age wise</th>
<th>Included Allergens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Child panel</td>
<td>Cow milk, Soybean, Peanut, Almond, Wheat, Egg white, Chick, Cat dander, Dog dander, Shrimp, Cat dander, Horse dander, Total IgE</td>
</tr>
<tr>
<td>Eczema panel</td>
<td>Egg white, Cow milk, Wheat, Rice, Peanut, Lentil, Carrot, Soya bean, Lemon, Banana, D fernae, D pteronyssinus, Dog dander, Chick pea, Cow dander, Shrimp, Cat dander, Horse dander, Total IgE</td>
</tr>
<tr>
<td>Rhinitis/Allergy</td>
<td>Cow milk, Soybean, Dog dander, Antigen, Dog dander, Cat dander, Aspergillus, Cockroach, Dog dander, Egg white, Lemon, D fernae, House dust mite, Total IgE</td>
</tr>
<tr>
<td>Asthma panel</td>
<td>Cow milk, Soybean, Dog dander, Cat dander, Dog dander, House dust mite, Antigen, Cat dander, Dog dander, Egg white, Lemon, D fernae, House dust mite, Total IgE</td>
</tr>
<tr>
<td>GIT panel</td>
<td>Egg white, Fish, Wheat, Peanut, Soyabean, Cow milk, Cod fish, Shrimp, Blue mussel, Salmon, Tuna, Almond, Coconut, Cashew nut, Peanut, Coconut, Cashew nut, Peanut, Tomato, Yeast, Garlic, Onion, Celery, Egg yolk, Chicken meat, Banana, Pineapple, Apple, Gluten, Total IgE</td>
</tr>
</tbody>
</table>

### Geographic

<table>
<thead>
<tr>
<th>Region</th>
<th>Included Allergens</th>
</tr>
</thead>
<tbody>
<tr>
<td>North India</td>
<td>Gynodorus, Soygum, Bacoo, Goosefoot, Ragweed, Adler, Eucalyptus, Saman, Ripe polian, D fernae, D pteronyssinus, Cockroach, Cat dander, Dog dander, Cow dander, Horse dander, P aspergillus, A mungus, Mungo, Paspalum, Total IgE</td>
</tr>
<tr>
<td>Eastern India</td>
<td>Eucalyptus, Common ragweed, Adler, Birch, Gynodorus, Mugwort, Cocklebur, D fernae, D pteronyssinus, A mungus, Mungo, Paspalum, Total IgE</td>
</tr>
<tr>
<td>Central India</td>
<td>Gynodorus, Chick pea, D fernae, D pteronyssinus, Cockroach, Cat dander, Dog dander, Cow dander, A mungus, Mungo, Paspalum, Total IgE</td>
</tr>
<tr>
<td>South India</td>
<td>Coconut, Almond, Cashew nut, Banana, Cat dander, Dog dander, Gynodorus, Comorion ragweed, A mungus, Mungo, Paspalum, Total IgE</td>
</tr>
</tbody>
</table>

### Food Panel

<table>
<thead>
<tr>
<th>Food panel</th>
<th>Included Allergens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food panel 1</td>
<td>Tomato, Yeast, Garo, Onion, Celery</td>
</tr>
<tr>
<td>Vegetable panel 2</td>
<td>Tomato, Paprika, Sprout, Cabbage</td>
</tr>
<tr>
<td>Non-vegetarian panel</td>
<td>Chicken, Mutton, Salmon, Tuna, Shrimp, Pork, Cod fish, Egg white, Egg yolk, Blue mussel, Total IgE</td>
</tr>
<tr>
<td>Vegetarian panel</td>
<td>Cow milk, Lemon, Pineapple, Peanut, Potato, Mustard, Wheat, Rice, Soyabean, Lentil, Olive, Total IgE</td>
</tr>
<tr>
<td>Vegetable panel 1</td>
<td>Tomato, Yeast, Garo, Onion, Celery</td>
</tr>
<tr>
<td>Non-vegetarian panel</td>
<td>Chicken, Mutton, Salmon, Tuna, Shrimp, Pork, Cod fish, Egg white, Wheat, Peanut, Soybean, Cow milk, Nut, Mungo, Common ragweed, Egg white, Cod fish, Shrimp, Tuna, Salmon, Blue Mussel, Pineapple, Potato, Lemon, Rice, Lentil, Mustard, Olive, Cocoa, Total IgE</td>
</tr>
<tr>
<td>Nut panel</td>
<td>Almond, Cashew nut, Peanut</td>
</tr>
<tr>
<td>Oil panel</td>
<td>Mustard seed, Ripe polian, Peanut, Coconut, Soybean, Olive</td>
</tr>
<tr>
<td>Fruit panel</td>
<td>Mango, Kiwi, Banana, Pineapple, Lemon, Blueberry, total IgE</td>
</tr>
<tr>
<td>Fish panel</td>
<td>Cod fish, Shrimp, Blue mussel, Salmon, Tuna</td>
</tr>
</tbody>
</table>

### Drug Allergy Panel

<table>
<thead>
<tr>
<th>Drug allergy panel</th>
<th>Included Allergens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mini</td>
<td>Antistin, Ampicillin, Acth, Penicillin, Penicillin</td>
</tr>
<tr>
<td>Human insulin</td>
<td></td>
</tr>
</tbody>
</table>

### Environmental Panel

- **Occupational allergy**: Acacia, wood dust, Castor bean, Cotton seed, Ethylene oxide, Fomam, Latex, Silk, Sunflower seeds, Total IgE
- **Trees panel**: Alnus incana, Common silver birch, Corylus avellana, Oak, Saltic caprea
- **Weeds panel**: Common ragweed, Mugwort, Plantain englisch, Goosefoot, Salicornia
- **Environmental panel**: Phasedrop, Cat dander, Dog dander, Horse dander, Cow dander, House dust mite, D fernae, D pteronyssinus, Cockroach, Alternaria, A mungus, Paspalum, Candida albicans, D fascihymenoides, Timothy grass, Johnson grass, Common ragweed, Mugwort, common Triticale, Common pigweed, Alf, Erch, Olive, Honey bee venom, Rape pollen, Total IgE

- **Grass panel**: Gynodorus, Poa, Common grass, Panicum, Lolium, Phleum
- **Animal panel**: Cat dander, Dog dander, Horse dander, Cow dander, Rabbits epithelium, Pig ear dropping
- **Dust panel**: House dust mite, D fernae, D pteronyssinus, Cockroach
- **Mild panel**: Alternaria, A mungus, Paspalum, Phomopsis, Candida albicans
- **Indoor panel**: House dust mite, D fernae, D pteronyssinus, Aspergillus, Candida, Cat dander, Dog dander, Cockroach
- **Outdoor panel**: Rye, Sweet vernal, Golden rod, English plantain, Alder, Birch, Lamb's quarter, Nettle
- **Insect allergy**: Honey bee venom, Cockroach, Mosquito
- **Vegetarian panel**: Cow milk, Lemon, Pineapple, Peanut, Potato, Mustard, Wheat, Rice, Soyabean, Lentil, Olive, Total IgE
- **Vegetable panel 1**: Tomato, Yeast, Garo, Onion, Celery
- **Vegetable panel 2**: Tomato, Paprika, Sprout, Cabbage
- **Non-vegetarian panel**: Chicken, Mutton, Salmon, Tuna, Shrimp, Pork, Cod fish, Egg white, Egg yolk, Blue mussel, Total IgE
- **Vegetarian panel**: Egg white, Wheat, Peanut, Soybean, Cow milk, Mutton, Chicken meat, Pork, Egg yolk, Cod fish, Shrimp, Tuna, Salmon, Blue Mussel, Pineapple, Potato, Lemon, Rice, Lentil, Mustard, Olive, Cocoa, Total IgE
- **Nuts panel**: Almond, Cashew nut, Peanut
- **Oil panel**: Mustard seed, Ripe polian, Peanut, Coconut, Soybean, Olive
- **Fruit panel**: Mango, Kiwi, Banana, Pineapple, Lemon, Blueberry, total IgE
- **Fish panel**: Cod fish, Shrimp, Blue mussel, Salmon, Tuna
Classical Fields of Neuroimmunology Research

Immune response to diseases of the nervous system

- Intra thecal humoral specific response to various antigens as diagnostic tool in - Demyelinating diseases (Central-MS, NMO, Peripheral -GBS),
  - Infections- viral.

- Aetiology for Encephalitides associated with antibodies to neuronal cell surface antigens (GAD, NMDA, VGKC)

- Aetiology for various paraneoplastic syndromes are antineuronal antibodies (Hu, Ri, Ma, CV2, amphiphysin).
**Isoelectric focusing of CSF.**

Case patient samples are in lanes 4 (CSF) and 4’ (serum). The CSF sample is positive for oligoclonal bands (arrows). The IgG index was 0.7 (reference interval 0.3–0.8) and did not suggest increased intrathecal synthesis. The serum/CSF albumin ratio was <9 indicating normal permeability of the blood brain barrier. Controls are indicated on the gel. Other patients are negative for oligoclonal banding shown in lanes 2, 3, and 5.

<table>
<thead>
<tr>
<th>Pattern</th>
<th>Bands Observed</th>
<th>Associated Diseases</th>
</tr>
</thead>
</table>
| 1       | Polyclonal pattern (no discrete bands) in both serum & CSF | Rare Multiple sclerosis  
Myelitis  
CNS vasculitis  
Paraneoplastic syndromes  
Systemic lupus erythematosis |
| 2       | Same number of OCB in serum & CSF | Myelitis  
CNS vasculitis  
Paraneoplastic syndromes  
Systemic infections  
Systemic autoimmune disease  
Lymphoproliferative disorders |
| 3       | OCB in both serum & CSF: CSF has at least 2 more bands than serum | Multiple sclerosis  
Most CNS infections |
| 4       | More than 2 OCB in CSF & polyclonal pattern in serum | Most Multiple sclerosis |
| 5       | Monoclonal band in both serum & CSF | Multiple myeloma  
MGUS, lymphoma |
Neuromyelitis Optica (NMO)

In India NMO diagnosed by Winger chuck et al. criteria 1999 is 5% (Pandit et al).

Aquaporin 4 antibodies by Indirect Immunofluorescence –

Aquaporin4 is an osmosis driven water channel protein present on astrocytes

a-Binding of serum AQP4-Ab to adult mouse cerebellum. Magnified images show staining of  b- the microvasculature, c - the Virchow–Robin spaces and d - the pia mater.

e–g | Binding of serum AQP4-Ab to the surface of cultured human embryonic kidney (HEK293) cells transfected with human full length AQP4, nontransfected control cells (panel g)

<table>
<thead>
<tr>
<th>Aquaporin 4</th>
<th>IIF - Mosaic with rat cerebrum, cerebellum, transfected HEK cells with aquaporin 4 &amp; non-transfected HEK cells</th>
<th>IgG NMO Rarely seen in SLE, Sjogren syndrome</th>
</tr>
</thead>
</table>
There is evidence that specific autoantibodies directed against neuronal proteins crucial to the control of neurotransmission are responsible for a proportion (~8% in one series) of such cases.

<table>
<thead>
<tr>
<th>Autoantibody</th>
<th>IIF or blot assay</th>
<th>Disease association</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAD</td>
<td>Cerebellar neuronal cytoplasm, granular positivity</td>
<td>Stiff syndrome</td>
</tr>
<tr>
<td>NMDA (NR1)</td>
<td>Mosaic with rat hippocampus &amp; cerebellum, transfected HEK cells with NMDA-NR1 &amp; non-transfected HEK cells</td>
<td>Limbic encephalitis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Psychiatric lupus</td>
</tr>
<tr>
<td>VGKC (caspr2,Ligi1)</td>
<td>Mosaic similar to NMDA</td>
<td>Limbic encephalitis</td>
</tr>
</tbody>
</table>
**Anti-neuronal antibodies – Paraneoplastic syndromes**

These antibodies can occur up to 5 years before the appearance of detectable tumour. Associated ANA can interfere in interpretation & Blot assay recommended in such cases to confirm antineuronal antibodies.

<table>
<thead>
<tr>
<th>Anti Neuronal Antibody</th>
<th>IIF / Immunoblot</th>
<th>Disease association</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANNA-1 (anti-Hu)</td>
<td>Cerebral &amp; cerebellar neuronal nuclei, intestinal meyenteric plexus</td>
<td>Subacute sensory neuronopathy – SCLC, neuroblastoma</td>
</tr>
<tr>
<td>ANNA-2(Anti- ri)</td>
<td>Cerebral &amp; cerebellar neuronal nuclei, Absent intestinal meyenteric plexus</td>
<td>Opsoclonus-myoclonus syndrome- Breast CA, SCLC</td>
</tr>
<tr>
<td>Anti- yo (anti-purkinje cell)</td>
<td>Cerebellar Purkinje cells cytoplasm</td>
<td>Diploplia, dysarthria, tremors- ovary, breast, uterus CA</td>
</tr>
<tr>
<td>Anti- tr (anti-purkinje cell)</td>
<td>Cerebellar Purkinje cells cytoplasm &amp; dendritic processes</td>
<td>Exact association unclear</td>
</tr>
<tr>
<td>Anti - amphyphysin</td>
<td>Cerebellar neuronal cytoplasm- granular positivity</td>
<td>Stiff person syndrome – SCLC, breast CA</td>
</tr>
<tr>
<td>Anti – CV2</td>
<td>Cerebellar neuronal cytoplasm- weak diffuse positivity</td>
<td>SCLC, thymoma</td>
</tr>
<tr>
<td>Anti - Ma</td>
<td>Cerebellar neuronal nucleoli positive</td>
<td>Brain stem encephalitis Anti Ma1- breast CA Anti Ma2/Ta- testis tumours</td>
</tr>
</tbody>
</table>
### Peripheral Nervous System - Autoantibody

<table>
<thead>
<tr>
<th>Clinical Syndromes</th>
<th>Specific Antibodies to</th>
<th>GM1</th>
<th>Asialo-GM1</th>
<th>GM2</th>
<th>GD1a</th>
<th>GD1b</th>
<th>GQ1b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guillain-Barré syndrome (GBS)</td>
<td>MAG SGPG</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>++ IgG</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(++)</td>
<td></td>
<td>IgM 6%</td>
<td>IgG 5%</td>
<td>IgG 2%</td>
<td></td>
</tr>
<tr>
<td>GBS variants:</td>
<td></td>
<td>+++</td>
<td>+</td>
<td>+++</td>
<td>+</td>
<td></td>
<td></td>
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<tr>
<td>AMAN and AMSAN</td>
<td></td>
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<tr>
<td>GBS with ophthalmplegia</td>
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<td></td>
<td></td>
<td>++ IgG</td>
<td></td>
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<tr>
<td>Ataxic GBS</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>++</td>
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<tr>
<td>CMV-related GBS</td>
<td></td>
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<tr>
<td>Miller-Fisher syndrome and related</td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td>+++ IgG &gt; 90 %</td>
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<tr>
<td>conditions</td>
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<tr>
<td>Multifocal motor Neuropathy (MMN)</td>
<td></td>
<td>+++ IgM 20-80%</td>
<td>(++)</td>
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<td>+</td>
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<td></td>
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<td>IgM 5%</td>
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<tr>
<td>Lower Motor Neuron Syndrome</td>
<td></td>
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<td>+</td>
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<tr>
<td>Neuropathy with anti-MAG/SGPG IgM-monoclonal gammopathy</td>
<td>MAG/SGPG</td>
<td>+++ M-IgM 50%</td>
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<tr>
<td>Motor neuropathy with IgM-monoclonal</td>
<td></td>
<td>+++ M-IgM 10%</td>
<td></td>
<td></td>
<td>+++</td>
<td></td>
<td></td>
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<tr>
<td>gammopathy</td>
<td></td>
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<tr>
<td>Sensory ataxic neuropathy and CANOMAD</td>
<td></td>
<td>+++ M-IgM</td>
<td></td>
<td>+++</td>
<td>+</td>
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<tr>
<td>Chronic inflammatory demyelinating Poly-</td>
<td></td>
<td>++ M-IgM</td>
<td>+</td>
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<td></td>
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<tr>
<td>neuropathy (CIDP)</td>
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</tbody>
</table>

**Line Blot Assay:**
- For antibodies against Gangliosides

![Line Blot Assay Diagram](attachment:image.png)
Neuromuscular disorders

Neuromuscular junction disease is a condition where the normal conduction through the neuromuscular junction fails to function correctly.

- **Post synaptic** -
  In *myasthenia gravis*, the End Plate Potential (EPP) fails to effectively activate the muscle fiber due to an autoimmune reaction against acetylcholine receptors, resulting in muscle weakness and fatigue.
  Most commonly by auto-antibodies against the acetylcholine receptor. It has recently been realized that a second category of gravis is due to auto-antibodies against **MuSK**.

- **Pre synaptic** –
  In *Lambert-Eaton myasthenic syndrome*, is usually associated with *presynaptic* antibodies to the voltage-dependent calcium channel.

These autoantibodies namely ACRAB, MUSK & VGCC can be tested in serum by immunoprecipitation reaction using Radioimmunoassay (RIA) technique.
# Myositis – serum markers

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Syndrome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mi2</td>
<td>DM</td>
</tr>
<tr>
<td>Jo1</td>
<td>Interstitial lung disease/anti-synthetase syndrome</td>
</tr>
<tr>
<td>Signal recognition particle</td>
<td>Severe PM</td>
</tr>
<tr>
<td>p-155</td>
<td>Juvenile DM</td>
</tr>
<tr>
<td>PM-Scl</td>
<td>Scleroderma + myositis</td>
</tr>
<tr>
<td>Ku</td>
<td>Scleroderma + myositis</td>
</tr>
<tr>
<td>Small nuclear RNP</td>
<td>Mixed connective tissue disease + myositis</td>
</tr>
<tr>
<td>Cytoplasmic RNP</td>
<td>Sjogren’s/ Systemic lupus erythematosis + myositis</td>
</tr>
</tbody>
</table>
Glomerulonephritis - Phospholipase A2 receptor (PLA2R) Antibody - IIF

- Primary Membranous Glomerulonephritis - The autoimmune mechanism of primary MGN, which was first discovered and described in 2009, is the result of autoantibodies reacting with phospholipase A2 receptors (transmembrane glycoproteins), which are expressed in human glomeruli on the surface of podocytes.
- They are involved in regulatory processes in the cell following phospholipase binding. Up until now two main groups of PLA2 receptors have been described (types M and N), with the M type being identified as the major target antigen of autoantibodies. The antigen/antibody complexes (immune deposits) are deposited in the glomerular basement membrane of patients with primary MGN & trigger complement activation with overproduction of collagen IV and laminin.
Autoimmune Liver Disease

Anti Smooth Muscle Antibodies - IIF

The BIOCHIP Mosaic consists of 6 substrates: human epithelial cells (HEp-2), primate liver, rat kidney, rat liver, rat stomach, VSM47 (F-Actin specific).

Thus, a broad spectrum of antigens is present, allowing not only targeted serological diagnoses, but also frequently yielding additional results with clinical relevance.

In the case of ASMA - the tunica muscularis, the lamina muscularis mucosa as well as the interglandular contractile fibrils fluoresce on the rat stomach. ASMA directed against the target antigen F-actin furthermore react with the cytoskeleton of HEp-2 cells and the bile canaliculi of primate liver. The substrate VSM47 reacts very specifically, showing a filamentous, needle-like fluorescence.
Fecal Calprotectin

- A 35 KDa Calcium and Zinc binding protein found in neutrophils, monocytes, and macrophages. Up to 60% of the total cytosolic protein content of neutrophils. Released from cells during cell activation or death.

FUNCTIONS-

- Binds to calcium and has antimicrobial & antitumoural properties.
- Reduces local zinc concentrations, and inhibits zinc dependent metalloproteinases
- Stable in faeces for several days (upto 5 days) after excretion

The main diseases that cause an increased excretion of Faecal Calprotectin:

- Crohn's disease, ulcerative colitis and neoplasms.
- Levels of faecal calprotectin are normal in patients with irritable bowel syndrome (IBS).

Specific indications for measuring Faecal Calprotectin are in:

- Identify organic bowel disease against functional bowel disease (IBS), and thus avoid the need for invasive tests such as colonoscopy.
- Assessing efficacy of IBD treatments.
- Predicting relapses or flares of IBD.
Patient <45 years with symptoms of (change in bowel habit, abd pain, bloating) for the past 3 months

TTG positive, treat as Coeliac disease.

Measure TTG + FBC, TSH and stool for Calprotectin at same time

If TTG, FBC, TSH & FC - NAD

Follow anaemia pathway.

Measure Faecal Calprotectin

Likely IBS

FC <50 ug/g, no evidence of active bowel inflammation. Symptoms highly likely to be due to IBS. If diarrhoea persists, or there remains clinical concern, consider referral for further investigations

FC >50 ug/g, Raised Calprotectin consistent with active bowel inflammation, further Gastroenterology investigations.
Reproductive hormones

Anti Mullerian Hormone

- Structurally related to inhibin and activin, and a member of the transforming growth factor-β (TGF-β) family. In female embryogenesis the absence of AMH allows for the development of upper vagina, uterus and cervix, and oviducts. Produced directly by growing primary, preantral & small antral ovarian follicles. Measuring ovarian aging-As number of antral follicles decrease with age, Anti-Müllerian hormone (AMH) serum levels also become diminished –undetectable(<0.3ng/ml) near menopause. Independent of menstrual cycle phase

- Evaluates fertility potential and ovarian response in IVF (<1ng/ml poor reserve). In anovulatory conditions including PCOS (> 6.7 ng/ml)

Inhibin B

- Inhibin B Test -predicts ovarian reserve, including egg quality and egg quantity. Inhibin B is produced directly by FSH-sensitive cohort of antral follicles, the amount of Inhibin B in the blood directly correlates to the number of eggs in the ovaries. Inhibin B can also predicts the ability of ovaries to produce more follicles. Low levels of Inhibin B(< 45pg/ml) are associated with: impaired ovulation, decreased success with IVF & lower pregnancy rates.

In male infertility in oligo/azoospermia, inhibin B (< 60 pg/ml)– favours spermatogenesis failure.

**Figure 3.** Circulatory pattern of inhibin B during the menstrual cycle of young healthy women. Day 0 = day of LH surge. Data are mean ± SD. *, P < 0.05 versus –14 time-point.

**Figure 4.** Circulatory pattern of anti-Mullerian hormone (AMH) during the menstrual cycle of young healthy women. Day 0 = day of LH surge. Data are Mean ± SD.
CA – 125 & HE 4 : ROMA

**HE4** - Human epididymis protein 4 (HE4), a relatively new marker for ovarian carcinoma, is the product of the *WFDC2 (HE4)* gene that is overexpressed in patients with ovarian carcinoma.

- Combination of HE4 and CA125 is better with 76.4% sensitivity and 95% specificity, making the combination more accurate than either test alone especially in Stage I, or early ovarian cancer.

Computing Risk of Ovarian Malignancy Algorithm (ROMA) classifies patients as being low or high risk for epithelial ovarian cancer.

<table>
<thead>
<tr>
<th>Premenopausal</th>
<th>ROMA &gt; 13% = High Risk for epithelial ovarian cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ROMA &lt; 13% = Low Risk for epithelial ovarian cancer</td>
</tr>
<tr>
<td>Postmenopausal</td>
<td>ROMA &gt; 28% = High Risk for epithelial ovarian cancer</td>
</tr>
<tr>
<td></td>
<td>ROMA &lt; 28% = Low Risk for epithelial ovarian cancer</td>
</tr>
</tbody>
</table>
Bone disorders - Osteoporosis

Bone Remodeling

**OC**
- Proteases
- Collagen
- Calcium
- Osteocalcin
- Hydroxyproline

**OB**
- Matrix
- TGF-B
- LTBP
- IGF-1
- IGF-2
- IGF-BPs

**RANK L**
- M-CSF
- IL-1, IL-6, IL-11
- TNF, TGF-B

**RANK**
- Ephrins
- D-Pyr
- CTx
- NTx
- EPHs

**Bone Remodeling**
- Activation
  - PTH, E2,
  - IL-1, IL-6, BMP
- Inhibition
  - Sclerostin, DKK1,
  - Leptin
  - Osteocalcin
  - BSAP

**Biochemical Markers of Bone Metabolism**
(Monitors of Bone Loss)

**Formation**
- Osteoblasts
  - AP / BAP (S)
  - Osteocalcin (S)
  - PICP / P1NP (S)

**Resorption**
- Osteoclasts
  - Crosslinks and crosslinked telopeptides
    - NTX, CTX (U, S)
  - Calcium (U)
  - Hydroxyproline (U)

**Markers of Bone Resorption:** Type I Collagen Crosslinks

- Free PYD and DPD (40%)
- Crosslinked C and N-telopeptides (60%)

**PYD** = pyridinoline; **CTX** = C-telopeptides of type I collagen
**DPD** = deoxypyridinoline; **NTX** = N-telopeptides of type I collagen
Flow Cytometry – CD34

CD34 Stem Cell Counts
Issues and Development

Non Specific Binding of CD34 Mab to Monocytes and Apoptotic Fragments

CD34- Heavily Glycosylated Molecule
Ab Epitopes with different sensitivity
Class I Mab
Class II Mab
Class III Mab
Preferred choice of Monoclonal Ab
Class II – PE conjugated or
Class III FITC or PE conjugated

Different Protocols for CD34 counting

Milan Protocol
\[
\% \text{CD34}^+ \text{cells} = \frac{\text{CD34}^+ \text{events}}{\text{FS vs. SS events}} \times 100
\]
Denominator may include debris, dead cells, nRBCs

SIHON Protocol
\[
\% \text{CD34}^+ \text{cells} = \frac{\text{CD34}^+ \text{events}}{\text{LDS 751}^+ \text{events}} \times 100
\]
Monocytes (CD14^+) and granulocytes (CD66^+) are excluded from the numerator to give purified CD34^+ population
Denominator includes only live cells (LDS 751^+ events) thus excluding debris, unlysed erythrocytes and platelets

ISHAGE Protocol
\[
\% \text{CD34}^+ \text{cells} = \frac{\text{CD34}^+ \text{events}}{\text{CD45}^- \text{ vs. SS events}} \times 100
\]
Numerator is a highly purified population which are bright CD34^+ , dim CD45^- and low FS & SS events
Denominator truly represents the leukocytes as only CD45^- are included and debris and nRBCs are excluded

Dual Platform: Based on Total WBC count from Cell Analyser. Variable Denominators in different protocols for assessing absolute CD34 counts

Single Platform: Bead Based Assays
Accurate and Precise
Bypass Denominator variability and are not affected by inaccuracy of cell analyser total WBC counts

A

Ungated
Gated on R1

R1
CD45 FITC
CD34 PE

R2


B

Gated on R4

R3
CD45 FITC

R4
GM-CSF Rα

CD34 PE
Blood Group - Automation

Column agglutination technology (CAT) - It offers many advantages over traditional methods:
- Improved sensitivity
- Standardized procedures
- Objectivity
- Increased productivity and ease of use
- Easy to automate

In addition a full automation platform, the Cassette may be used manually, with a workstation consisting of a centrifuge, incubator, precision pipettes and work
Infections - HCV

**Importance of HCV Genotype -**
HCV Genotype information is important because of the role it plays in HCV medical treatment. The treatment duration, dose of ribavirin and whether an HCV protease inhibitor will be added to pegylated interferon and ribavirin therapy are also affected by genotype.

**HCV Genotype Distribution**
1, 2, 3  Worldwide
4  Middle East, Africa
5  South Africa
6  Southeast Asia

**Genotype and Treatment Duration**
Generally, genotypes 2 and 3 are treated for 24 weeks. Treatment duration for HCV genotype 1 is dictated by response-guided therapy—that is, depending on the how quickly someone becomes HCV RNA negative will determine if treatment duration will be 24, 36 or 48 weeks. HCV genotype with type 1 or 4 have lower sustained virological response (SVR) rates than those infected with type 2 or 3.

**HCV & IL28B**
Genetic variation in the interleukin 28B (IL28B) gene has been associated with the response to interferon- alfa/ribavirin therapy especially in HCV genotype 1 infection. The importance of three IL28B single nucleotide polymorphisms (rs8099917 CC, rs12980275 CT and rs12979860 TT) for HCV genotype 2/3-infections is unknown.
Infections - TB

The effective treatment of MDR-TB is a life-saving intervention

Early diagnosis of both TB and DR-TB are the key for an effective TB control

Novel technologies for rapid screening of anti-TB drug resistance have become a priority in Tuberculosis research
Infections - TB

TRANSFORMING THE DIAGNOSIS OF TUBERCULOSIS

Conventional Culture

PCR

Algorithm for diagnosis of tuberculosis

Sample Type

Pulmonary

- All other Patients
  - AFB Smear
    - Positive
    - Negative
  - Xpert MTB/RIF

Extra Pulmonary

- AFB Smear
  - Positive
  - Negative
- Culture + TMA (rRNA testing)
  - Positive
  - Negative
- Negative specimen
  - AFB Identification and MDR screen by molecular genotyping
  - Resistance pattern (RIF & INH)
  - If MDR
    - AFB - identification and XDR screen by molecular genotyping
Infections - TB

In addition to being sensitive and specific, it is also endorsed by WHO

“A Globally Established, WHO endorsed test for diagnosis of TB within 24 hours”

- Mycobacterium Tuberculosis (MTB) Complex Identification
- Identification of Mono-Resistance
- Identification of MDR

Detects resistance by demonstration of mutations in

- rpoB gene: Rifampicin resistance
- katG gene: high level Isoniazid resistance
- inhA gene: low level Isoniazid resistance

Can be used for Sputum and Non-Sputum Samples

Helps definitive diagnosis

Hain’s Test

Allow decision on treatment initiation

Report in ONE day

Resistance - R+I I R+I R+I R+I

R = Rifampicin
I = Isoniazid
Infections - HIV

- HLAB*5701 associated HCP5rs2395029 genotyping for Abacavir hypersensitivity-
  The assay detects presence of HCP5rs2395029  T>G (valine to glycine) variant through linkage disequilibrium which predicts the presence of HLAB*5701. Approximately 5% individuals can develop life threatening hypersensitivity to abacavir, hence prior testing is suggested before starting therapy.

-HIV- I And HIV- II RNA Quantitative PCR

- HIV I Drug resistance - virtual phenotype
  This test is really a method of interpreting genotypic test results. First, genotypic testing is done on the sample. Phenotypic test results for other virus samples with a similar genotypic pattern are taken from a database. These matched samples tell how the virus is likely to behave. The virtual phenotype is faster and less expensive than a phenotypic test.
Cervical cancer screening

- Pap Smears
- Thin prep / Liquid based cytology
Cervical cancer - HPV Tests -

1. Real time PCR -

2. Hybrid Capture Assay –
An In Vitro Nucleic Acid Hybridization Assay with Signal Amplification using Microplate Chemiluminescence for the Qualitative detection of Human Papillomavirus (HPV) serotypes in Cervical Specimens.

<table>
<thead>
<tr>
<th>Selected Human Papillomavirus (HPV) Types</th>
<th>According to Risk Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Risk status</td>
<td>HPV type number</td>
</tr>
<tr>
<td>High-risk or oncogenic</td>
<td>16, 18, 31, 33, 35, 45,</td>
</tr>
<tr>
<td>HPV types</td>
<td>51, 52, 56, 58</td>
</tr>
<tr>
<td>Low-risk HPV types</td>
<td>6, 11, 42, 43 and 44</td>
</tr>
</tbody>
</table>
# Multiplex IHC Tests

The most advanced multiplex technology available for any IHC laboratory
- Increase predictive value by combining highly sensitive & highly specific antibodies on one slide
- Conserve precious patient tissue, reduce labor + reagent costs by > 50%

Solving complex clinical problems & simplifying interpretation

Allows for differentiation of CIS from benign lesions (breast, prostate & bladder)
- Simultaneously test for morphologically distinct markers > superior diagnostic data
- Eliminate multiple slides to evaluate antigen ratios such as Kappa:Lambda

<table>
<thead>
<tr>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung Adeno-2™ (TTF-1 + Napsin A)</td>
<td>Lung Squamous-2™ (p63 + CK5)</td>
<td>Kappa + Lambda</td>
</tr>
<tr>
<td>4</td>
<td>5a</td>
<td>5b</td>
</tr>
<tr>
<td>CD4 + CD8</td>
<td>Glypican-3 + CK19</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D2-40 + KI-67</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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Proven IHC Staining Platform – The Autostainer™ platform has been delivering reliable IHC results globally for over a decade

Broad Capabilities – A fully open platform for maximum flexibility to program individual slide IHC protocols. MultiPlex and Routine products while simultaneously performing your research projects and other custom protocols. Flexibility in staining software allows optimization of dispense volumes and incubations

**Multiplex IHC Tests**

1. Lung adenocarcinoma: TTF-1 (DAB) + Napsin A (FR)
2. Lung squamous cell carcinoma: p63 (DAB) + CK5 (FR)
3. Neoplastic lymphoma: Kappa (DAB) + Lambda (FR)
4. Large cell lymphoma: CD4 (DAB) + CD8 (FR)
5a. Hepatocellular carcinoma b) Cholangiocarcinoma: Glypican-3 (DAB) + CK19 (FR)
Karyotyping
- Targets **metaphase** cell nuclei
- Requires cell culture & sample pretreatment hence more time consuming
- It utilizes various **staining techniques** (G-banding, C-banding, Q-banding, T-banding, R-banding)
- Upto 20 cells screened
- The major advantage of karyotyping is that it gives a very thorough picture of an individual's chromosomes.

FISH
- Targets **interphase** cell nuclei
- Does not require cell culture & sample pretreatment hence faster
- It utilizes target **DNA probes**
  (Types- Locus specific, Centromere repeats specific, Whole chromosome specific)
- Upto 100 cells screened
- Advantage of identifying mosaicism
- Since the key to FISH is knowing the base pair sequence and/or location of a gene, it cannot be used as a general screening tool

- **Interphase & Metaphase FISH sample**
- **Trisomy 21** → Down Syndrome
- **Chromosomal paint**: 21 (red); X (green)
DNA Sequencing -

- During the last 25 years, a number of meaningful DNA-based diagnostic tests have been available to aid in the diagnosis and subsequent treatment of heritable disorders, especially neurological and metabolic & hematologic disorders.

- These tests have targeted a limited number of genes and are often ordered in serial testing strategies in which results from one preliminary test dictate the subsequent test orders.

### HBB Sequence in Normal Adult Hemoglobin (Hb A):

<table>
<thead>
<tr>
<th>Nucleotide</th>
<th>CTG</th>
<th>ACT</th>
<th>CCT</th>
<th>GAG</th>
<th>GAG</th>
<th>AAG</th>
<th>TCT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amino Acid</td>
<td>Leu</td>
<td>Thr</td>
<td>Pro</td>
<td>Glu</td>
<td>Glu</td>
<td>Lys</td>
<td>Ser</td>
</tr>
<tr>
<td></td>
<td>1/3</td>
<td>1/6</td>
<td>1/6</td>
<td>1/9</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### HBB Sequence in Mutant Adult Hemoglobin (Hb S):

<table>
<thead>
<tr>
<th>Nucleotide</th>
<th>CTG</th>
<th>ACT</th>
<th>CCT</th>
<th>GTG</th>
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<td></td>
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<td>1/9</td>
<td>1/9</td>
<td>1/9</td>
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</table>
Thank you