



# INSIGHT

News Letter



## Dr Lal PathLabs

Volume 2, Oct. 2005



### From the Director's Desk:

Quality is the keyword in Laboratory Medicine and is the major driving force contributing to performance improvement. The entire process of laboratory services must be controlled by Quality performance indicators, setting targets to be achieved annually. This helps in establishing a continual quality improvement program, which is a must for good laboratory practice. Commitment to quality is applicable to all branches including the field of surgical pathology.

Surgical pathology laboratory initiates a complex series of events that culminates in the issuance of the final report. Application of quality indicators in this field is of paramount importance. Monitoring the work being carried out in the surgical pathology laboratory for the purpose of detecting inadequacies, updating procedures, improving the final product is an important responsibility of a laboratory. The steps, which should be undertaken to establish benchmarks for establishing quality in surgical pathology laboratory, are:

- Identify an indicator or process to improve**
- Measure current level of performance for that process**
- Determine the desirable level of performance for that process**
- Re-evaluate level of performance for that process**
- Repeat the above quality steps till the target is achieved**

Quality control is a process control and in surgical pathology it means controlling laboratory accessions, gross & microscopy techniques, reporting and retention of submitted specimens. It also requires documentation of temperatures of water baths, cryostats & room in specially created logs, running of positive & negative controls for special stains, peer review, turnaround time and customer feedback.

At LPL, quality processes are well defined and highly developed. This can be ascertained by the fact that Dr. Lal Pathlabs Pvt. Ltd. is the highest accredited laboratory in India conforming to stringent quality standards specified by the National Accreditation Board for Testing and Calibration Laboratories ( NABL ), College of American Pathologists & ISO 9001:2000 British Standards Institution.

**Dr. Vandana Lal**  
Executive Director  
Dr Lal Pathlabs Pvt. Ltd.



## ESSENTIALS OF IMMUNOFLUORESCENCE IN DERMATOLOGICAL PRACTICE

Immunofluorescence (IF) studies are the mainstay in the diagnosis of immunologically mediated bullous diseases. IF testing alone can lead to the appropriate diagnosis for the vast majority of patients with bullous diseases. The relative simplicity of the technique and its wide availability make IF an unavoidably powerful technique in the diagnosis of bullous diseases. Even in situations in which the histopathological findings seem characteristic of a specific bullous disease, IF testing can add to the certainty of the diagnosis, sometimes modify and occasionally reveal a different diagnosis.

### DIRECT IMMUNOFLUORESCENCE

Demonstration of tissue bound immuno-reactants by **direct immunofluorescence** is a valuable parameter in the diagnosis of various autoimmune skin diseases; including vesiculo-bullous, collagen vascular and vasculitic disorders.

Reliable diagnosis involves:

#### **Adequate Skin/Mucosal Biopsies**

**Proper Transportation to preserve the immunoreactants.**

#### **Recognition of Immunofluorescence Patterns by expert observers**

1. The **choice** of taking a biopsy in Autoimmune Skin Diseases is critical and needless to say an improperly taken or inadequate biopsy can lead to diagnostic and treatment errors. An ideal and most practical approach is to obtain two skin specimens, one for histologic study and the second for Immunofluorescence studies. An excisional biopsy is generally preferred as punch biopsies may lead to sloughing of adjacent skin and disintegration of the blister. The specimen for IF should be taken from the normal appearing skin adjacent to the blister. It is safest to obtain the biopsy within few mm. to upto 1cm. from the blister edge. IF is less likely to yield results if the skin adjacent to the blister is erythematous or urticarial. In such cases ideally two specimens should be taken for IF studies, the first is from nonblistered inflamed skin and the other from normal appearing skin immediately adjacent to the inflamed skin.

In cases of **vasculitis**, the specimen should be taken 10mm from an early lesion (less than 24 hours old). Preferred biopsy site in **Bullous SLE** is sun exposed lesional skin

2. Proper **transportation** is very vital to reach a Diagnosis.

The adequately taken biopsies are immediately put in the transport medium labelled, with the patient's details, sealed tightly and sent to the laboratory at 2-8 degrees Celsius.

The recommended transport media are:

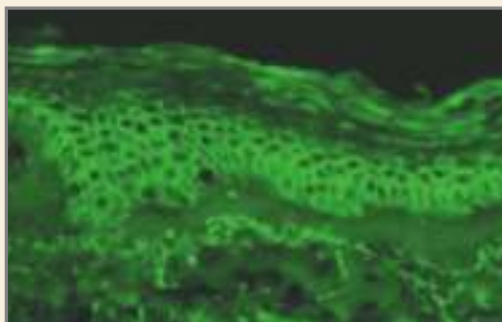
- a) **MICHEL'S MEDIUM** (Ammonium sulfate, N-ethylmaleimide, magnesium sulfate in citrate buffer, Ph 7) Ammonium sulfate is a liquid fixative which prevents degradation of tissue and the immunoreactants. The tissue enzymes are inhibited without irreversibly damaging the fixed molecular components.
  - b) **ISOTONIC SALINE** (0.9% saline) A few recent reports have shown that the specimens received in saline yielded almost similar results as when sent in Michel's medium.  
*(Michel's medium may be obtained free of cost from LPL, Hanuman Road/LPL Diagnostic Centres)*
3. Fresh Frozen sections are incubated with the antibodies to immunoglobulins (IgG/IgA/IgM)/complement (C3)/fibrinogen. These antibodies are then linked to a fluorescent label to allow visualisation using a fluorescent microscope.

### INDIRECT IMMUNOFLUORESCENCE (IIF)

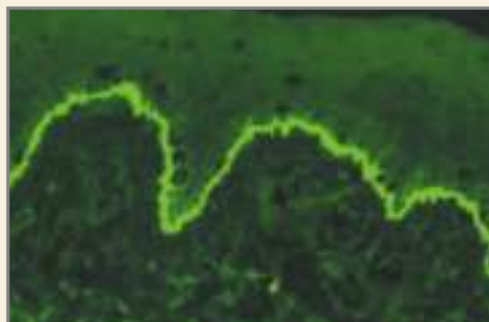
IIF test is performed on serum to detect the presence and amount of circulating IgG, IgM and/IgA antibodies to epidermal/epithelial antigens. Semiquantitative levels of antibodies are reported as titles.

#### **SAMPLE REQUIREMENT:**

Blood specimens should be drawn in a plain, red top tube or a serum separation tube. At least 2 ml. of serum is required to perform the test. Label the tube with the patient's details, seal tightly and ship the tube to the laboratory at 2-8 degrees Celsius, with a detailed clinical history. The sample should never be frozen as freezing and thawing can lead to hemolysis and rejection of the sample. The substrates (guinea pig lip/esophagus, rabbit lip/esophagus, rat bladder) is incubated with the serum in serial dilutions and then tagged with fluorescein labeled specific anti immunoglobulin antibody. The slide is then viewed under a fluorescence microscope.



**IgG cell surface  
(intercellular  
substance) staining  
(40x)**



**IgG linear  
basement  
membrane  
zone (20x) and  
C3 linear  
basement  
Membrane**

## IMMUNOFLUORESCENCE PATTERNS IN AUTOIMMUNE DERMATOSES

S.N.Disorder	Light Microscopy	DIF/IIF
1 <b>P.vulgaris</b>	Supra-basilar blister, acantholysis, Row of Tombstone appearance	Intercellular IgG +C3 <b>Lace like appearance</b>
2 <b>Paraneoplastic Pemphigus</b>	Supra-basilar blister, cell vacuolation exocytosis, dyskeratosis	Intercellular and basal BM IgG+C3 <b>Lace like appearance(DF) Substrate used in IIF is rodent epithelium</b>
3 <b>IgA Pemphigus</b>	Sub-corneal vesiculo-pustules with minimum acantholysis / Intraepidermal Vescicopustules with small to moderate no. of neutrophils.	intercellular IgA <b>Linear dep</b>
4 <b>Bullous Pemphigoid /cicatrical Pemphigoid</b>	Subepidermal blister; mixed inflammatory infiltrate	IgG,C3 at DEJ(DF) <b>Linear dep Salt split tech(IIF)</b> using sal split human skin. Dep. At the roof of the split.
5 <b>Herpes Gestationis</b>	subepidermal blister perivascular infiltrate Marked papillary edema	C3 at DEJ <b>Linear dep(DF) Herpes Gestationis Factor(IIF)using indirect complement fixation tech.</b>
6 <b>Dermatitis Herpetiformis</b>	Neutrophilic abscesses in dermal papillae, Dermal infiltrates of neutrophils, eosinophils, subepidermal vesicles	IgA at tips of dermal papillae <b>Granular dep.(DF) Endomysial antibodies (IIF)(70-80% pts)</b>
7 <b>Linear IgA Dermatoses</b>	Sub-epidermal bullae with infiltrate of neutrophils at Basement membrane, Dermal papillae	IgA along DEJ +/- IgG,C3 <b>Linear dep.</b>
8 <b>Henoch schonlein Purpura</b>	eucytoelastic vasculitis,subepidermal blister(pustular vasculitis)	IgA in vessel wall
9 <b>Bullous LE</b>	broad based subepidermal blister asso. with papillary dermal edema, Papillary dermal abscesses	LUPUS BAND TEST(continuous linear or granular dep. of IgG,C3, IgM at DEJ) mod.intensity

DIAGNOSIS
Disorders Excluded with Negative DIF
CHARACTERIZATION
IgA pemphigus Pemphigus Bullous pemphigoid DLE SLE IgA vasculitis/Henoch-Schonlein Purpura

**Dr. LalPathLabs** is dedicated to serving dermatologists by providing expert Immunofluorescence examination in skin biopsies and serum samples. Direct Immunofluorescence in skin biopsies may be requested for IgG, IgA, IgM, C3 either as separate immunoglobulin or combined as a panel. Also available are Endomysial antibody (IIF), ANA (IIF), Anti ds-DNA (IIF).

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## MYCOSURE- an innovative PCR Test for detection of TB.

**Tuberculosis (TB)** is the most common cause of infection related death worldwide. According to **WHO more than 8 million cases of TB occur each year**. Currently **19-43% of the world's population is infected with Mycobacteria**. Developing countries including India, China, Pakistan, Philippines, Thailand, Indonesia, Bangladesh and the Democratic Republic of Congo account for nearly 75% of all cases.

Early confirmation of the diagnosis of **Tuberculosis is a challenging problem especially in case of paucibacillary and extra pulmonary forms**. Conventional methods available for diagnosis are limiting in their scope a problem. Immunology is often not conclusive as antibodies and delayed type hypersensitivity response persists for a long time after subsidence of sub-clinical or clinical disease. Sputum smear microscopy requires **10,000 to 1,00,000 organisms/mL and AFB** positivity could be due to any **pathogenic or saprophytic Mycobacteria**. Identification through culture remains the yardstick for diagnosis, but the time required and the frequent negative results in paucibacillary specimens limits its usage. Development of systems such as **BACTEC, MGIT (Mycobacteria Growth Indicator Tube), Septicheck** and reporter phages besides other methods have reduced the turn around time but sensitivity remains an issue.

The advent of molecular era has seen a rapid reduction in turn around time to a few hours with unparalleled sensitivity and specificity. Most of these techniques rely on polymerase chain reaction technology (PCR) based *in-vitro* amplification of specific stretches of DNA isolated from the organism. There are a variety of genes which may be amplified to detect the presence of Mycobacteria and each has its limitations. They can be broadly classified as genus specific and species specific. The species specific PCR amplify the insertion sequence **IS 6110** or gene for **MPB 64** while the genus specific PCR include amplification of gene coding for **16S rRNA** and **65kD** a protein among others.

While there have been reports that the **IS 6110** insertion sequence is absent in some of the Indian strains, an extensive comparison between **IS 6110** and **MPB 64** based PCR revealed that both gave the same result. The positivity rate was 7-10% for the samples received by the lab. In contrast, the positivity jumped to 40% when a genus specific PCR like 16S rRNA was used. This spurt in the positivity could be attributed to non-tuberculous mycobacteria (NTM's) or Mycobacteria other than tuberculosis (MOTT). The primers used for the amplification of 16S rRNA could also amplify DNA from closely related species like Corynebacterium, Nocardia and Rhodococcus as was apparent by obtaining a band on an agarose gel.

Thus, there was a need to go a step further than conventional agarose gel electrophoresis and be able to segregate the true Mycobacterium positive samples from the 16S rRNA positive ones and secondly to pinpoint whether these samples are MOTT or are positive for Mycobacterium tuberculosis complex (*M. tuberculosis, M. africanum, M. bovis, M. microti*). This was done by using Southern hybridization technique. Post amplification, rather than using gel electrophoresis for detection, DNA probe based technology was used in **"MYCOSURE LPL's TB test"**. The amplified product was subjected to two probes. The first probe was specific for Mycobacterium genus. Thus, amplification product from other non-mycobacterial species (Corynebacterium, Rhodococcus etc.) were eliminated. The other probe was specific for Mycobacterium tuberculosis complex (MTC) and was thus able to pick out the true MTC positive samples.

The sensitivity of the assay was checked by serially diluting a culture corresponding to **MacFarlandt 1**. The assay was positive upto two **Bacilli/PCR**. Similarly DNA isolated from a culture was serially diluted to 10fg (corresponding to two bacilli) and was found to be positive. The assay has been evaluated for its specificity by amplifying 101 mycobacterial strains from 33 species. DNA could be amplified from all of them. Of the 31 non-mycobacterial strains belonging to 17 genera which were tested, although DNA could be amplified from closely related species as evident by agarose gel electrophoresis, none of the PCR products hybridized to Mycobacterium specific or the species specific probe.

The assay has been tested for a variety of pulmonary and extra-pulmonary samples including sputum, BAL, urine, endometrium biopsy, blood, menstrual blood, pleural fluid, ascitic fluid, tissues etc. 6565 samples have been done using this technology so far with 22% samples being positive for Mycobacterium genus alone and 7.25% being positive for MTC. Research is going on so as to identify more species using this test. **Report on 3<sup>rd</sup> working day : cost Rs. 1500**

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## CLINICAL APPLICATIONS OF COLOR DOPPLER, MUSCULOSKELETAL AND OCULAR ULTRASOUND AT A GLANCE.

(Dr Satish Puri, Consultant Radiologist,  
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### Indications for Abdominal Vascular examination:

- Hypertension resistive to medical therapy
- Abdominal bruit
- Abdominal angina
- Abdominal aortic aneurysm
- Vena cava obstruction
- Pelvic vein thrombosis
- Cirrhosis and portal hypertension
- Evaluation of intra-abdominal graft patency
- Renal transplant rejection

### Hepatobiliary Applications of Colour Doppler:

- Portal Vein Hypertension
- Portal Vein Thrombosis
- Budd-Chiari Syndrome
- Hepatic Vein changes
- Vena cava obstruction

### Indications for Colour Doppler in Gynae/Obstetrics:

- Fibroids
- Adnexal Flow, PID
- Corpus Luteal Cyst vs Ectopic Pregnancy
- Retained Products of Conception
- Ovarian Masses
- Pre-eclampsia
- IUGR
- Fetal Echo

### Cerebrovascular Applications:

- Plaques
- Stenosis/Occlusion
- AV fistulas
- Aneurysms
- Subclavian Steal
- Vertebro-basilar Insufficiency

### Indications for Peripheral Venous Evaluation:

- Limb Swelling
- Leg Pain
- Varicose Veins
- Superficial Thrombosis
- Suspected Deep Vein Thrombosis
- Evaluation of post-phlebitic syndrome

- Vein Suitability-Coronary bypass
- In-situ saphaneous bypass

### Indications for Peripheral Arterial Evaluation:

- Claudication
- Rest Pain
- Cutaneous Ischaemic Lesions
- Acute Arterial Emboli/Thrombi
- Diabetic small vessel disease
- Amputation level
- Arterio-venous fistulas
- Femoral Popliteal Aneurysms
- Upper Extremity Aneurysms
- Popliteal entrapment
- Arterial Graft patency assessment

### Musculoskeletal Applications:

- **Shoulder:** Rotator cuff pathology, Biceps tendon pathology, Effusions
- **Elbow:** Unossified loose bodies, Epiphysis, apophysis and growth plate injuries in children
- **Hand & Wrist:** Ganglion, Effusions, Tendon trauma, Carpal Tunnel Syndrome
- **Hip:** Acetabular dysplasia in neonates, effusions
- **Knee:** Peripheral meniscal tears, Patellar tendinosis, Collateral ligament injuries, Effusions
- **Ankle & Foot:** Ganglion, cysts, Tenosynovitis, Tendon tears, Effusions

### Ocular Applications:

- Dislocated lens
- Vitreous Pathologies- Hemorrhage, exudates, floaters, detachment
- Retinal detachment, complications
- Choroidal pathologies
- Intraocular foreign bodies, trauma
- Optic disc pathologies



## LETTER FROM THE EDITOR

I am writing to you after a very good response from many of the readers of the first edition of the newsletter which was launched in June this year . We at Dr. Lal Path. Labs have been looking all the time to in crease our test menu and to reach out to more patients so that diagnosis correctly made is half the job done.

Screening services are also increasing as they are specifically aimed at the preventive aspect of healthcare. World health services are moving in various dimensions curative, preventive, promotive, rehabilitative, restorative etc. and we also must move on rapidly in these directions.

We want to continue improving so that we can manage the cost, quality and improve the turn around time for different tests. With time we have improved the productivity meaning the testing turnover from a vast range of tests. Another area we have identified which has and will help us in excelling is the capture, analysis of complaints and feed backs. Data collection from such resources cover the number of complaints and their corrective action after proper classification with the nature of complaints.

Before I sign off I would like to show you the funny side of our line of work.

### Popular Pathology Requisitions: The Hidden Meaning

**For urgent processing:** pleeeaaase, the boss is breathing down my neck, and I haven't the faintest clue what this is, save me!

**Diffuse mildly enlarged thyroid:** I didn't feel it, the boss did.

**Ill defined nodularity in the upper, outer quadrant of the right breast for FNAC:** I saw you joblessly loitering in the canteen, so I'm sending some business your way; enjoy the hide and seek!

**22 year old woman with vaginal bleeding every 28 days, to rule out endometrial pathology:** now, I am jobless.

**FNAC benign, frozen section to rule out malignancy:** Buddy, I don't trust you.

**Please issue duplicate slides for further management:** I still dont trust you.

**2500 ml of urine from a dipsomaniac, to look for malignant cells:** I didn't know how to discard it.

**5 x 0.5-cm axillary node for FNAC, patient is HIV positive:** I don't have the nerve to biopsy it.

**Please look for Helicobacter pylori:** The consumer forum president has become flatulent; tummys no trouble; dig into that haystack, find a needle, and save us all from the ordeal.

**Request for a complete necropsy on a patient who died 420 days after an aspirate from a lipomatous swelling on his right little toe:** We don't know why he died, but we are hoping to pin the blame on you.

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