



Microbiology Newsletter

Sir Ganga Ram Hospital

Volume 13, No. 1

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DENGUE NS1 ANTIGEN TEST

A 28 year old male patient was admitted with a history of high fever and severe headache for two days and blood sample was sent for Dengue IgM antibody ELISA test. As the patient had an acute onset of fever with severe headache a CSF sample was examined on the same day and the biochemical parameters were within normal range. The blood sample tested negative for IgM dengue antibody. His platelet count was 80,000/ μ l. Sample was subsequently tested for NS1 dengue antigen which tested positive. After four days the patient was again investigated for, NS1 antigen and IgM dengue antibodies. The sample tested negative for NS1

antigen but was now positive for dengue IgM antibody. The patient was diagnosed as suffering from dengue fever and was treated symptomatically. Platelet count rose to 1.2 lakh within a couple of days and was subsequently discharged after 5 days of hospital stay. The early detection of NS1 antigen prevented further unnecessary investigations and treatment.



Dr. Iqbal R. Kaur (Secretary), Dr. J.C. Samantaray (President), Dr. B.K. Rao (Chairman, Board of Management, SGRH), Dr. C. Wattal (Chairman, Organising Committee). Release of SGRH Microbiology Newsletter by Dr. B.K. Rao

NS1 is a highly conserved glycoprotein which appears essential for virus viability, although no precise function has yet been assigned to it. During cell infection, NS1 is found associated with intracellular organelles or is alternatively transported through the secretory pathway to the cell surface. A soluble hexameric form may be released in a glycosylation-

dependent fashion from infected mammalian cells but not from vector-derived mosquito cells.¹

Enzyme-linked immunosorbent assay (ELISA) directed against the NS1 antigen has demonstrated high concentrations of this antigen in the sera of dengue virus-infected patients during the early clinical phase of this disease.

Dengue NS1 Ag test (BioRad) and MAC ELISA (Focus Diagnosis) are currently routinely used for the serodiagnosis of dengue fever; however, the IgM antibodies do not become positive before 5 days of fever. NS1 is detected concomitant with viremia and may be detectable even when RT-PCR is still negative suggesting that NS1 protein may circulate at higher levels than virus particles.² NS1 appears in the sera before IgM antibodies.²

Though NS1 antigen and IgM antibodies both develop during the acute phase, but from day1 to day3, NS1 in our hands, was found to be more sensitive as seen in the case discussed above.³

The use of NS1 detection as a first-line test during the first 4 days of fever could help in earlier diagnosis of dengue fever.⁴

Dear Doctors & Patrons,

The Department of Clinical Microbiology at SGRH can look back with satisfaction at the progress of their Microbiology Newsletter since 1995 and every attempt is made to share it with you all. Our strengths in other fields of clinical microbiology have also progressed all these years & probably have come of age to share data other than that of bacteriology with you all. We have changed the format of our Microbiology Newsletters from six monthly to yearly database reviewing, though the Newsletter will continue to be produced every six months. The current issue has combined yearly data of Mycology, Mycobacteriology, Microbial Immunology and Parasitology. As a result of this two kinds of Newsletters will alternate every year. We are hopeful to generate useful data in other fields of Clinical Microbiology from our hospital.

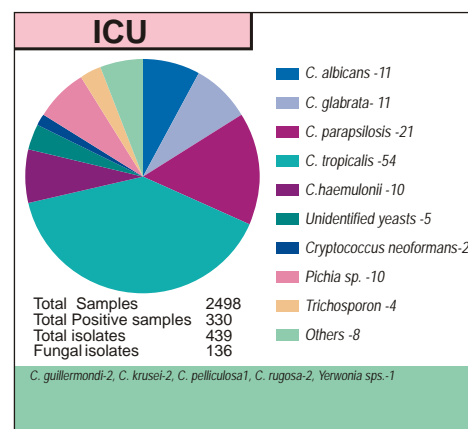
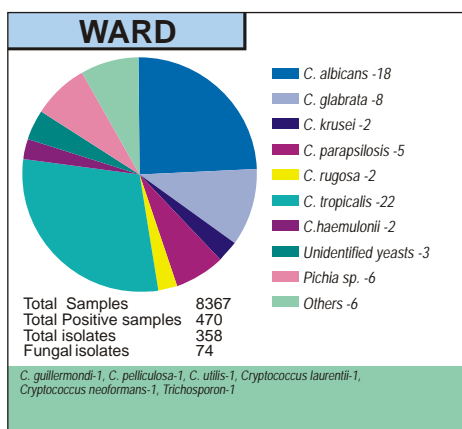
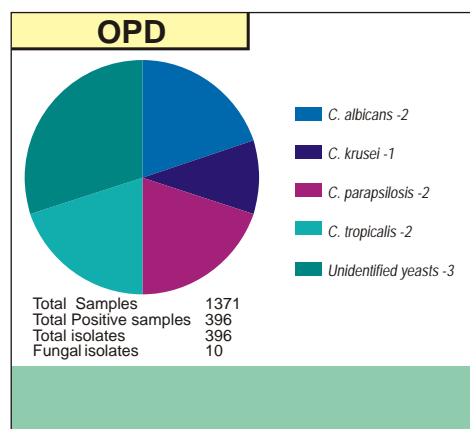
Trust you will appreciate the changed format. Your comments as before shall be welcome and in fact has helped us improve the quality of our Newsletter.

Best wishes,

Dr. C.WATTAL

contd. on page 12

Blood - Fungal Isolates (Jan. - Dec. 2006)



% Resistance* in Yeasts Fungi **

Species (Total no. of isolates tested)	No. of Blood isolates tested	Flucytosine	Amphotericin B	Fluconazole	Itraconazole
<i>C albicans</i> (36)	25	0	0	0	2.7
<i>C tropicalis</i> (64)	53	6.2	0	7.8	31.2
<i>C parapsilosis</i> (34)	29	3	0	24.2	15.1
<i>C glabrata</i> (18)	17	0	0	27.7	83.3
<i>Pichia anamola</i> (7)	7	14.2	0	0	57.1
<i>Trichosporon sp.</i> (6)	6	33.3	0	16.6	100
<i>C. neoformans</i> (3)	3	33.3	0	0	33.3
<i>C. krusei</i> (4)	3	100	0	100	100
<i>Pichia ohmeri</i> (8)	8	0	0	75	75
<i>C. pelliculosa</i> (1)	1	0	0	0	0
<i>C.haemulonii</i> (10)	10	0	0	100	100
<i>C. utilis</i> (1)	1	0	0	0	50
<i>C guilliermondii</i> (2)	2	0	0	0	50
<i>C rugosa</i> (3)	3	0	0	33.3	66.6

Candida spp. have become one of the most common blood isolates as well as one of the leading causes of nosocomial blood stream infections. Candida spp. was the most common isolate from blood cultures in ICU patients (30.9%).

Increasing prominence of Candida species other than *C. albicans* (82.5%) in our isolates is notable and still more is the increasing resistance to the triazole antifungal agents such as fluconazole & itraconazole in these isolates. Another interesting observation is the emergence of a new species *C. haemulonii* with frank resistance to triazole and higher MIC to even Amphotericin B, but susceptible to 5-flucytosine with varying susceptibility to voriconazole (*C. haemulonii*- an emerging pathogen, Microbiology newsletter, SGRH, July 2007). The wide spread azole use could be one of the reasons for the emergence of species other than *C. albicans* as the cause of blood stream infections.

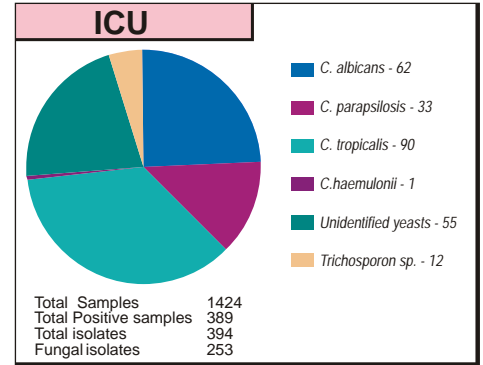
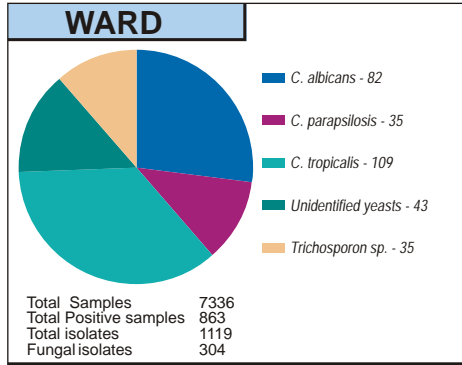
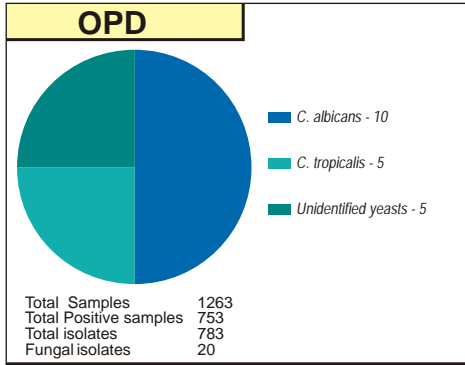
The present data suggests proper identification and susceptibility testing of Candida spp. is mandatory for effective therapy.

*Includes R & S-DD (Sensitive - Dose Dependent) Categories

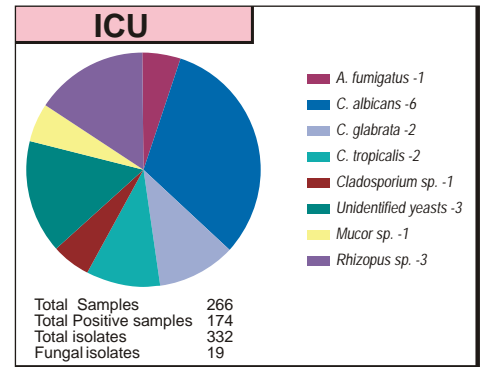
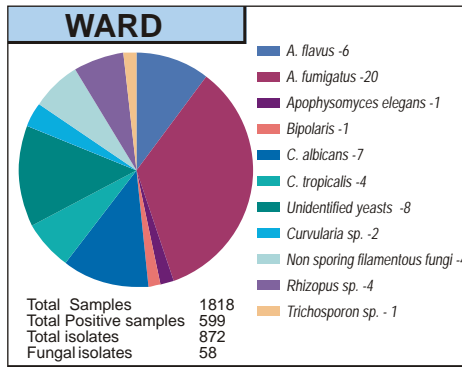
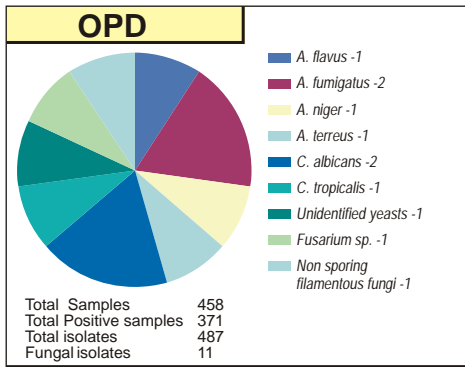
**ATB fungus 2 (bioMerieux, France)

Fungal Isolates other than Blood (Jan. - Dec. 2006)

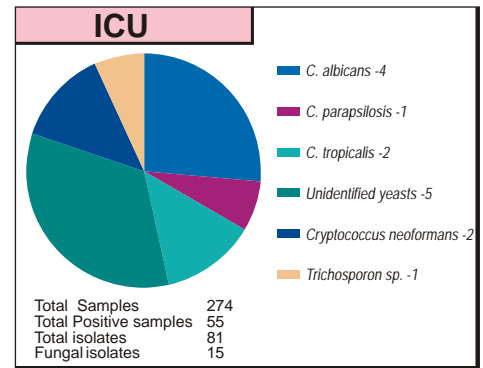
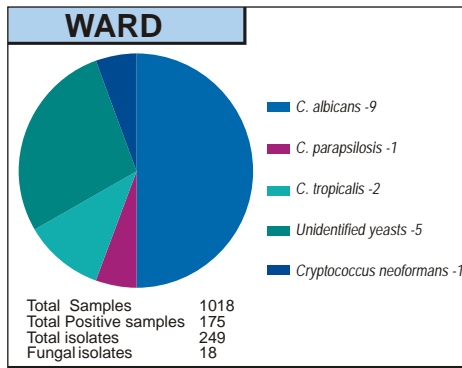
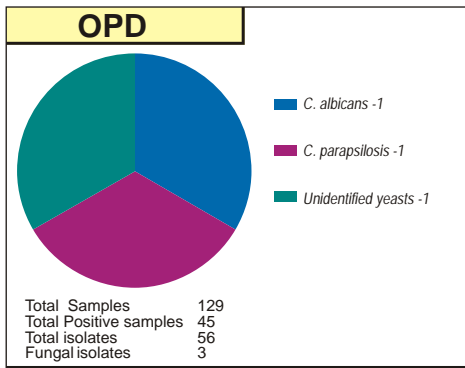
Urine



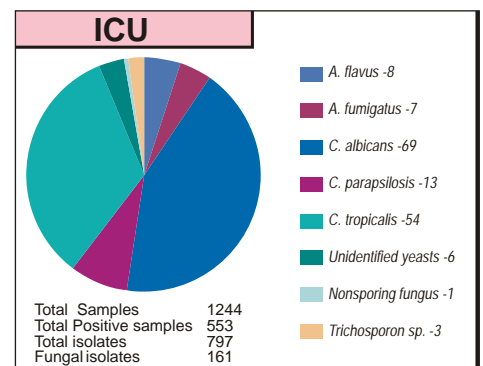
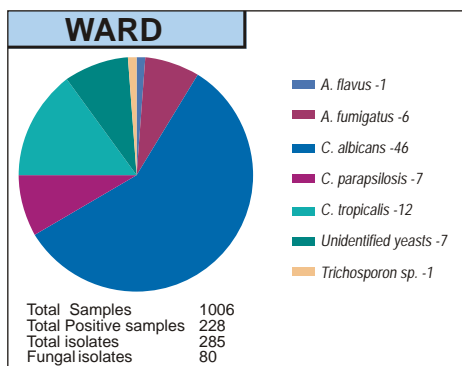
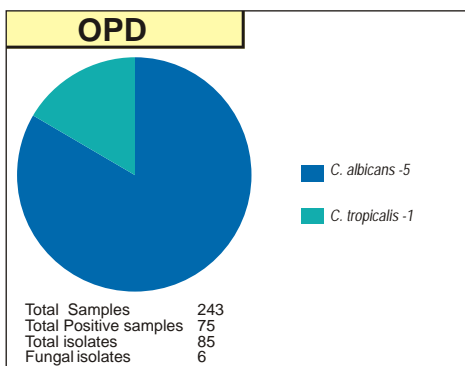
Pus & Tissue



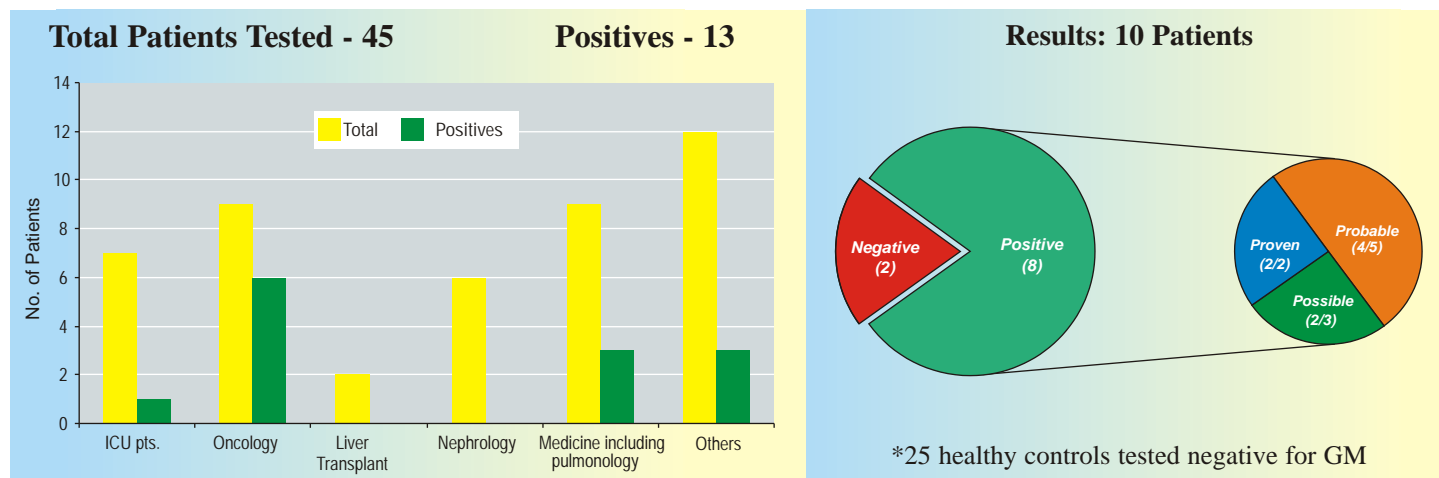
Body Fluids



Respiratory Isolates



Galactomannan Antigen Assay



Recently, we have introduced an ELISA based galactomannan antigen detection assay (*Platelia Aspergillus* EIA, BioRad laboratories) for the diagnosis of invasive aspergillosis (IA). The test detects the presence of galactomannan in serum. FDA (US Food & Drug Administration) approved. Galactomannan (GM) is a heat stable heteropolysaccharide cell-wall component of *Aspergillus* produced in-vivo and can be detected in biological fluids. As little as 1 ng/ml of galactomannan can be detected using an ELISA method in the serum of the patients. In the dataset evaluated by FDA, the overall sensitivity and specificity of the method were 80.7% and 89.2%, respectively.

GM assay should be routinely used when there is a high pretest probability of IA, such as in high-risk populations with neutropenia and malignancy or patients having undergone transplantation. The test can also be used as a prognostic marker, as galactomannan levels decrease on institution of appropriate and effective antifungal treatment.

The assay has been used widely across the globe but in India, it is being performed routinely only at 2 centers. Initially standardization was difficult, but those issues were resolved. In our hands this assay is showing a good clinical correlation. Out of the 45 patients tested so far between March-December 2006, case details of 10 were available, in whom the test was positive in 2/2 proven, 4/5 probable and 2/3 possible cases of IA, showing sensitivities of 100%, 80% and 66% in IA, respectively. 25 healthy controls also tested were negative. These being preliminary results, we hope to analyze its effectiveness in detail in the next issue with improved number of cases.

Cryptococcal Antigen Assay (Jan. - Dec. 2006)

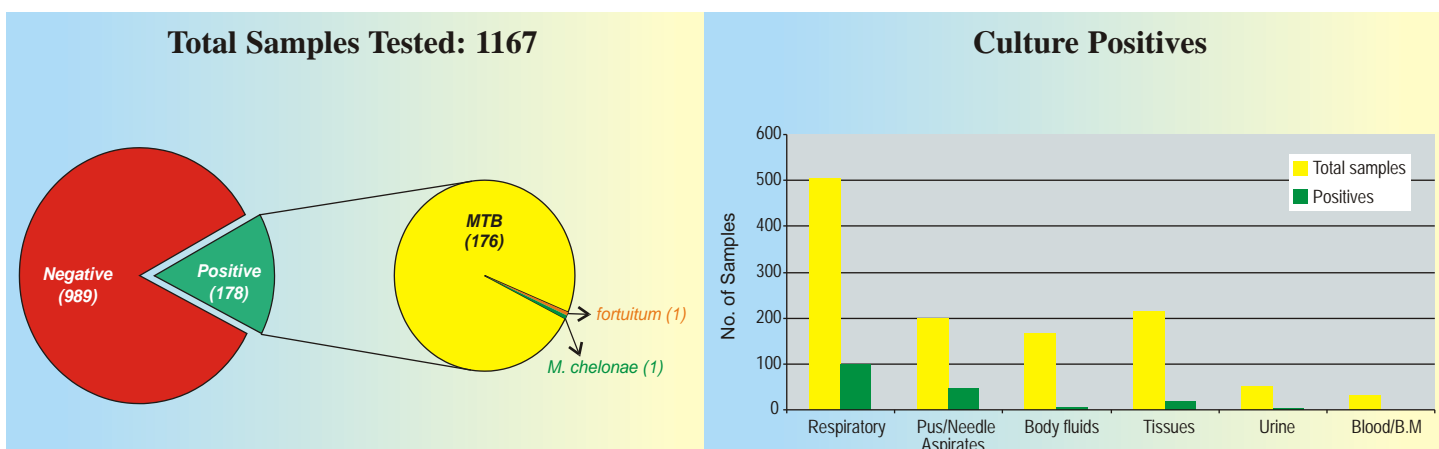
	Total Samples	Positive Samples
CSF	159	6
Serum	1	1

Cryptococcus neoformans capsular polysaccharide antigen assay latex agglutination test (Crypto-LA Test, Laboratories Fumouze)

The detection of cryptococcal capsular polysaccharide antigen is one of the most valuable rapid serodiagnostic tests for cryptococcal infection performed on a routine basis. The latex agglutination test has gained widespread appeal because of its ease of use and greater sensitivity compared to other conventional immunodiagnostic methods. False-positive cases related to rheumatoid factor and other factors like infection due to *Trichosporon* spp., contamination during pipetting in the laboratory, etc. have been described. False-negative reactions are also occasionally noted and may be due to the prozone effect, infection with a poorly encapsulated strain, low organism burden, or problems with the test kit.

Mycobacteriology at SGRH

AFB CULTURE (Jan. - Dec. 2006)



AFB CULTURE

BacT/ALERT-3D v/s LJ medium

Total Samples Cultured (both media): 954
Total isolates: 138

Mycobacterial sps (n)	Positive specimens detected by	
	BacT/ALERT 3D	LJ
<i>M. tuberculosis complex</i> (137)	135(98.5)	108 (78.8)
Smear positive (101)	99	82
Smear negative (36)	36	26
NTM (1)	1 (100)	1 (100)
<i>M. fortuitum</i> (1)	1	1
Total (138)	136 (98.5%)	109 (78.9%)

Time to Positivity

Total Isolates Recovered on both Systems: 107

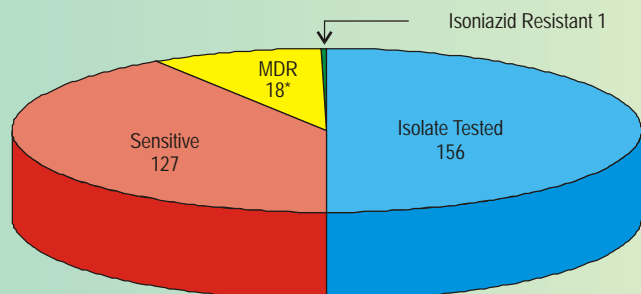
Sample	Smear status	Mean time to positivity (days)	
		BacT/ALERT 3D	LJ
Pulmonary	Positive	15.1	32.3
	Negative	19.6	38.3
Extra Pulmonary	Positive	16.25	28.6
	Negative	21.1	40.4
All samples	Positive	15.4	31.3
	Negative	20.9	39.9

Conventional culture of mycobacteria on solid media is labour intensive and time consuming. Substantial improvement in time to detection and total number of positive cultures can be achieved if automated or semi-automated liquid culture systems that use Middlebrook broth are used. In our experience, we have found the nonradiometric MB BacT/ALERT 3D system to be a rapid and sensitive method to isolate mycobacterial species from both pulmonary and extra-pulmonary clinical specimens. 954 samples were cultured on both the MB BacT/ALERT 3D system and Lowenstein-Jensen (LJ) medium, out of which 139 were positive in either or both systems. as seen in table. MB BacT/ALERT 3D system detected mycobacteria more rapidly than the LJ medium. Smear positive samples with higher mycobacterial load, i.e. 3+ positive are usually positive within 10-12 days.

Identification of mycobacteria by growth characteristics and conventional biochemical tests is time-consuming (3 to 6 weeks). Several systems exist for the rapid identification of mycobacterial species once isolated. The Accuprobe (GenProbe, USA) system which includes tests for the identification of MTB complex, *M avium* complex, *M avium*, *M intracellulare*, *M kansasii* and *M gordonae* is available and in our experience, the system appears to be promising. It is highly specific and identifies mycobacterial species within 2 hours. Out of the 178 mycobacterial isolates identified by AccuProbe, 176 belonged to MTB complex and 2 still remained unidentified even after using all the five probes mentioned above. They were identified as *M. chelonae* and *M. fortuitum* by conventional techniques.

Sensitivity of *M. tuberculosis*

(by 1% Proportion Method)



* No clear distinction between primary and acquired resistance was possible to determine, though 11 patients were previously on ATT.

Mycobacteriology Assays available at SGRH

- AFB smear (ZN/DF).
- AFB culture (BacT/ALERT-3D & LJ).
- Mycobacterial Identification by AccuProbe.
- Genprobe - Direct Nucleic Acid amplification.
- Quantiferon TB Gold Assay.
- Mantoux test.

Parasitology data (Cryptosporidium, Giardia, Entamoeba, etc) will be presented in the next issue.

Forth coming issue:

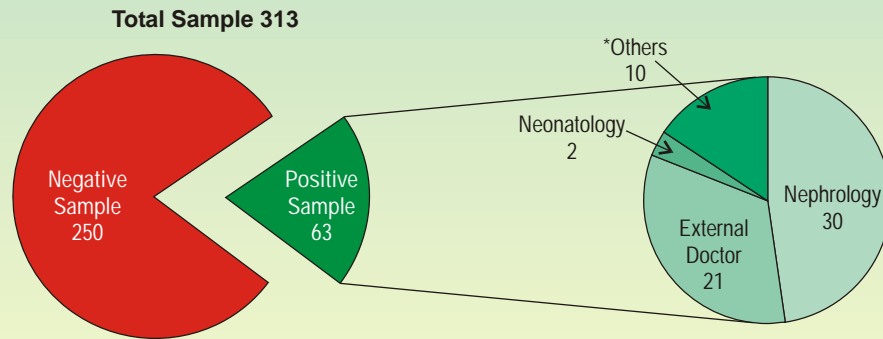
1. Quantiferon TB Gold Assay
2. Direct Nucleic Acid Detection for *M. tuberculosis* (GenProbe)

Virology at SGRH (Jan. - Dec. 2006)

NASBA

NucliSENS CMV pp67 measures replication of CMV in blood and body fluids. Using the well-established NASBA RNA amplification technology, this assay detects messenger RNAs coding for the matrix tegument protein pp67 of CMV, a true late protein, which is only expressed during viral replication. The NASBA technology selectively amplifies RNA in a DNA background and allows direct testing in whole blood. The NucliSENS CMV pp67 assay offers both the diagnostic laboratory and the clinician a new tool for CMV diagnosis - for both diagnosis of active CMV infection and monitoring treatment efficacy.

It is a FDA approved assay for the qualitative detection of pp67mRNA in patients having CMV viraemia and HIV quantization.

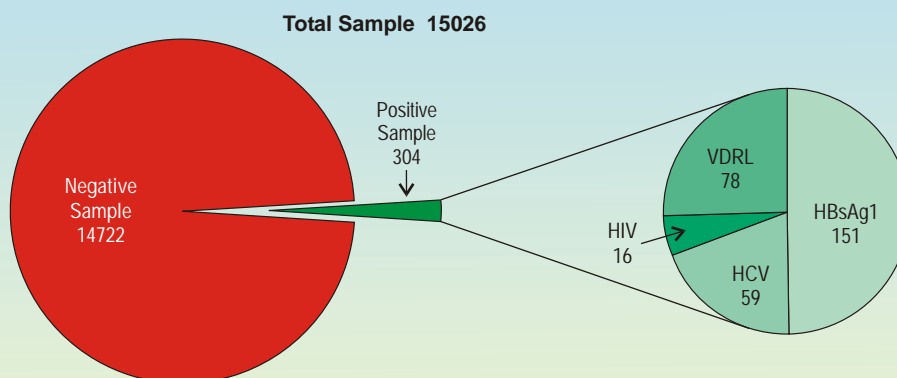


Total Sample	Positive	Nephrology	External Doctor	Neonatology	*Others
313	63	30 (48%)	21 (33%)	2 (3%)	10 (16%)

*(-Cardiology •Male General Ward •Paediatrics •Surgical Gastroenterology)

Serostatus of Blood Donors at SGRH (Jan. - Dec. 2006)

Screening of blood donors for transfusion transmissible infections is an important tool to achieve the goal of safe blood transfusion. The status of these infections among blood donors at Sir Ganga Ram Hospital, is presented below:



Total Sample	Positive	HBsAg	HCV	HIV	VDR1
15026	304	151 / 15026 (1%)	59 / 15026 (0.39%)	16 / 15026 (0.10%)	78 / 15026 (0.51%)

All blood bank donors have a pre and post test counselling and necessary follow up is provided to seropositive donors. We have started screening for total Hepatitis B core antibody from 2007 and will reflect the data in subsequent newsletter.

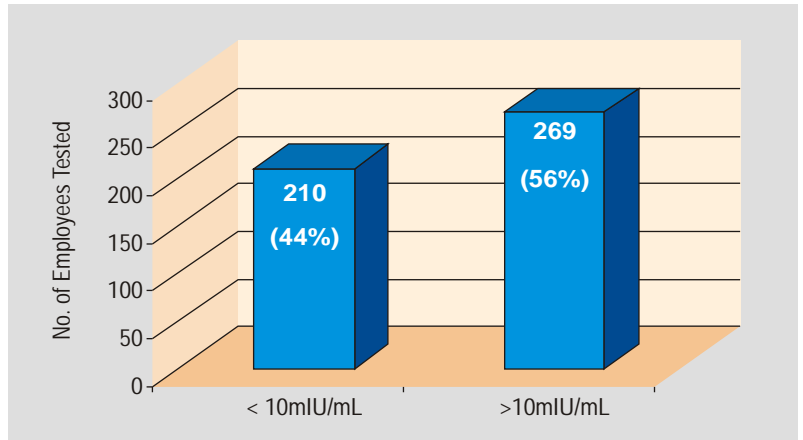
(NAT testing to be started soon.)

Pre-vaccination Screening for "a" specific Anti HBV Antibody

An anti-HBs level of greater than or equal to 10mIU/ml is recommended by ACIP (Advisory committee on immunological practices) as standard for demonstrating post vaccination protection for hepatitis B virus.

Total hospital employee 479 screened between Jan 06 -Dec 06 of which 269 had protective levels. The rest 210 needed vaccination. The HBs Ag screening test was avoided on the patients with protective levels thus decreasing the expense on the cost per assay, vaccination and the work load of the department.

Total Employee screened 479, 43.84% needed vaccination and 56.15% had protective antibody levels.



Advantages of pre-screening test before vaccination.

- To differentiate those who require vaccination from those who do not.
- Those having protective anti HBs titre do not require a HBs Ag screening test.
- Thus money saved by not screening HBsAg screening was Rs 80,700 .
- Money saved on unnecessary vaccination was Rs.2,71,087 .

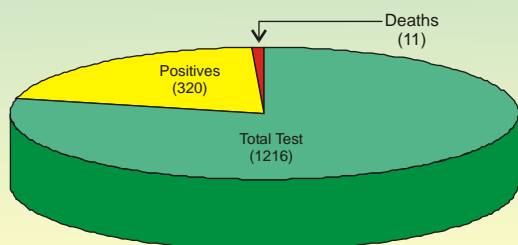
TORCH Assay (Jan. - Dec. 2006)

Seroassays have been one of the commonly used assays to study the seroprevalence of this group of organisms commonly associated with congenital infections. These assays are being conducted by our department since 1990, however, the current data is of the previous year . An overview of the seroprevalance of these organisms amongst mostly the pregnant population attending our OPD is presented here in the table.

Name of Test	Total	Obs. & Gyn.	IgM (%) Positive		IgG (%) Positive	
Toxo	927	756	2	0.26%	66	8%
CMV	2245	1280	32	2.50%	998	77%
Rubella	1330	1260	1	0.08%	659	52%
Herpes	1202	800	202	25%	178	22%

(Technology stands upgraded to MiniVidas.)

Dengue (Jan. - Dec. 2006)*



Total tests done	1216	%
Total positives (IgM)	320	26.31%
Total number of deaths	11	3.40%

*IgM - Dengue Assay

Bacterial Serology at SGRH

Tests for Syphilis - (RPR & TPHA)

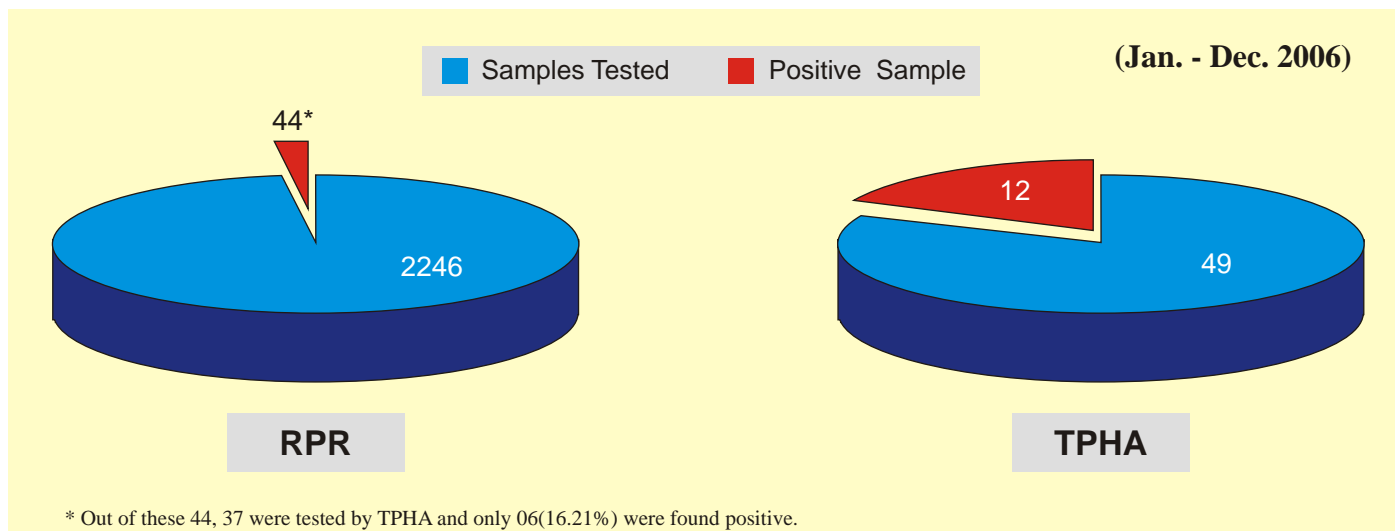
Rapid Plasma Reagin (RPR)

Rapid Plasma Reagin (RPR) Test is a macroscopic non-treponemal flocculation test for the detection and quantitation of antilipoidal antibodies. Non-Treponemal tests like RPR/VDRL are of great value when used for screening and follow up of therapy. Recommended for screening of blood donors and pregnant women for presumptive diagnosis of syphilis. RPR test is more sensitive than VDRL test and is equally specific. The test is known to give false positive reactions in conditions such as tuberculosis, leprosy, malaria, typhoid, certain viral diseases, pregnancy and autoimmune disorders. Overall specificity of this test is 98%

In 2004, we replaced the conventional VDRL test with RPR for the screening of blood donors and pregnant women. In the year 2006 a total of 2246 samples obtained from mainly Obstetrics and Gynaecology, department of our hospital were screened. Out of them 44(1.95%) were found reactive using RPR assay.

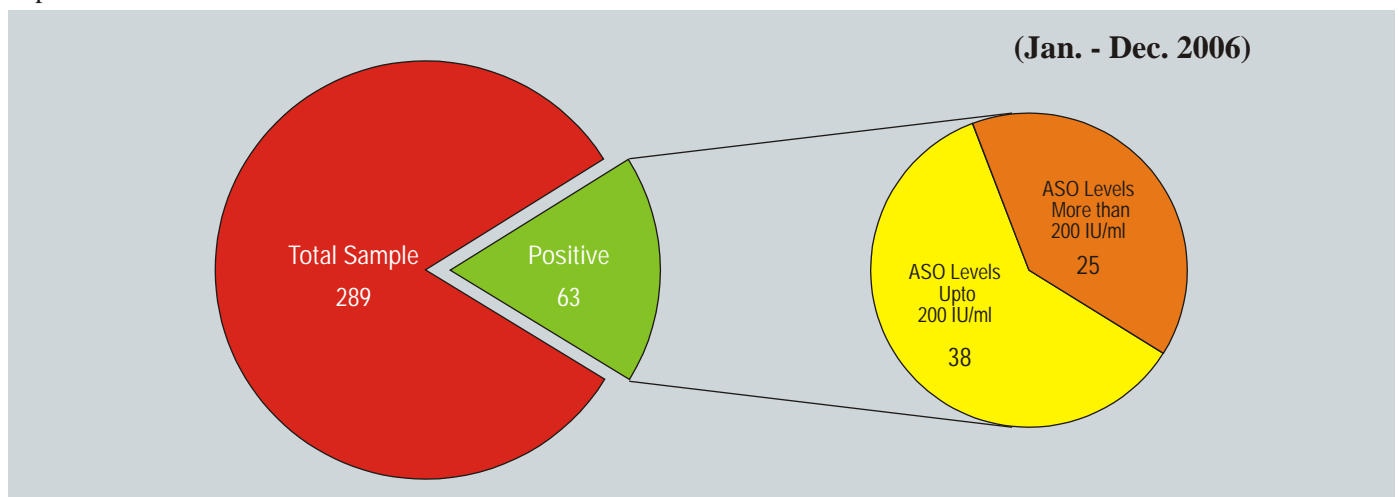
Treponema Pallidum Haemagglutination (TPHA)

TPHA is a test for the detection of antibodies to *Treponema pallidum* in serum. All RPR reactive patients should be tested by TPHA for the confirmation of syphilis. In the year 2006, only 49 samples were tested by TPHA, of which 12 were found positive for *Treponema pallidum* antibodies.



Anti-Streptolysin O-Test (ASO)**

ASO is a latex agglutination slide test for detection of anti Streptolysin O antibodies in human serum. The Group A - Streptococci produce various exotoxins such as Streptolysin O and Streptolysin S that can act as antigens. The affected individuals produce specific antibodies against Streptolysin O, namely Anti - Streptolysin O. Determination of these antibodies is very useful for the diagnosis of Streptococcal infections and their relative effects such as Rheumatic fever and acute Glomerulonephritis. Elevated levels of ASO (> 200 IU/ml) indicate acute infection. ASO levels are also monitored for impact of treatment.



**From June 2007, the ASO testing using latex agglutination has been replaced by Nephelometry detection system.

Diagnosis of Rheumatoid Arthritis

Rheumatoid Factor (RF) Slide Test and Anti-CCP ELISA Test

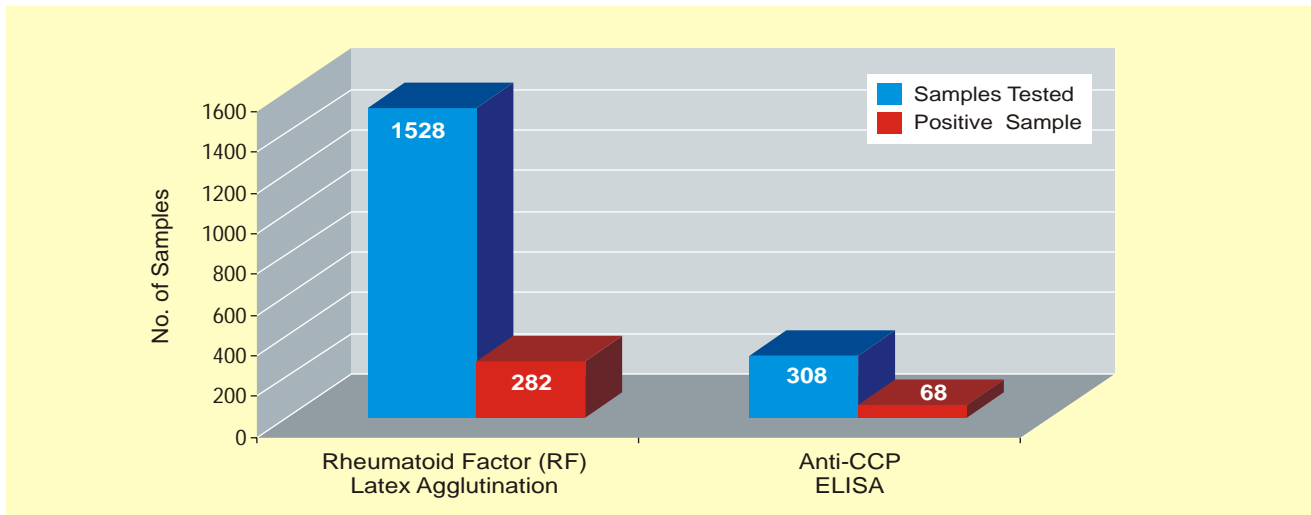
Diagnosis of Rheumatoid Arthritis (RA) depends primarily on clinical manifestations of the disease. The only serological test routinely done is detection of IgM Rheumatoid factor (RF) in the serum. Although RF is found in majority of RA patients and is reasonably sensitive (75-80%), but lacks specificity (60-80%) due to its presence in other diseases. Even upto 15% of healthy individuals may test positive for RF. Moreover, the absence of RF positivity in majority of early cases of RA, is a major diagnostic limitation of this assay.

The discovery of anti cyclic citrullinated peptides (Anti-CCP) based assays that are specifically recognized by autoantibodies in sera of RA patients has brought a revolution in rheumatology. Anti-CCP antibodies have been found to be more specific marker of disease, especially for the detection of the disease at an early stages i.e. much before the appearance of clinical symptoms. Anti-CCP has 96% specificity and 78% sensitivity for RA and also occurs in 70% of early RA synovitis. Anti-CCP is also predictive of erosive disease.

Furthermore, the concomitant presence of RF and Anti-CCP positivity has 99.5% specificity for RA. Therefore, a combined testing for Anti-CCP antibodies and RF may be optimal.

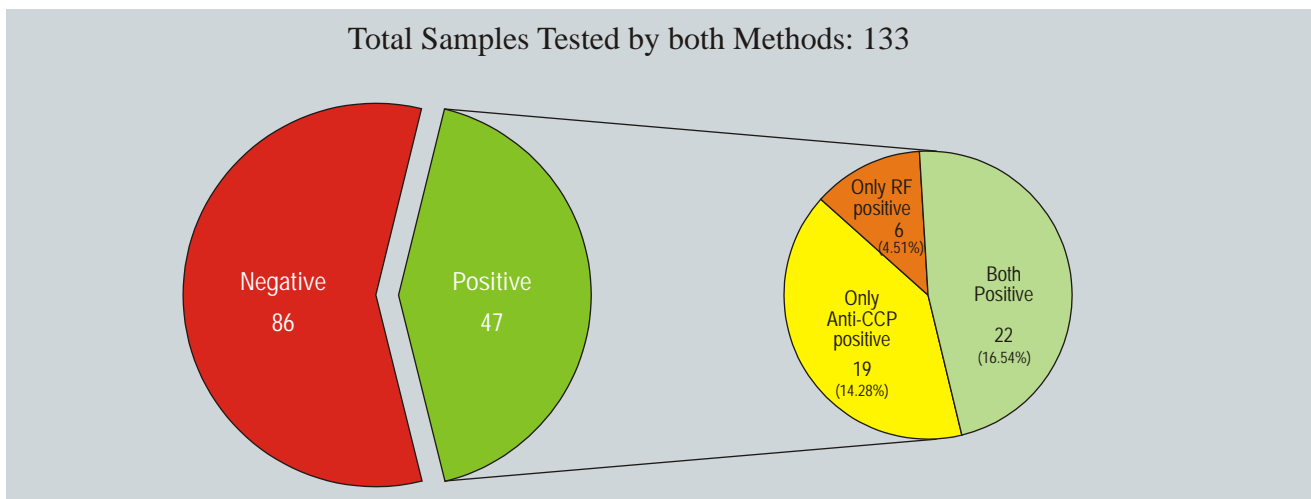
Considering the importance of Anti-CCP as an early marker of the disease and a test with higher specificity, we introduced an ELISA test for the detection of Anti-CCP antibodies in suspected RA patients in the year 2005. In the year 2006, a total no. of samples received for RF and Anti-CCP were 1528 and 308 respectively.

RF* and Anti-CCP ELISA (Jan. - Dec. 2006)



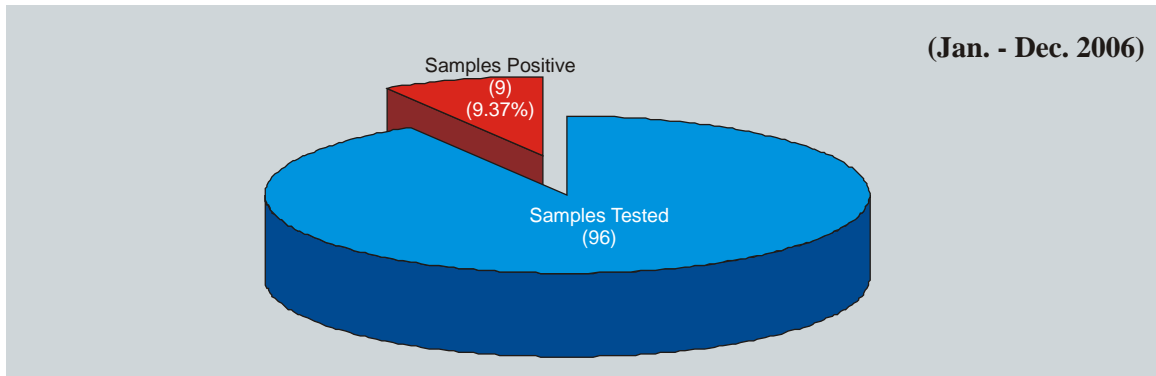
*Slide Latex Agglutination

Anti-CCP ELISA versus RF Factor (Jan. - Dec. 2006)



Lepto Tek Dri-Dot

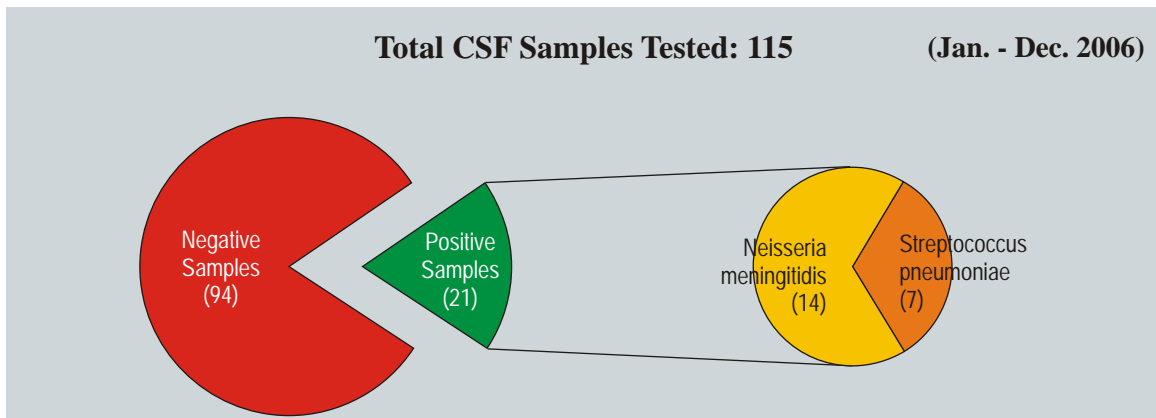
The Lepto Tek Dri-Dot assay is aimed at the detection of *Leptospira*-specific antibodies, i.e. total immunoglobulin, in human sera. It has been developed by bioMerieux in collaboration with The Royal Tropical Institute in Amsterdam for rapid screening for leptospirosis. Sensitivity and Specificity of this test claimed has been 91.2% and 91.0% respectively.



Bacterial Antigen Test

Meningitis has a wide variety of potential causes, either infectious or noninfectious. To check the fatality due to bacterial meningitis, a quick diagnosis is needed for immediate treatment of such cases. Bacterial Antigen test is a latex agglutination test for qualitative detection of antigen from *Streptococcus* group B, *Haemophilus influenzae* type b, *Streptococcus pneumoniae*, *Neisseria meningitidis* groups A, B, C, Y and W135 and *E.coli* K1 present in the CSF.

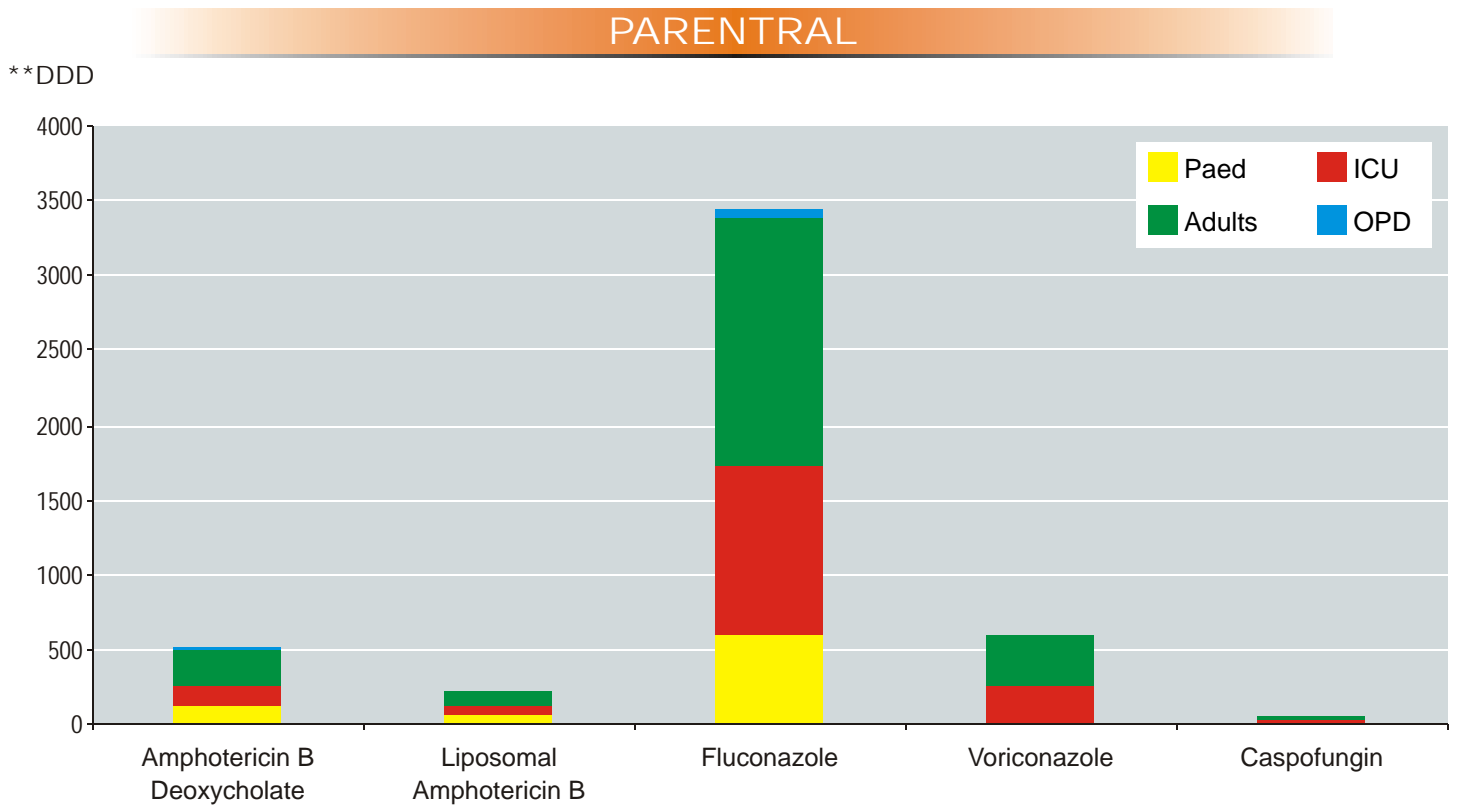
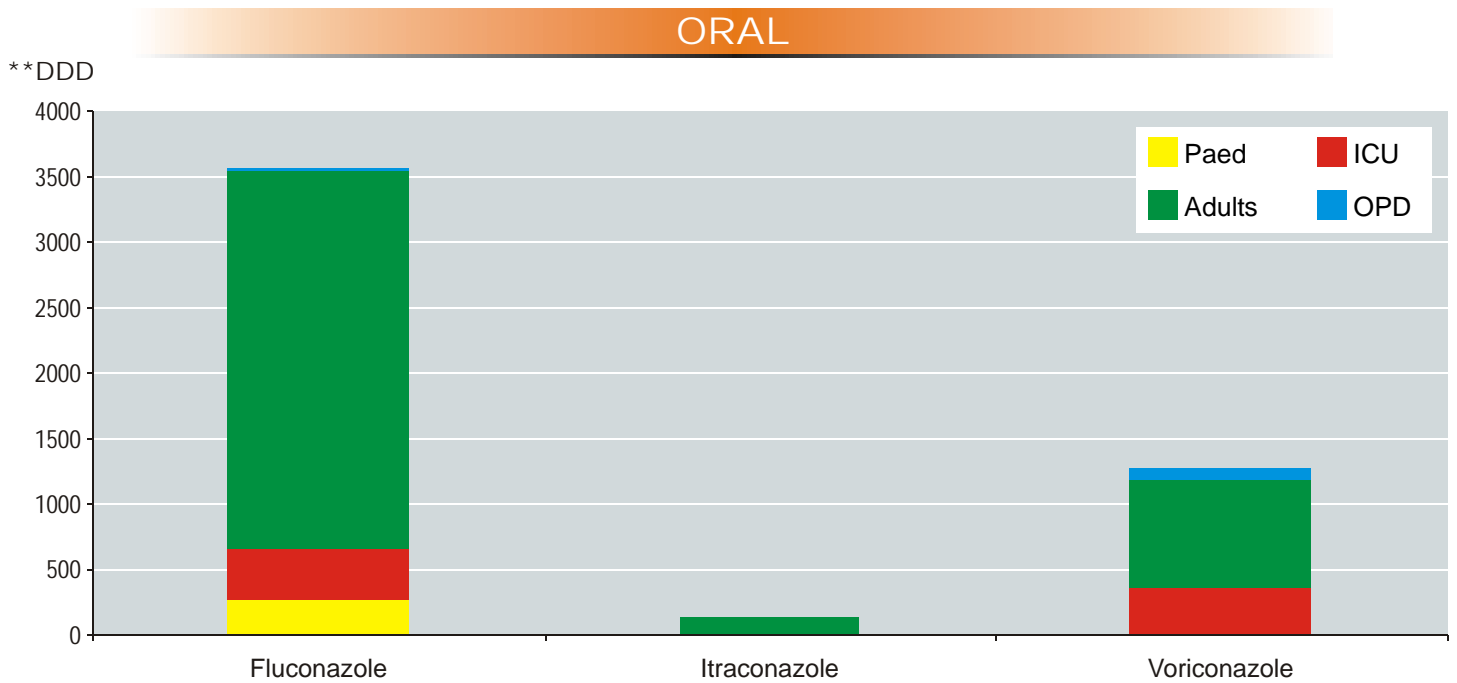
The sensitivity of this test in detecting bacterial antigen in CSF (according to kit) is 67% for *Streptococcus* group B, 97% for *Haemophilus influenzae* type b, 88% for *Streptococcus pneumoniae*, 71% for *Neisseria meningitidis* groups A, B, C, Y or W135 and 65% for *E.coli* K1. The specificity of all the five tests on CSF is > 98%.



EQUAS

Assessment of our department by External Quality Assurance Scheme organised by Deptt. Of Clinical Microbiology Amla Institute of Medical Sciences, Thrissur & L&T Research Centre Shanker Nethralaya, Chennai and scored on three occasions (April, July and October 2007) showed a score of 23, 25 and 25 respectively, out of a total of 25.

New Methodology i.e. Nephelometry has been introduced for the detection of CRP, RF, ASO, C₃ and C₄ in serum

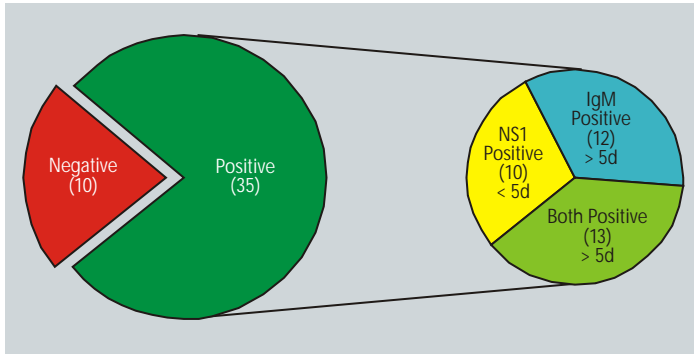


* Based on the hospital pharmacy data of the antifungals dispensed.

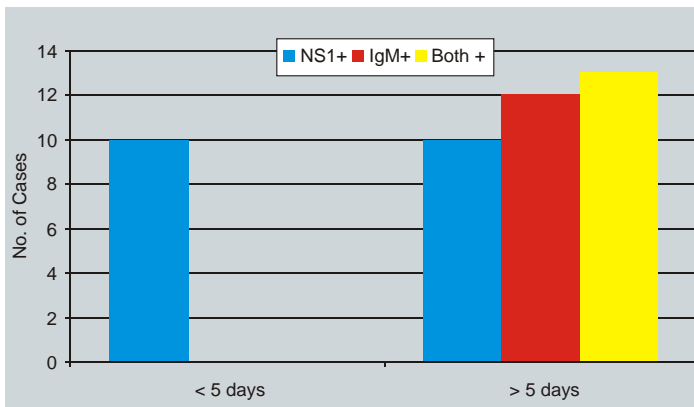
** DDD - Daily Defined Doses; have been calculated as per the "Guide to antimicrobial therapy" by Sanford et al, USA (2007).

The Platelia Dengue NS1 Ag assay (Biorad) is a one-step microplate enzyme immunoassay. It is simple, rapid, subject to quality assurance, and can strongly discriminate between positive and negative samples.

Total 45 samples screened for NS1 and IgM Dengue



Dengue Assays and Days to Positivity



A diagnostic strategy combining Platelia Dengue NS1 Ag testing of serum samples collected within 5 days of the onset of fever and MAC-ELISA for serum samples collected in the early convalescent phase would potentially make it possible to diagnose 91.9% of dengue virus infections.⁵

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Dr. Peter Hawkey addressing the delegates

The Department of Clinical Microbiology, Sir Ganga Ram Hospital, New Delhi organised a Delhi Chapter Meet of IAMM on 4th August 2007 along with a pre-conference workshop. The theme of the workshop & conference was Antimicrobial Resistance with the focus on **“Detection of β Lactamases in GNB - a practical approach”**.

23 Doctors from all over the country attended the workshop. A hands on training for the detection of ESBLs (disk diffusion, Etest, Vitek, MicroScan), Detection of Metallo β Lactamases & detection of Amp C mediated resistance was provided. Prof. Peter M. Hawkey, Professor of Clinical & Public Health Bacteriology and Honorary Consultant, University of Birmingham & Heath Protection Agency, UK assisted in conducting the workshop.

At the end of the workshop all attendees were provided with the ESBL positive ATCC quality control strain to ensure standardization for detection of ESBLs by them at their respective place of work.

Prof. Peter Hawkey delivered a guest lecture on **“Molecular epidemiology of nosocomial infections – Bench to Bedside”**, at the conference, which was held at India Habitat Centre, Lodhi Road. Free posters as well as poster for Meghna Kishan Baweja Best Poster award on Pediatric Infectious Diseases were displayed. Eighteen posters were presented and were judged by Dr. Krishna Ray and Dr. Sarman Singh. The conference was inaugurated by the chief guest, Dr. B.K. Rao, Chairman, Board of Management, Sir Ganga Ram Hospital, New Delhi and more than 180 delegates & guests attended the conference.

Dr. R. Raveendran, delivered another lecture on blood stream infections and central line related blood stream infections (BSI & CRBSI) - SGRH experience. There was a presentation made by Dr. Karthik Anantharaman (MSD) on **“Resistant Bugs and a new drug: Indian Perspective”**. The profile of Ertapenem was discussed. The events were appreciated by one and all. We thank our comrades to have made the events a success.

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