

**DIVISION OF MICROBIOLOGY AND
CLINICAL PATHOLOGY**

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Microbiology

The division extended microbiological diagnostic support to various intramural and extramural projects of NARI. The etiology of the infectious /opportunistic diseases was determined by performing diagnostic tests on specimens sent for bacterial, fungal, viral & parasitic infections and determining the antimicrobial sensitivity of the microorganisms that were isolated. Pathogens were isolated from stool, sputum/oral swabs, urine, genital swabs and skin scrapings collected from HIV infected patients attending NARI clinics and identified. Isolation of *Neisseria gonorrhoea* strains to study the trend of antibiotic sensitivity pattern was continued. We contributed to *Chlamydia trachomatis* and *Neisseria gonorrhoea* diagnosis by Nucleic acid amplification assays.

A. DETECTION AND ISOLATION OF ORGANISMS

Clinical specimens received from our clinics are tested to determine the etiology of infection.

A1. Isolates from sputum / oral swab specimens

A total of 44 sputum/oral swabs (HPTN -13, ACTG - 07 & Referred - 24) were collected from HIV infected symptomatic patients and processed for bacterial and fungal cultures. Pathogens were identified in 26 specimens by API 20 system. They were *Streptococcus spp.* (5), *Klebsiella pneumoniae* (5), *Hemophilus spp.* (6), *Pseudomonas aeruginosa* (4), *Escherichia coli* (1), *Staphylococcus spp.*(1), *Candida albicans* (4).

A2. Isolates from stool specimens

Stool samples (51) were collected from HIV infected patients presenting with diarrhea enrolled in various projects (HPTN - 10 , ACTG - 17, CPI - 02 & Referred patients - 22) of NARI. These samples were processed for routine/microscopy by saline or iodine mount and special staining like modified ZN or Trichrome. Forty-two samples did not yield any pathogen while enteric parasites were identified in 7 samples, *E. histolytica / E. dispar* (3), *Strongyloidosis stercoralis* (1), *Cyclospora spp.* (1) and *Isospora spp.* (2).

A3. Isolates from urine specimens

Urine specimens collected from 31 (HPTN - 05, ACTG - 13, CPI - 01 & Referred patients - 12) symptomatic patients were processed for bacterial culture. *Escherichia coli* (5), *Staphylococcus aureus* (3) were isolated from these specimens.

A4. Resistance pattern in *N. gonorrhoeae*

In order to continuously study the pattern of drug resistance, isolation, identification and antibiotic susceptibility testing of *N. gonorrhoeae* isolates obtained from symptomatic patients, attending NARI clinics, is being routinely undertaken at this division from the year 1996. Two *Neisseria gonorrhoeae* isolates were obtained in the year 2007. Antibiotic sensitivity testing was

conducted by the Kirby Bauer method. MICs for these strains were performed by E test for Ciprofloxacin, Penicillin, Ceftriaxone, Tetracycline and Spectinomycin. Both strains were sensitive to Spectinomycin and Ceftriaxone, intermediate sensitive to Penicillin & resistant to Ciprofloxacin and Tetracycline.

A5. Mycobacterium tuberculosis

A total of 131 sputum samples from 63 patients with respiratory symptoms enrolled in various studies at (NIV, SGH, Talera and NARI) NARI clinics were received to screen for acid fast bacilli (AFB). All the samples were processed for Acid Fast smear microscopy by Ziehl Neelsen staining. Eight (12.6%) patients were positive on microscopy. Of these, sputum samples from 12 patients (2 AFB positive and 10 AFB negative) were cultured on LJ medium on clinician's requisition. *Mycobacterium tuberculosis* was isolated from 2 AFB positive samples

NARI is conducting TB-HIV studies entitled "Evaluation of diagnostic algorithm for HIV positive pulmonary tuberculosis suspects who are initially smear negative, in Pune (Smear Negative TB-HIV study)" and "Nevirapine versus Efavirenz-based highly active antiretroviral therapy regimens in antiretroviral-naive patients with HIV and Tuberculosis infection in India" at Talera hospital, Chinchwad. A total of 253 sputum samples from 120 patients attending the TB OPD were screened for sputum AFB smear microscopy for enrollment in these studies. Fifty six (46.6%) patients were positive for AFB smear microscopy.

Under the Smear Negative TB-HIV study, 124 sputum samples from 30 patients were collected and processed for AFB smear microscopy and culture received from TB OPD of Talera Hospital, Chinchwad. Of these 3 (10%) patients were positive for AFB microscopy, which were also culture positive.

Apart from sputum samples other specimens were also referred from NARI clinics for ZN staining, 5 pus (1 cervical lymphadenitis, 1 right thigh wound, 1 peri-anal lesion and 2 anal fistula) 10 stool, 3 urine, 1 ascitic fluid samples were also received for AFB smear microscopy. All samples except pus sample from one patient with cervical lymphadenitis were negative for AFB microscopy.

A6. Detection of Neisseria gonorrhoeae and Chlamydia trachomatis by Nucleic Acid Amplification Assay

As a part of screening, 188 samples from HPTN 052 study were tested by Nucleic acid Amplification Assay for chlamydial (CT) and gonococcal (NG) infection. All specimens were negative for NG/CT.

B. SEROLOGICAL ASSAYS

Potential study participants are tested for syphilis, Hepatitis B surface antigen (HBsAg) and Hepatitis C (HCV) antibody in order to determine their eligibility for enrollment. Additionally samples from clinically suspected cases are received and tested for Toxoplasma, Cytomegalovirus (CMV) and Cryptococcal infections.

B1. Syphilis Serology

Syphilis serology was conducted on serum samples from patients screened for enrollment in the various projects conducted at NARI. Samples were first tested in RPR and reactive samples (Titer > 1:8) were confirmed by TPHA test. These tests were done at the respective NARI clinic. The Microbiology laboratory performs quality control check on 10% of the totally processed samples from each NARI clinic. Of the 416 samples, 54 were RPR reactive. All RPR reactive samples were positive by TPHA.

B2. HBsAg and HCV

In order to determine the eligibility of subjects for enrollment in the studies, a total of 349 samples were screened for presence of Hepatitis B virus surface antigen, and 25 samples were tested for the presence of antibody to Hepatitis C virus (Table 5.1).

Table 5.1: Samples tested for HBsAg and Anti-HCV

Project	Samples tested		Samples positive (%)	
	HBsAg	Anti-HCV	HBsAg	Anti-HCV
AACTG	6	2	0	0
HPTN (059 & 052)	239	9	8 (3.3%)	0
Referred	104	9	6 (5.8%)	0
NeuroAIDS	0	5	0	0

B3. HSV2 IgG

Of the 304 specimens from HPTN study participants tested for Herpes simplex virus IgG antibody, 61 (20%) were found to be positive.

B4. Toxoplasma IgG & IgM

A total of eight specimens from patients were tested for Toxoplasma IgG & IgM antibody. Six specimens were found to be positive for Toxoplasma IgG and none was positive for IgM antibody.

B5. CMV IgG & IgM

Two specimens from patients were tested for CMV IgG and IgM, out of which both were positive IgG and none for IgM.

B6. Cryptococcal antigen

A total of 34 specimens from patients were tested for Cryptococcal antigen, out of which 2 were positive.

C. PROFICIENCY PARTICIPATION

The laboratory is enrolled for CAP Programmes for proficiency testing of Gram stain, Culture and sensitivity (bacteria, mycobacteria and fungi), Syphilis serology, Viral markers, Parasitology, Blood parasites and NAAT for Chlamydia (CT) and gonorrhoea (NG) infection. All panels were successfully passed during the period.

Clinical Pathology

Clinical Pathology laboratory of NARI provide support for Haematology and Biochemistry investigations in NARI Clinical Trial protocols and other research studies, as diagnostic service to patients attending NARI clinics for clinical care. Additionally, OPD based labs at Sassoon and Gadikhana clinics of NARI also process haematology samples of referred patients. Haematology testing is done on Sysmex KX- 21 and Coulter Act5 diff. Biochemical investigations are done on Olympus AU400. Electrolyte analysis is done on two Roche 9180 machines.

Table 5.2: Samples Processed For Tests

Investigation type	Number of samples analysed
Haematology	6816
Biochemistry	6122
Urine analysis	2000
Coulter at NARI	117

PROFICIENCY PARTICIPATION

The laboratories are enrolled for CAP, NHLS and RCPA programmes for Haematology, Biochemistry and Urine analysis proficiency testing. All panels were successfully passed during the period.