

**DIVISION OF MICROBIOLOGY  
AND CLINICAL PATHOLOGY**



### MICROBIOLOGY

**D**ivision of Microbiology extended microbiological diagnostic support to both intramural and extramural projects of NARI. The projects were supported to determine the etiology of the infectious /opportunistic diseases by performing diagnostic tests on specimens sent for bacterial, fungal, viral & parasitic infections & determining the antimicrobial sensitivities of the microorganisms that were recovered. Pathogens were isolated and identified from stool, sputum/oral swab, urine, genital swabs & skin scrapings collected from HIV infected patients attending NARI clinics. Isolation of *Neisseria gonorrhoeae* strains to study the trend of antibiotic sensitivity pattern was continued. HPTN 059 and Dr. Reddy's Condom study was initiated at NARI this year. We contributed to Chlamydia and gonorrhea diagnosis by Nucleic acid amplification assays for these studies. Integrated Behavioral & Biological Assessment (IBBA) study in HIV high-risk population in six HIV prevalence states was conducted by NARI during the year. Various clinical specimens received from different districts in Maharashtra & other states were processed for serological assays (HIV, HSV, Syphilis). Nucleic acid amplification test for detection of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* was performed on urine samples.

#### A. Detection and Isolation of Organisms

##### A1. Enteric Pathogens in HIV infected patients

Stool samples (57) were collected from symptomatic HIV infected patients enrolled in various projects [HPTN (5), ACTG (20), CPI (1) and referred patients (31)] carried out at NARI. Forty-six samples did not yield any pathogen, while enteric parasites were identified in 11 samples; *E. histolytica* / *E. dispar* (6), *Ascaris* (2), *H. nana* (1), *Cryptosporidia* (2) and *Isospora* (1). Mixed infection was found in one sample (*Ascaris* and *Isospora*).

##### A2. Isolates from Sputum / Oral swabs

A total of 27 oral / sputum specimens [HPTN 034 (2), ACTG (19) and referred (6)] were collected from HIV infected symptomatic patients and processed for bacterial and fungal cultures. Pathogens were identified by API 20 system. *Candida albicans* (5), *Staphylococcus aureus* (3) and *Streptococci* group B (2), *Streptococcus sanguis* (4), *Streptococcus oralis* (1), *Streptococcus* species (8), *Klebsiella pneumoniae* (1) were isolated from these specimens. Four of the 7 *Streptococcus* strains and 1 of the *Staphylococcus aureus* strain were found to possess resistance to multiple antibiotics.

##### A3. Urine Culture

A total of 35 urine specimens collected from symptomatic patients were processed for bacterial culture. *E. coli* (4), *Staphylococcus aureus* (3) and other nonfermenter species (1) were isolated from these specimens. Antibiotic susceptibility testing revealed that all strains were resistant to Nalidixic acid, 4 were resistant to Norfloxacin and Nitrofurantoin, 2 to Trimethoprim and 1 to Ampicillin.



### A4. *Neisseria gonorrhoeae* and *Chlamydia trachomatis* by Nucleic acid Amplification Assay

As a part of screening, 28 urine samples from the colposcopy study, 27 from the HPTN 052 study, 282 from HPTN 059 study and 6 from Reddy's female condom study were tested by Nucleic Acid Amplification Test for chlamydia (CT) and gonorrhea (NG) infection. All specimens were negative for NG / CT.

### A5. *Mycobacterium tuberculosis*

A total of 107 sputum samples from clinically suspected pulmonary tuberculosis patients were received from the TB OPD of Sassoon General Hospital, Pune. Of these, 87 were from HIV negative and 20 were from HIV positive individuals. All the samples were processed for Acid Fast smear microscopy by Ziehl Neelsen staining and were cultured on to LJ media after decontamination by Petroff's method. Cultures were grown from 58 HIV negative and 16 HIV positive patients. 230 sputum samples were also received for sputum AFB smear microscopy from Talera Hospital, Chinchwad during the routine surveillance of HIV in tuberculosis patients in Pimpri- Chinchwad area. Of these, 207 samples were from HIV negative and 23 were from HIV positive patients. Overall 100 (42%, 6 from HIV positive & 94 from HIV negative patients) samples were positive for AFB smear microscopy.

## B. Diagnostic Serological Support

### B1. Syphilis Serology

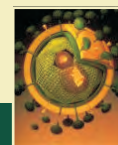
Syphilis serology was conducted on serum samples from patients screened for enrollment in the various projects conducted at NARI [HPTN 052 study (25), HPTN 059 study (106)]. Samples were first tested in RPR and reactive samples (Titer > 1:8) were confirmed by TPHA test. Two (1.5%) were found to be positive and were treated before enrollment. Of the 3758 patients, with high-risk behaviour, referred to NARI clinics, 62 (1.6%) were found positive. All 62 RPR reactive samples were found to be positive by TPHA.

### B2. Other Serological Tests

Samples from participants screened for various studies were tested for Hepatitis B virus surface antigen and presence of antibody to Hepatitis C virus. Further, samples collected from patients referred to NARI clinics were also tested

**Table 5.1: Samples tested for HBsAg and Anti-HCV.**

	Samples tested for		Positive	
	HBsAg	Anti-HCV	HBsAg	Anti-HCV
ACTG Project (HIV +ve)	115	6	3 (5.2%)	0 (0.0%)
HPTN 059 Project (HIV -ve )	234	-	3 (1.3%)	-
Referred patients (HIV +ve)	77	59	2 (2.5%)	1 (1.6%)



A total of 29 specimens received from patients suffering from genital ulcer disease were tested for Herpes simplex virus IgG antibody.

**Table 5.2: Herpes simplex virus IgG antibody positivity**

	No. tested	Positive
ACTG Project	15	13 (86.6%)
Referred patients	14	11 (78.5%)

A total of 10 specimens from patients suspected of suffering from Toxoplasma infection were tested for Toxoplasma IgG and IgM antibody. Three specimens were found to be positive for Toxoplasma IgG and none was positive for IgM antibody.

### C. Proficiency Testing

Microbiology laboratory is participating in a proficiency program implemented by College of American Pathologists (CAP), National Health Laboratory Systems (NHLs), and WHO Gonococcal Antibiotic Surveillance Program for South East Asia region (GASP) and Central Lab (JHU, USA). The performance rating was 80 percent or more in all panels except one.

### D. Integrated Behavioral & Biological Assessment study (IBBA)

*[Principal Investigator Dr. R.S. Paranjape]*

Integrated Behavioral & Biological Assessment of HIV high-risk population was initiated in six high HIV prevalence states to measure the major outcomes & impacts of the Avahan interventions by collecting behavioral and biological trend data in populations targeted by the interventions. Various clinical specimens received under the study from different districts in Maharashtra, were tested at NARI. Additionally, quality control testing was done for the specimen collected in other 5 states.

Serum samples were tested for HIV (J.Mitra / Genedia), Syphilis (RPR, Span Diagnostics, Surat India, TPHA, Syphagen, Barcelona) & HSV2 antibodies (Focus Technologies). Urine samples were tested in GeneAptima Combo II assay for presence of NG/CT.



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**Table 5.3 District wise specimens received**

Sr.no.	District Name	HIV 1 <sup>st</sup> test	HIV 2 <sup>nd</sup> test	RPR	TPHA	HSV-2	Gen-Aptima
1	Kolhapur	120	41	Nil	32	12	122
2	Pune	918	273	918	257	96	951
3	Thane	803	95	Nil	51	78	803
4	Yavatmal	560	98	Nil	109	69	562
5	Parabhani	758	74	758	56	85	773
6	Mumbai	1212	283	Nil	140	131	1230
7	Nagaland*	Nil	Nil	Nil	Nil	Nil	1287
8	Manipur*	Nil	Nil	Nil	Nil	Nil	839
<b>Total</b>		<b>4371</b>	<b>864</b>	<b>1676</b>	<b>645</b>	<b>471</b>	<b>6567</b>

\* Dried blood spot

