

## STUDY REPORT

Title: Venereal Diseases in Thailand

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**Objective** Initially the primary objective of this study was to investigate causes of therapeutic failures in the treatment of gonorrhea in SEATO troops and their contacts. Earlier studies indicated that therapeutic failures were caused by organisms other than Neisseria gonorrhoeae and that all N. gonorrhoeae isolates were sensitive in vitro to obtainable blood levels of penicillin or tetracycline. Studies during the current reporting period have emphasized evaluation of laboratory techniques for diagnosis of cases and continued surveillance of clinical isolates for changes of their in vitro sensitivities to antibiotics.

**Description** Studies comparing laboratory diagnostic procedures were carried out on Thai females who reported for periodic pelvic examinations at Thai venereal disease clinics. Rapid diagnostic procedures evaluated were the Pathotec Cytochrome Oxidase paper strip and the fluorescent antibody techniques. Also evaluated were modifications of culture media to increase isolation rates of N. gonorrhoeae. Other studies already in progress were sensitivity determinations of gonococci to antimicrobials, examinations of specimens from U.S. nationals in the Bangkok area, and serological tests for syphilis.

**Progress** 1. Comparison of culture media for the isolation of Neisseria gonorrhoeae from female out-patients at Ban Chiwi VD Clinic, Bangkok, Thailand.

Diagnosis of gonorrhea in the female is difficult and fallible. A number of new media—mostly incorporating antimicrobials—have been reported in recent years. One such new medium, developed by Baltimore Biological Laboratories (BBL), was reported by Martin et. al\* to be superior to Difco GC medium (DF), which had been used routinely in this Laboratory for the last two years. A study was designed to compare these two media. Additional studies on the same specimens included further evaluations of the Pathotec Cytochrome Oxidase (CO) paper strip and the fluorescent antibody (FA) techniques for rapid diagnosis of gonorrhea.

\* Martin, J.E., Billings, T.E., Hackney, J.F., and Thayer, J.D.:  
Primary isolation of N. gonorrhoeae with a new commercial medium. Public Health Rep. 82:361—363,  
April 1967.

Cervical specimens were obtained from 206 self-referred outpatients at the Ban Chiwi Clinic in Bangkok. These patients are usually checked weekly and theoretically do not necessarily represent suspected cases of gonorrhoea. However only those individuals with vaginitis, vulvitis, cervicitis or urethritis were cultured for this study. Every patient received a weekly prophylactic injection of penicillin or streptomycin, which could alter normal vaginal flora and cause clinical symptoms from over-growth of other microbial agents. Before obtaining swabs for culture and smear, the CO paper test was performed with cervical discharge by direct contact. The test papers were placed immediately in screw cap test tubes and results were read between 10–30 minutes later. Immediately afterwards specimens were cultured on both BBL and DF media and slides were prepared for the direct FA technique and for gram stains.

Results in Table 1 indicate that the BBL medium was superior to the DF medium both in terms of positive cultures and the numbers of N. gonorrhoeae colonies per plate. While this is an improvement, there were also instances where N. gonorrhoeae were isolated on DF but not BBL medium. Comparisons of the cytochrome oxidase technique with other procedures are shown in Table 2. Assuming growth of N. gonorrhoeae on BBL medium as a base there were 44.9% false positive and 51.5% false negative specimens with the cytochrome oxidase test and there were 37 instances where the cytochrome oxidase test was the only one positive. The suspicion that this test is not specific was confirmed by demonstrating that pus specimens from infected wounds were frequently (but not predictably) positive for cytochrome oxidase. This test is not a reliable procedure for diagnosis of gonorrhoea.

Table 1. Comparison of Media for the Isolation of Neisseria gonorrhoeae from Female Out-patients at Ban Chiwi VD Clinic, Bangkok, Thailand

Medium	No. of specimens	Specimens Positive for <u>Neisseria gonorrhoeae</u>					
		Total	< 1+*	1+	2+	3+	4+
BBL**	206	68	7	21	21	12	7
Difco***	206	47	7	19	12	5	4

\* <1+ Less than ten colonies per plate  
 1+ 10–25 colonies per plate  
 2+ 25–100 colonies per plate  
 3+ 100–200 colonies per plate  
 4+ > 200 colonies per plate

\*\* Each ml BBL medium contained 3 units vancomycin, 7.5 micrograms sodium colistimethate and 1.25 units of nystatin.

\*\*\* Each ml DF medium contained 25 units of polymyxin B and 10 mcg of ristocetin.

Table 2. Comparison of Various Techniques for Diagnosis of Gonorrhea in Females

Culture medium	Number of specimens	Cytochrome Oxidase		Direct FA		Gram Stain for Gram Negative Diplococci		
		+	-	+	-	Intracellular	Extracellular	Negative
BBL +	68	33	35	33	35	17	30	21
BBL -	138	62	76	43*	94*	11	30	97
Difco +	47	25	22	27	20	13	22	12
Difco -	159	70	89	57*	101	13	36	110

\* One slide not available for FA

With the direct FA technique there were 31.4% false positive and 48.5% false negative results. False negative results are not unusual and a delayed FA test in which 6 hour cultures rather than direct smears are used, is generally recommended. Under the criterion that positive smears must contain gram negative intracellular diplococci, these results are discouraging. Even if gram negative extracellular diplococci are considered diagnostic of gonorrhea, enough false positives and negatives occurred to indicate that this test is of questionable reliability for use with females.

Reports in the literature indicate that alcohol consumption increases the frequency of complications of gonorrhea. Alcohol is considered a leading cause of chronicity and reportedly can activate latent or sub-clinical gonorrhea. The possibility that this effect of alcohol could be due, in part, to its action on the causal organism prompted a study on the in vitro effect of alcohol on isolation rates of N. gonorrhoeae from females. Three cervical swabs were obtained from each of 109 out-patients at Ban Chiwi Clinic. One swab was streaked directly on BBL agar; one was placed for 3 hours in a screw cap test tube of 2.0 ml trypticase soy broth containing 0.25% ethanol, after which it was streaked on BBL agar; and one was streaked to BBL agar containing 0.25% ethanol. Results (Table 3) indicate alcohol did not effect the number of positive isolates but heavier growth occurred on the alcohol-free medium.

Table 3. Effect of Incorporation of Ethanol on Isolation of Gonococci from Females

Medium	No. of specimens	Specimens Positive for <i>Neisseria gonorrhoeae</i>					
		Total	<1+*	1+	2+	3+	4+
BBL	109	50	3	6	4	10	27
BBL containing 0.25% Ethanol	109	50	4	4	6	12	24
TSB containing 0.25% Ethanol followed by streaking to BBL	109	50	7	2	8	20	13

\* < 1 + = 1-10 colonies per plate  
 1 + = 11-25 " " "  
 2 + = 26-100 " " "  
 3 + = 100-200 " " "  
 4 + = > 200

## 2. Gonorrheal Survey, Korat

Thirty-two male patients with urethritis from various U.S. military units in Korat and 43 Thai national females were examined for gonorrheal infection in June 1967.

Sixteen of the male patients tested by the cytochrome oxidase rapid paper strip technique were positive. Of these, 14 were also positive by the FA technique, 13 had gram negative intracellular diplococci (GNID) demonstrated by smear, 2 had gram negative extracellular diplococci (GNED) and 11 yielded positive cultures for Neisseria species on DF medium. These could not be further identified because of failure in subculture.

Urethral exudates were cultured from 32 males and concurrent smears for gram staining made from 30 of them. Of the total male specimens examined, 18 had GNID and 5 GNED, 21 were positive by the FA technique, Neisseria spp were isolated from 11, and Mima—Herellea organisms were isolated from 2. Other organisms isolated included diphtherids, staphylococci (coagulase positive and negative), Pseudomonas, and Aerobacter. The onset of symptoms ranged from 1 to 14 days after the last admitted contact. Twelve of the patients indicated during interview that they had used condoms. Of these, 6 said that the condom was not intact on completion of the contact.

No Neisseria were isolated from any of the 43 females examined. Of these, 17 had GNED and 5 were positive by FA. Mima—Herellea were isolated from 12. Other isolates included coagulase negative staphylococci, diphtheroids, alpha and beta streptococci, Pseudomonas and coliforms.

### 3. Susceptibility of gonococci to antimicrobials

The results of plate dilution tests for in vitro sensitivities of N. gonorrhoeae to oxytetracycline and penicillin G are shown in Table 4. (Results are expressed micrograms/ml rather than units/ml of penicillin G.) None of the strains tested were resistant in vitro to readily achievable blood levels of these antibiotic during the last 2 years.

Table 4. Antibiotics Sensitivities of Neisseria gonorrhoeae Isolated in Thailand  
(1 April 1967—31 March 1968)

Period	Penicillin G (mcg/ml)			Oxytetracycline (mcg/ml)		
	# of cultures	Range	Median	# of cultures	Range	Median
1 April 1967—30 June 1967	15	0.12—0.60	0.36	16	1.0—2.8	1.4
1 July 1967—30 September 1967	16	0.18—0.72	0.48	19	1.0—2.6	2.4
1 January 1968—31 March 1968	24	0.12—0.60	0.24	35	0.8—4.0	2.0
Totals 1 April 1967—31 March 1968	55	0.12—0.72	0.36	70	0.8—4.0	2.3

### 4. Bacteriological examination of urethral or vaginal discharge specimens from U.S. personnel in the Bangkok area.

Results of cultural examinations of urethral and vaginal discharges from U.S. personnel submitted by the U.S. Embassy Medical Unit, the 5th Field Hospital and other local units are summarized in Table 5. N. gonorrhoeae were isolated from 20 of 127 specimens from males and from 5 of 87 specimens from females. Staphylococcus epidermidis and diphtheroids were most frequently isolated. Numbers of the Mima—Herellea group were isolated from 5 males and no females.

### 5. Serological Tests for Syphilis (VDRL)

VDRL tests were performed on 677 sera from Thai and 542 sera from U.S. personnel during this reporting period. Most of the latter specimens were from U.S. soldiers prior to their departure from

Thailand and rarely represented suspected syphilis. Other specimens from U.S. and Thai nationals were submitted as a part of routine physical examinations or as a result of suspected venereal contacts. Data in Table 6 indicate that syphilis was not prevalent among the U.S. personnel tested.

Summary A new commercial culture medium supplemented with a chemically defined enrichment and made selective by adding antimicrobials was tested for primary isolation of gonococci from females. Recovery of gonococci was better than on the medium previously used. Incorporation of 0.25% ethanol in the new medium did not decrease isolation rates but numbers of colonies per specimen were reduced. The cytochrome oxidase test and direct fluorescent antibody technique were found unreliable for rapid diagnoses of gonorrhea in females. In vitro sensitivity studies showed that all isolates were sensitive to readily achievable blood levels of penicillin and oxytetracycline. Results of VDRL tests of sera submitted as part of routine physical examinations indicate that syphilis was not prevalent among U.S. personnel in Thailand.

Table 5. Organisms Isolated from U.S. Personnel with Suspected Gonorrhea

	No. of isolates from	
	127 Males	87 Females
<i>Neisseria gonorrhoeae</i>	20	5
<i>Neisseria species</i>	4	3
<i>Staphylococcus aureus</i>	5	6
<i>Staphylococcus epidermidis</i>	49	17
Diphtheroids	41	23
Alpha hemolytic streptococci	12	8
Non-hemolytic streptococci	12	11
Beta hemolytic streptococcus (Group A)	1	0
<i>Micrococcus tetragenus</i>	31	13
Mima-Herellea group	5	0
<i>Candida albicans</i>	0	7
<i>Candida species</i>	0	6
<i>Proteus mirabilis</i>	0	1
<i>Proteus species</i>	1	6
<i>Streptococcus fecalis</i>	3	5
<i>Escherichia coli</i>	6	6
<i>Klebsiella-Aerobacter</i>	0	1
<i>Pseudomonas aeruginosa</i>	0	1
<i>Pseudomonas species</i>	2	3
<i>Haemophilus species</i>	3	1
Enterococcus	8	10
<i>Paracolobactrum species</i>	2	4
<i>Bacillus species</i>	3	2
Total isolates	208*	139*

\* More than 1 isolate from most specimens

Table 6. Results of VDRL Test on Serum Specimens 1 April 1967—31 March 1968

Group	Period	No. of specimens	Negative	Positive							
				Undiluted	1:2	1:4	1:8	1:16	1:32	1:64	1:128
Thai Nationals	April—June 1967	122	109	3	3	4	—	1	—	1	1
	July—September 1967	101	91	2	2	4	2	—	—	—	—
	October—December 1967	277	261	5	1	1	6	3	—	—	—
	January—March 1968	177	154	7	4	4	3	4	1	—	—
	Totals	677	615	17	10	13	11	8	1	1	1
U.S. Nationals	April—June 1967	123	118	4	—	—	1	—	—	—	—
	July—September 1967	235	234	1	—	—	—	—	—	—	—
	October—December 1967	104	104	—	—	—	—	—	—	—	—
	January—March 1968	80	79	—	1	—	—	—	—	—	—
	Totals	542	535	5	1	—	1	—	—	—	—