

EVALUATION OF THE SCREENING TEST RESULTS IN ASYMPTOMATIC PROSTITUTES  
FOR GONORRHEA AND SYPHILIS BY USING ENZYME IMMUNOASSAY-GRAM STAIN AND  
SRCT-TPHA FROM IZMIR STD CLINIC

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A commercial enzyme (EIA) (Gonozyme; Abbott Laboratories) for detection of gonococcal antigens in endocervical specimens; SRCT (Modified VDRL) and TPHA tests for syphilis have been used. Forty eight specimens were collected at sexually transmitted diseases (STD) clinic from registered prostitutes in Izmir. Additional 48 specimens were collected at Dokuz Eylül University Hospital Obstetrics and Gynecology Department from patients supposed not having gonorrhoea or syphilis. Eight of 48 (17 %) prostitutes were positive for gonococcal antigen with enzyme immunassay (EIA) technique and only 4 of those 8 (50 %) were positive with Gram stain. Sera of those prostitutes were tested for reagin and Treponema pallidum antibodies. Five sera (10 %) were positive with SRCT, 7 sera (14 %) were positive with TPHA, but only two sera were positive (4 %) for both techniques. None of 48 females attending university clinic (as a control group) were found positive with all the tests used for prostitutes.

KEY WORDS : Gonorrhoea, syphilis, enzyme immunoassay, Treponema pallidum hemagglutination.

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ÖZET Enzim immunoassay-Gram Boyama, SRCT-TPHA Tekniklerini Kullanarak İzmir'de Aseptomatik Genelev Kadınlarında Gonore ve Sifilis Tanınma Sonuçlarının Değerlendirilmesi.

Ticari bir enzim immunoassay, EIA Gonozyme; (Abbott Laboratuvarları) ile endoserviks'te Gonokok antijeni ile serumlarda SRCT ve TPHA deneyleriyle de Sifilis aranması yapılmıştır. İzmir'de venereal hastalıklar dispanserinde kayıtlı 48 genelev kadınından ve Dokuz Eylül Üniversitesi Hastanesi Kadın Hastalıkları ve Doğum Kliniğinde Gonokok veya Sifilisi olmadığı kabul edilen kontrol 48 materyal alınmıştır. Kırksekiz genelev kadınının 8'inde enzim immunoassay yöntemi ile

gonokokkal antiijen olumlu (% 17) bulunmuş, bu pozitif 8 örneğin sadece 4'ünde (50 %) gram boyası ile Neisseria gonorrhoeae görülmüştür. Bu kadınların serümlerinde reagin ve Treponema pallidum antikorları aranmış ve 8 (% 10) SRCT (Modifiye VDRL) 7 (% 14) TPHA ile pozitif reaksiyon varmışlardır. Sadece iki serum (% 4) her iki teknikle SRCT ve TPHA ile olumlu bulunmuştur. Üniversite hastanesi kliniklerinde muayene olan 48 kadında (kontrol grubu olarak) bu deneylerin hiç biri olumlu bulunmamıştır. Gonorrhoea and Syphilis are still the main focuses among the sexually transmitted diseases (STD). Efforts to control their spread including screening of asymptomatic females seem to be a problem in Government clinics for STD. They must be diagnosed in a short time for treatment and preventing new cases. Control of gonorrhoea is difficult because almost 50 % of infected females and 10 % of infected males are asymptomatic (1,2). Presumptive diagnosis of urogenital gonorrhoea in asymptomatic males may be made on the basis of Gram staining. In females which mostly are clinically inapparent, Gram staining is less sensitive than culturing. However culturing is not an ideal diagnostic way for gonococcal infection : there are four main disadvantages :

- a- It needs labor, experienced technicians and organized laboratory (3,6,7).
- b- Takes minimum of 48 hours,
- c- Loss of viable gonococci associated with improper specimen handling (5), or using external germicide by infected persons (in cases of registered prostitutes),
- d- Specimen containing N.gonorrhoeae mixed with other microorganisms (3).

Diagnosis of inapparent syphilis may firstly be done by VDRL test than any positive result should be confirmed by using specific Treponema antibody test. A person with syphilis may be in reagin negative state, consequently SCRT test will be negative. Presumptive diagnosis of Syphilis could be made by using both. SCRT and TPHA tests and other definitive methods may be used in case of TPHA positivity.

#### MATERIAL AND METHODS

##### Specimen Collection :

Endocervical specimens were collected from both registered prostitutes at Izmir STD clinic during regular examination and Dokuz Eylül University Hospital ob-gyn. clinic complaining of vaginal discharge or attending for check-up . Total specimens were 96 and each group consisted of 48. All specimens were taken by the specialist using a sterile speculum without being soaked in germicide. Two swab samples were taken from each person, one was taken with special Rubazyme STD-EZE swab and transported in storage reagent (provided by Abbott laboratories). They were stored in refrigerator till EIA-test was performed not later than 3 days. Second material was taken with ordinary cotton tipped swab to prepare a smear of the exudate as soon as possible.

Blood specimens were obtained from patients with disposable syringe not using any coagulant. Sera were separated and stored in -20°C till serological tests were performed. All sera were tested within 2 months.

Gram staining : After standard Gram staining, smears were examined microscopically for *Neisseria gonorrhoeae*. Positives were graded as one to four plus.

EIA (Enzyme-Immunoassay) Procedure : Immediately after endocervical swab specimens for the EIA were secured, they were placed into 100 ul of a specimen storage reagent (Gonozyme, Abbott Laboratories) (8,9,10,11) and stored at + 4°C for up to 5 days before testing. To the vial containing the swab 1 ml of Gonozyme specimen dilution buffer was added and immersed in it for 2 minutes, vortexed vigorously for 20 seconds and then squeezed to remove excess fluid. The swab was discarded. A 200-ul of the controls and specimens from patients were incubated with treated-beads (capable of binding *N.gonorrhoeae* antigens) in a 37° C waterbath. Unbound material was aspirated and the beads were washed with distilled water. Anti-*N.gonorrhoeae* rabbit serum (200 ul) was added to each bead. The beads were incubated at 37°C and washed. Goat anti rabbit immunoglobulin serum coupled with horseradish peroxidase (200 ul) was added in the same manner above, the incubation-and-washing cycle was repeated. The beads were immediately transferred to assay tubes and 300 ul freshly prepared O-phenylene diamine-2 hydrochloride was added. An orange color reaction is produced in proportion to the amount of enzyme attached to the beads. This reaction was stopped by the addition of 1 ml of 1 N H<sub>2</sub>SO<sub>4</sub>. Absorbance at 492 nm was determined with a Quantum (Abbott Laboratories) photometric analyzer.

SRCT (Syphilis Reagin Card Test) : The Cambridge Biomedical Syphilis Reagin Card Test is a macroscopic non-treponemal flocculation technique that is used to detect reagin antibodies. It is a presumptive diagnosis for syphilis. Antigen is a modification of VDRL Antigen which contains microparticulate carbon.

TPHA (TREPONEMA PAALIDUM HEMAGGLUTINATION) TECHNIQUE : It is a micro hemagglutination assay. Test kit is used for detecting the specific *Treponema pallidum* antibodies.

#### RESULTS

The results of Gram stain (GS) is compared with results of EIA (Table 1) for *Neisseria gonorrhoeae* infection. Eight of 96 specimens gave positive reaction with EIA and three of the specimens had greater than 2 absorbance value (Table 2). Only 4 GS were positive for gonococci which were also positive with EIA, the mean correlation is 50 %. All of those positive samples were in the prostitute group and none of the controls gave any EIA or GS positive result.

TABLE 1 : Results of EIA (Enzyme Immunoassay) for Gonococcal antigen and GS smear microscopic examinations of 96 specimens from endocervix of asymptomatic females.

Number Examined		EIA	GS
Total	96		
Prostitute (48)		8	4
ObGyn-cont (48)		0	0
Positive cases		8/96	4/96

TABLE 2: EIA values and GS examination of 8 positive specimens

Lab. No.	1157	1165	1168	1173	1182	1183	1190	1199
EIA <sup>*</sup> (Gonozyne)	2	1.268	0.581	2	0.467	0.601	2	0.807
GS <sup>**</sup>	N	N	++	++	+	N	++++	N

\* Means of negative control

" " positive "

" " cut of value : 0.306

\*\* N : Negative ; positives were graded as one to four plus.

Results of serodiagnostic tests for syphilis yielding five positive with SRCT (Non specific), 7 positive with TPHA (specific) were obtained from 48 prostitutes, none of control gave any reaction with both techniques. Two TPHA positive specimens were also positive with both techniques. Two positive specimens were also positive with SRCT test. Only positive results have been obtained from one prostitute by TPHA, Gonozyne and GS (TABLE 3).

TABLE 3 : Comparison of EIA, GS, VDRL and TPHA positive results of specimens taken from 48 prostitutes.

Lab. No.	(Gonozyne) EIA	GS	VDRL	TPHA
1157	2	-	-	-
1158	-	-	+	-
1163	-	-	+	+
1165	1.268	-	-	-
1166	-	-	+	-
1168	0.581	++	-	+
1169	-	-	-	+
1173	2	+	-	-
1179	-	-	-	+
1182	0.467	+	-	-
1183	0.601	-	-	-
1189	-	-	+	-
1190	2	++++	-	-
1196	-	-	-	+
1197	-	-	-	+
1198	0.807	-	-	-
1703	-	-	+	+

Total : 96, prostitute group 48

Positive cases : Gonozyne 8/48, GS 4/48, VDRL 5/48, TPHA 7/48

#### DISCUSSION

Gram staining technique is used in most of the STD clinics in many countries and Turkey for the screening of gonorrhoea for registered prostitutes (4,8). Many asymptomatic females carrying *Gonococcus* are missed, because of the shortage of time and technician. Registered prostitutes have scheduled date for check-up in Izmir and they apparently use germicide washing before coming to the examination. Therefore obtaining proper specimens for GS and culture become very difficult because most bacteria may be lost.

We tried to obtain specimens from endocervices using steril speculums not being soaked in any germicide solution. To search for Gonococcal antigen with EIA technique is more reliable and a highly sensitive method because even when *Gonococci* are not alive, antigen will also be detected. Gram stain in females seems missing some of carriers. Stamm et al (4) obtained 48 % of sensitivity with GS in females which is very near to our results. Sensitivity of EIA can be used as a better technique than GS in the STD patients to control the spread of gonorrhoea. For the diagnosis of syphilis among the prostitutes in the first step, VDRL or SRCT (Modified VDRL) may be used but some syphilis cases can be missed among those risk groups. TPHA must be used for all the patients because person may be in the reagin negative state and SRCT gives negative result or cross-reaction with other diseases. We recommend the use of diagnostic tests EIA and TPHA for searching gonorrhoea and syphilis respectively in the routine control examination of registered prostitutes attending STD clinics in Turkey.

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