

# Enzyme-linked immunosorbent assay for detecting gonococcal antibodies using two antigenically different gonococcal pili as antigen

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**SUMMARY** Antibodies to pilar antigens of two gonococcal strains isolated in Rotterdam (6650 and 1443) were detected using enzyme-linked immunosorbent assays (ELISA). Paired sera (the first sample taken at the first examination (D1) and the second 11-22 days later (D2)) from women with and without gonorrhoea attending a sexually transmitted disease (STD) clinic were studied. The sensitivity of the ELISA using gonococcal pili 6650 as antigen (ELISA 6650) was significantly higher than that using gonococcal pili 1443 as antigen (ELISA 1443). The specificity of the two tests differed little. On D1 the sensitivity in women with uncomplicated gonorrhoea was 69% in the ELISA 6650 and 45% in the ELISA 1443; the corresponding values in asymptomatic infected women were 75% and 57% respectively. The agreement (both in positive and in negative results) between the two tests was less than might have been expected ( $\kappa = 0.41$ ).

## Introduction

Antigenic heterogeneity of gonococcal pili to gonococcal antibodies in human sera was first detected by Reimann *et al*<sup>1</sup> using an indirect haemagglutination (IHA) reaction. Three antigenically different pilar preparations were used. The sensitivity of the IHA using one of these pilar preparations as antigen (6650) was significantly higher than that using the antigenically different 2686 pili as antigen. The sensitivity of the enzyme-linked immunosorbent assay (ELISA) using gonococcal pili 1443 as antigen virtually equalled that of the IHA using gonococcal pili 2686 as antigen.<sup>2</sup>

The aim of this study was to establish whether the use of gonococcal pili 6650 as antigen in the ELISA would also increase the sensitivity compared with that using pili 1443 as antigen.

## Material and methods

We studied sera from 104 women with uncomplicated gonorrhoea and 28 women who requested a routine

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examination for STD. Sera from blood donors ( $n = 119$ ) and from healthy children aged 10-12 years ( $n = 58$ ) were used as control sera. All sera used in this study belonged to the material previously studied in detail.<sup>1,2</sup>

## GONOCOCCAL STRAINS AND PREPARATIONS OF PILAR ANTIGEN

The gonococcal strains used were 1443 and 6650, both isolated in Rotterdam. Pili 1443 were purified as described by Robertson *et al*<sup>3</sup> and pili 6650 according to Hermodson *et al*.<sup>4</sup> In the first case the gonococci were homogenised and purified over a caesium chloride gradient after differential centrifugation. In the last case gonococci were pre-processed in the same way but purified by precipitation with ammonium sulphate. The pilar preparations looked equally pure by electron microscopy.

## ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA)

The ELISA procedure was carried out as described.<sup>2</sup> In the same way as in the ELISA using gonococcal pili 1443 as antigen (ELISA 1443), the cut-off point in the ELISA with pili 6650 as antigen (ELISA 6650) was determined as  $A \geq 0.85$  ( $A =$  absorbance). The cut-off point was chosen so that all the sera of 58 healthy children aged 10-12 years were negative.

Results with absorbance values between 0.75 and 0.85 were regarded as dubious and those with absorbance values between 0.85 and 0.95 as weakly positive in the ELISA 6650. In both assays the coefficient of variation within the test (plate-to-plate) and between tests (day-to-day) was less than 15%; for unmistakably negative sera only was this coefficient higher.

#### STATISTICAL ANALYSIS

Fisher's test<sup>5</sup> was used to compare the percentages of positive results in the two different groups of patients; the two-tail probability was computed as proposed by Cox and Hinkley.<sup>6</sup> McNemar's test<sup>5</sup> was used to compare the percentages of positive results in two consecutive serum samples from the patients. The coefficient of agreement  $\kappa^{7,8}$  was used to measure the agreement between two serological tests.  $\kappa$  equals zero under statistical independence, and  $\kappa$  equals one when there is perfect agreement.

#### Results

##### UNCOMPLICATED GONORRHOEA

The percentage of positive sera in both ELISA for women with uncomplicated gonorrhoea (n = 104) is given in table I. At the date of the first examination

(D1) the ELISA 6650 was positive in 69% of the sera and 11-22 days later (D2) it was positive in 67% of the sera.

The 104 patients were divided into women with asymptomatic infections (n = 56) and with symptomatic infections (n = 48). The sensitivity of the ELISA 6650 was higher in the former (D1, 75%; D2, 70%) than in the latter group (D1, 63%; D2, 65%). This difference was not significant. In neither group did the testing of a second serum sample show an increased sensitivity. Generally, the sensitivity of ELISA 6650 exceeded that of ELISA 1443. This difference in sensitivity was significant except on D2 in the women with asymptomatic infections.

##### SPECIFICITY

The specificity of ELISA 6650 and ELISA 1443 for women without gonorrhoea who requested a routine examination for STD (n = 28), for blood donors (n = 119), and for healthy children aged 10-12 years (n = 58) is shown in table II. There was little difference in specificity with the two antigens. For the blood donors the ELISA 1443 was positive in none of the sera and dubious in 2%, the corresponding figures for the ELISA 6650 being 8% and 5% respectively. For the children the ELISA 1443 was dubious in 12% of the sera while the ELISA 6650 was dubious in none.

TABLE I *The sensitivity of the ELISA using 6650 pili and 1443 pili as antigens for sera from 104 women with uncomplicated gonorrhoea. (Absolute numbers are given in parentheses)*

	%Sensitivity of the ELISA in relation to time of examination:					
	Women with asymptomatic gonorrhoea (n = 56)		Women with symptomatic gonorrhoea (n = 48)		Total group of women with uncomplicated gonorrhoea (n = 104)	
	D1*	D2*	D1	D2	D1	D2
Pili 6650	75 (42)	70 (39)	63 (30)	65 (31)	69 (72)	67 (70)
Pili 1443	57 (32)	61 (34)	31 (15)	44 (21)	45 (47)	53 (55)
McNemar's test	p<0.05	NS	p<0.01	p<0.01	p<0.01	p<0.01

\*D1 = date of first examination; D2 = 11-12 days after D1  
NS = not significant (p>0.1)

TABLE II *The specificity of the ELISA using 6650 pili and 1443 pili as antigen for sera from women without gonorrhoea, blood donors, and children aged 10-12 years. (Absolute numbers are given in parentheses)*

	%Specificity of the ELISA in relation to time of examination:					
	Women without gonorrhoea having routine STD examination (n = 28)		Blood donors (n = 119)	Children (n = 58)		
	D1*	D2*				
Pili 6650	71 (20)	73† (19)	92 (110)		100 (58)	
Pili 1443	75 (21)	82 (23)	100 (119)		100 (58)	
McNemar's test	NS	NS	p<0.01		NS	

\*D1 = date of first examination; D2 = 11-22 days after D1  
†Only 26 serum samples tested  
NS = not significant (p>0.1)

## DEGREE OF AGREEMENT BETWEEN ASSAYS

The agreement between the assays was measured using the coefficient of agreement  $\kappa$ . The agreement or non-agreement between the assays in 208 determinations in 104 women with uncomplicated gonorrhoea is shown in table III. Agreement was observed in 147 tests ( $n_o$ ). The number of agreements expected under statistical independence is 105 ( $n_c$ ). The ratio  $(n_o - n_c)/(N - n_c)$  is known as the coefficient of agreement  $\kappa$ . For agreement between the two tests  $\kappa = (147-105)/(208-105) = 42/103 = 0.41$ .

TABLE III Agreement between assays using different gonococcal pili (6650 or 1443) as antigen for sera from 104 women with uncomplicated gonorrhoea at date of first examination (D1) and 11-22 days later (D2). (Number to be expected under statistical independence given in brackets)

	Pili 6650		
	+	-	
Pili 1443			
+	93 (72)	12	} $\kappa = 0.41$ SE = 0.06
-	49	54 (33)	

+ = Positive; - = negative; SE = standard error;  $\kappa$  = coefficient of agreement

## Discussion

Serological tests using gonococcal pili as antigen for detecting gonococcal antibodies have been variously described.<sup>9-13</sup> The high sensitivity obtained by Buchanan<sup>10</sup> using a radioimmunoassay (RIA) (86% in women with uncomplicated gonorrhoea) was never obtained in any of the other studies.<sup>9 11-13</sup>

Differences in sensitivity obtained in the various studies could have been due to differences in patient material, the test system used, and the different gonococcal pili used as antigen. Reimann *et al*<sup>1</sup> obtained significantly different sensitivities when using pili from strains 6650 and 2686 as antigens in the IHA test on the same sera as were used in this study (66% with 6650 pili and 58% with 2686 pili on D1).

The present study was undertaken to establish whether the use of 6650 pili as antigen would increase the sensitivity of the ELISA compared with that obtained using 1443 pili as antigen. This was indeed the case (69% with 6650 pili and 45% with 1443 pili on D1). The specificities differed little. Using the

coefficient of agreement  $\kappa$ , a lower degree of agreement was found between the two assays ( $\kappa = 0.41$ ) than might have been expected. In fact this agreement was even less than the  $\kappa$  (0.44) between the ELISA 1443 and the IHA using gonococcal pili 2686 as antigen.<sup>2</sup> This was probably entirely due to the heterogeneity of the pili preparations. It would be interesting to use a pool of these two, or more, pili preparations as antigen in the ELISA. Combination of pili 6650 with pili 2686 in the IHA did not lead to the desired improvement.<sup>1</sup> The heterogeneity of gonococcal pili when using human sera in the test system should be borne in mind when antigens are chosen for serological tests or for vaccination.

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