

# Enzyme-linked immunosorbent assay (ELISA) to detect antibodies in gonorrhoea using whole cells

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**SUMMARY** The choice of an antigen that will adequately differentiate between infected and non-infected patients has been a major problem in detecting gonococcal antibodies for diagnosis. We have used the sensitive technique of ELISA to test various serotypes of *Neisseria gonorrhoeae* for their suitability as antigens. Whole cells of each serotype were attached to polystyrene plates using poly-L-lysine. *N gonorrhoeae*, strain H1 type 1 was used to detect antibodies in patients with known clinical history and then as a standard to evaluate the ability of different serotypes to differentiate between infected and non-infected groups.

The most satisfactory method of diagnosing an infection is to culture the organism. However, there are situations in which this is difficult or impossible. There may be intrinsic problems in growing the organism, the patient may have received antibiotics or the attempt at diagnosis may be retrospective of infection already subsided. In all these serological methods may be useful. In sexually transmitted diseases, particularly in screening programmes, patients may find it more acceptable to provide a blood sample than a specimen for culture.

In recent years many serological tests for gonorrhoea have been described but none is in routine use. The key factor is the antigen used and an antigen of adequate specificity has not yet been isolated. Preparation of pure antigens involves relatively complex isolation procedures and this can be tedious if it is desired to compare many strain differences. While the ideal of purified antigens with well characterised determinants must not be neglected, the use of whole bacteria, as in classical agglutination tests, would be very convenient in comparing antibody responses to different strains using the sensitive technique of the enzyme-linked immunosorbent assay (ELISA).

Sheep erythrocytes can be attached to polystyrene Petri dishes using poly-L-lysine.<sup>1,2</sup> We have modified this method in order to attach whole gonococci to microtitre polystyrene trays for use as an antigen in ELISA.

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Various strains were examined to determine which gave the optimum differentiation between infected and non-infected groups of patients.

## Material and methods

### BACTERIA

Of the strains of *N gonorrhoeae*, H1 was originally isolated from a patient at St Mary's Hospital. Colony types 1 and 4 have been kept by repeated selective subculture. F62 types 1 and 4 are descendants of a strain of F62 given by Dr DS Kellogg. Strains A1, B2, C3, D4, G7, H8, N10, S12, T13 and V15 are all colony type 4 and were given by Dr K Johnston. Each possesses an outer membrane protein of different serotype.<sup>3</sup>

The reference strains of *N meningitidis*, NCTC 10025 serogroup A, NCTC 10026 serogroup B, NCTC 8554 serogroup C, were obtained from the National Collection of Type Cultures (Colindale, England).

All strains were grown in Petri dishes on GC Base Agar (Difco) with supplements of dextrose,  $\alpha$  glutamine, cocarboxylase, and ferric nitrate<sup>4</sup> but no antibiotics.

After 18 h incubation in a carbon dioxide incubator (7% CO<sub>2</sub>) at 36°C, growth from each plate was scraped into 2 ml phosphate-buffered saline (PBS) (0.15 M, pH 7.4). Suspensions were ultrasonicated (15 s at 25 kHz) to break up clumps and adjusted to an optical density (OD) of 0.5 ( $\lambda = 700$  nm).

### ANTIGEN TITRATION

Each strain was tested by ELISA at increasing

concentrations against a positive and a negative control serum. All gave curves with a plateau over the range OD 0.2-1.0. A standard working concentration of OD = 0.5 was therefore used for all strains. The antigen excess ensured that even quite large variations in concentration had little effect.

#### ANTIBODY DILUTION

At the antigen concentration chosen, positive and negative control sera were titrated. The dilution giving optimum differentiation between the control sera were used for all sera tested.

#### PREPARATION OF TRAYS

Flat bottomed polystyrene microtitre trays (Dynatech) were used. All the wells in the trays were filled with poly-L-lysine (Sigma), at 50 µg/ml in PBS, and left at room temperature for 30 min. They were then emptied and washed three times with PBS for 3 min each time.

To half the wells in each tray 100 µl bacterial suspension (antigen) was added and left for 30 min at room temperature. The bacteria were then removed by suction into disinfectant, care being taken to avoid bench contamination and production of aerosols. The trays were then washed three times with PBS as before, and filled with 0.2% glutaraldehyde. They were then washed and stored in 0.1 M glycine at 4°C in moist conditions.

#### ELISA USING POLY-L-LYSINE-ANTIGEN COATED TRAYS

The trays already prepared were allowed to reach room temperature and washed three times with PBS containing 1% gelatin to prevent non-specific sticking of serum proteins. To appropriate wells with and without antigen 100 µl of serum at a dilution of 1/400 was added and left for 1 h at 30°C. Unattached serum proteins were then removed by washing three times with PBS-gelatin. The assay was continued with alkaline phosphatase-conjugated anti-human IgG as already described.<sup>4,5</sup>

#### POPULATIONS STUDIED

Sera were taken from four groups.

- (1) Males who were known to be smear- or culture-positive for gonorrhoea.
- (2) Females who were all culture-positive for gonorrhoea.
- (3) Male volunteer medical students as normal controls.
- (4) Females from the abortion clinic known to be culture-negative for gonorrhoea, or patients attending the antenatal clinic, who were presumed not to have gonorrhoea.

In initial studies using one strain of *N gonorrhoeae*,

H1, colony type 1, there were 100 in each group. The gonococcal patients were of defined clinical history.

In subsequent studies using the other strains of *N gonorrhoeae*, there were groups of 25 people, again defined above but whose detailed clinical history was not known.

#### IODINATION OF BACTERIA

For some experiments bacteria were iodinated with <sup>125</sup>I by means of the lactoperoxidase technique.<sup>6</sup> In these experiments separate wells (Dynatech) were used instead of trays. After the ELISA reaction had been read the wells were counted in a γ counter (LKB).

#### Results

##### DETECTION OF ANTIBODIES TO *N GONORRHOEAE* H1, TYPE 1

The 400 sera, as described in Methods, were tested for IgG antibodies using strain H1, type 1. The results were expressed in arbitrary units (E<sub>cr</sub>) based on corrected extinction values.<sup>4</sup>

There was a significant difference between the control and infected groups, the infected patients having a mean four times that of the controls (Table 1). Male and female control groups gave the same mean antibody concentration.

Table 1 *IgG antibody to N gonorrhoeae (strain H1 type 1) in patient and control groups*

	Male	Female
	Geometric mean	
STD patients with proven gonorrhoea	11.5*	13.9
Normal controls	2.96	2.98
p (Student's <i>t</i> test)	<0.001	

\*In corrected extinction units (E<sub>cr</sub>).

The potential of this strain to diagnose gonorrhoea can be seen from the cumulative frequencies in each of the groups at different antibody concentrations (Table 2).

When this technique was used to detect IgG antibodies, there was no residual effect of a previous history of gonorrhoea either in males or females even after ten or more separate infections.

There was also no correlation between the antibody concentration, patient age, the presence or absence of symptoms, syphilis or other sexually transmitted diseases. The meningococcal carriage in these groups was not known.

#### IGG ANTIBODY AGAINST DIFFERENT STRAINS

The 10 strains of *N gonorrhoeae* with different

**Table 2** % positives at different chosen antibody concentrations

Arbitrary normal upper limit ( $E_{cr}$ )	% Positive			
	Males		Females	
	Patients	Controls	Patients	Controls
5	73	29	76	32
10	57	20	64	17
15	45	12	56	11
20	35	6	44	6
25	30	2	37	2

serotypes were tested against four groups of 25 sera. The geometric mean antibody level of each group varied greatly between strains (Table 3).

The differential between patients and controls was generally greater in males than females, the controls being higher in the female group. The possibility that these variations were due to differences in the sticking ability of individual strains was considered. However, radiolabelling studies showed that percentage sticking was not related to the ELISA results obtained (Table 3).

**Table 3** IgG antibodies against *N gonorrhoeae*. (Each strain has an outer membrane protein of different serotype)

Serotype strain (all colony type 4)	ELISA ( $E_{40s}$ )* Geometric mean		ELISA ( $E_{40s}$ ) Geometric mean		% sticking
	Males		Females		
	Patients	Controls	Patients	Controls	
A1	15.3	2.3	18.5	4.6	2.6
B2	6.8	2.9	9.3	3.8	4.5
C3	33.7	1.2	31.9	4.9	3.3
D4	25.2	8.3	28.9	19.9	6.4
G7	21.8	3.7	24.1	8.1	4.1
H8	11.7	1.6	7.8	6.8	4.6
N10	12.6	3.8	10.9	11.1	3.1
S12	22.6	2.9	12.3	7.9	2.6
T13	14.8	1.6	13.8	7.3	3.3
V15	9.3	1.2	6.6	4.3	5.0
†SE (range)	0.3-0.9	0.3-2.1	0.4-1.0	0.4-2.0	0.4-2.2

\*Results given in crude uncorrected extinction units.  
†Individual standard errors are not given to simplify the table.

#### IGG ANTIBODIES AGAINST DIFFERENT COLONIAL TYPES

Antibody activity against the strains H1, and F62 was measured using whole cells of both colony types 1 and 4. There was significantly more antibody detected against type 1 than 4 (Table 4). This is probably due to the presence of pili on colony type 1, as the variations were not due to differences in sticking.

#### CROSS-REACTIONS

*Neisseria meningitidis* was also used as an antigen

**Table 4** Geometric mean IgG antibody in 25 patients and 25 controls tested against *N gonorrhoeae* of different colony types

Strain (colony type)	ELISA ( $E_{40s}$ )* Males		ELISA ( $E_{40s}$ ) Females		% sticking
	Patients	Controls	Patients	Controls	
	H1 T1	31.5	8.1	48.4	
H1 T4	15.8	7.3	28.6	12.7	1.75
F62 T1	18.1	8.6	14.3	9.1	2.92
F62 T4	9.8	7.8	7.7	7.1	1.87
†SE (range)	0.3-0.8	0.5-0.7	0.7-0.9	0.4-0.8	0.1-1.2

\*Results given in crude uncorrected extinction units.  
†Individual standard errors are not given to simplify the table.

and detected IgG antibodies in patients with proven gonorrhoea (Table 5). By means of a whole cell technique, cross-reactions between species of the same genus are not unlikely.

**Table 5** Geometric mean IgG antibody levels in 25 patients with gonorrhoea and 25 controls tested against serogroups of *N meningitidis*

Strain	ELISA ( $E_{40s}$ )* Males		ELISA ( $E_{40s}$ ) Females		% sticking
	Patients	Controls	Patients	Controls	
	<i>N meningitidis</i>				
(A)	14.7	4.5	14.0	7.4	2.8
(B)	13.3	1.1	11.2	4.2	2.0
(C)	20.6	2.6	7.2	5.2	2.8
†SE (range)	0.4-0.6	0.5-1.2	0.5-0.8	0.5-0.8	0.5-1.5

\*Results given in crude uncorrected extinction units.  
†Individual standard errors are not given to simplify the table.

#### Discussion

Initial studies showed it is possible with a single strain of *N gonorrhoeae* to differentiate between infected and non-infected groups. Using a cut-off level of 15 corrected extinction units ( $E_{cr}$ ), approximately 50% of patients with acute gonorrhoea could be diagnosed and this is comparable to studies with purified antigens.<sup>4,7</sup>

The aim of the present work was to determine if any particular serotype would differentiate between groups more efficiently. Of the 10 serotypes studied, it would appear for our geographical area, that strain C3 may be particularly useful. It was not possible for us to correlate the serotype of strain C3 with that of our infecting strains, as their outer membrane protein serotype was not known.

It is interesting that these strains with different serotypes which came from America gave very low antibody levels with our normal male controls.

The whole cell technique has the advantage of

using an antigen which was easy and quick to prepare. The trays can be prepared in bulk and stored in moist conditions for at least three months without deterioration. The test could be used with two or more strains to increase the predictive value,<sup>8</sup> in a similar way that two different antigens are used in the serodiagnosis of syphilis. The possible application of this technique to other bacteria is being investigated. Preliminary results have shown that antibody can be detected to  $\beta$ -haemolytic streptococci (group B) and we have detected IgG antibodies in a patient known to be a carrier.

Salmonella and *Escherichia coli* attached to poly-L-lysine coated trays have been used in our department to detect both human and mouse antibodies. So too has *Staphylococcus aureus* though it is important to use a protein A-deficient strain—for example, Wood 46.

Clinical bacteriology has exploited new quantitative and sensitive techniques such as ELISA much less than some other disciplines such as virology and parasitology.<sup>8</sup> Most bacteria have a negative surface charge and the ability to attach them readily to polystyrene trays via poly-L-lysine should encourage further development.

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