Comparison of antisera in the fluorescent antibody test for detection of *Bacteroides* spp in clinical specimens

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SUMMARY Twenty-two known strains of the *Bacteroides fragilis* group of organisms and 67 clinical specimens from a variety of sites were examined by fluorescent antibody test (IFA) using two different antisera for the rapid detection of *B fragilis* group of organisms.

A previously reported Barts' pooled antisera was compared with a commercially produced Fluoretec kit antisera and the findings were related to routine anaerobic culture and gas liquid chromatography for short chain fatty acids.

The Barts' antisera was more sensitive (88 %) but less specific (88 %) than the kit (sensitivity 50 %, specificity 98 %). This indicates that Barts' antisera picks up more positive cultures than the kit. The predictive value of a positive test was 82 % for Barts' antisera and 93 % for the kit. There were higher numbers of false-negatives with the kit (13/26) than with the Barts' (3/26). The predictive value of a negative test was 92 % for Barts' antisera and 75 % for the kit, indicating that a negative IFA test with Barts' antisera is a reliable index of the absence of the *B fragilis* group of organisms from clinical specimens. The implications for the use of this test in a routine laboratory are discussed.

With improved laboratory techniques anaerobic bacteria have been increasingly shown to be of great importance in a wide range of infections.¹⁻³ The *Bacteroides fragilis* group of organisms is the commonest anaerobic species isolated from purulent material.^{4,5} Treatment of such infections is often effective if instituted early but cultural techniques are slow and may not give a clinically useful result in time. A rapid and reliable method of diagnosis which can be incorporated into the routine procedures of most clinical bacteriology laboratories is required. Immunofluorescence may provide such a method using equipment readily available to many laboratories.⁶⁻⁸ The use of two antisera for the detection of *B fragilis* infections by the indirect fluorescent antibody (IFA) test has been reported by this laboratory at St Bartholomew's Hospital.⁹ Recently a commercial IFA test kit (Fluoretec-F) has been marketed and evaluated in the USA¹⁰ and Belgium.¹¹ We have tested the kit in parallel with our pooled Barts' antisera previously studied in this laboratory.⁹

Material and methods

BACTERIAL STRAINS

Twenty-two known *Bacteroides* strains belonging to the *B fragilis* group were used in this study.

These included:

- 13 strains of *B fragilis* (5 NCTC strains and 8 clinical isolates)
- 5 strains of *B distasonis* (ATCC 8503 and 4 faecal isolates)
- 2 strains of *B vulgatus* (NCTC 10583 and 11154)
- 1 strain of *B thetaiotaomicron* (NCTC 10582)
- 1 strain of *B ovatus* (ATCC 8483)

A heavy inoculum of each strain was taken from an overnight growth on DST agar (Oxoid), added to 10 ml peptone-yeast-glucose (PYG) broth and incubated anaerobically at 37°C for 24 h. This culture was centrifuged and washed three times in phosphate-buffered saline (PBS) pH 7.2.

CLINICAL SPECIMENS

Sixty-seven clinical specimens were examined. These were collected from patients in St Bartholomew's Hospital and St Thomas' Hospital, London, in whom

an anaerobic component of infection was suspected (see Table 2).

All specimens were cultured aerobically (horse blood agar and MacConkey) and anaerobically (neomycin 70 mg/l, blood agar or nalidixic acid 10 mg/l blood agar, both supplemented with menadione and haemin). Anaerobic culture was carried out in a glove box with an anaerobic atmosphere (nitrogen 80%, hydrogen 10% and carbon dioxide 10%). Identification of organisms was carried out according to methods in the VPI manual.¹² Bacterial culture and immunofluorescence were carried out by separate workers and results correlated at the end of the study. Clinical specimens were also analysed by gas-liquid chromatography (GLC) for the presence of short chain fatty acids.

IMMUNOFLUORESCENCE

Fluoretec-F kit (Pfizer Inc, New York) was used according to the manufacturer's instructions. A light smear of the washed organisms or purulent specimens was made with a swab on a clean slide over an inscribed circle of 1 cm diameter. After drying in air the smear was fixed by very gentle heating and treated with rhodamine-conjugated globulin prestrain for 15 min at room temperature in a moist chamber. The prestrain was removed by blotting and replaced with anti-*B fragilis* group polyvalent conjugate. After incubation for 30 min at room temperature this was removed by washing in distilled water. The slides were allowed to dry and mounted under a cover slip.

Smears were also made on a Teflon-coated slide bearing 6 mm diameter wells (C A Hendley (Essex) Ltd). These smears were also fixed by gentle heating and treated with Barts' pooled preparation of whole-cell antisera¹³ of different *B fragilis* somatic serotypes in a dilution of 1/32, as previously described.⁹ The smears were incubated at room temperature in a moist chamber for 30 min and washed in PBS for 10 min. Goat antirabbit immunoglobulin conjugated with fluorescein isothiocyanate (Behringwerke AG, Marburg) diluted 1/10 in PBS was then added to each well and incubated for a further 30 min at room temperature. After thorough washing in PBS for 10 min and drying, the slides were mounted with buffered glycerol pH 8.5 under a coverslip (22 × 56 mm).

MICROSCOPY

Both sets of slides were examined for fluorescent organisms using a Leitz Ortholux II incident light fluorescent microscope illuminated by a 50 W ultra-high pressure mercury vapour lamp with BG38 red suppression filter and KP500 selective excitation filter. All specimens were viewed with × 40 dry

objective, and the IFA kit slides were also read using × 100 oil immersion objective as recommended by the manufacturers.

Statistical analysis were determined by the method of Vecchio.¹⁴

Sensitivity = culture-positive specimens with positive IFA/all culture positive specimens tested.

Specificity = culture-negative specimens with negative IFA/all culture negative specimens tested.

The predictive values of a positive and a negative test were also determined by the above method.¹⁴

Results

The results of the study of 22 known stock strains are shown in Table 1. The kit and the Barts' pooled whole-cell antiserum show broadly comparable results. The kit failed to detect the two strains of *B vulgatus* while the Barts' antiserum missed one of these and one strain of *B distasonis*.

Table 1 Results of immunofluorescence on 22 known strains of the *B fragilis* group

Species	No strains tested	No of stains IFA-positive	
		Barts' pooled whole-cell antiserum	Fluoretec-F kit
<i>B fragilis</i>	13	13	13
<i>B thetaiotaomicron</i>	1	1	1
<i>B vulgatus</i>	2	1	0
<i>B ovatus</i>	1	1	1
<i>B distasonis</i>	5	4	5
Total	22	20	20

Of the 67 clinical specimens, 35 were negative on IFA and culture for *B fragilis* group of organisms. Twenty-six yielded *B fragilis* or *Bacteroides* spp on culture (Table 2). The latter group includes the five members of the *B fragilis* group of organisms but excludes the *B melaninogenicus* group, *B oralis* and *B ureolyticus*.

The Barts' antiserum had the greater sensitivity (88%) compared with the kit (50%) suggesting that the Barts' antisera picks up more positives than the kit (Table 3).

The kit had higher specificity (98%) compared with the Barts' antisera (88%), but the kit gave 13 false-negative results and one false-positive, whereas the Barts' antisera failed to detect three culture-positive specimens but gave false-positive results with five specimens.

The predictive value positive (PV-pos) that is, the likelihood that a subject with a positive IFA test will actually have infection with the *B fragilis* group, was 82% and 93% for Barts' and kit antisera

Table 2 Results of immunofluorescent assay (IFA), gas-liquid chromatography (GLC) and bacteriological culture on 32 clinical specimens

Specimen		GLC		IFA using:		Organisms isolated	
No	Source			Barts' antisera	Kit antisera	<i>B fragilis</i> group	Other organisms
1	Paracolic abscess	+	+		+	<i>B fragilis</i>	—
2	Abdominal wound	+	+		+	<i>B vulgatus</i>	<i>B asaccharolyticus</i>
3	Appendix abscess	ND	+		+	<i>B thetaiotaomicron</i>	<i>C ramosum</i> <i>Bacteroides</i> (not <i>fragilis</i> group)
4	Groin abscess	—	+		+	<i>B fragilis</i>	—
5	Diverticular abscess	+	+		+	<i>B vulgatus</i>	<i>Ps anaerobius</i> <i>Peptococcus</i> <i>E alactolyticum</i> <i>C ramosum</i> <i>B asaccharolyticus</i> <i>Ps anaerobius</i> <i>B eggerthii</i> <i>Peptococcus</i>
6	Loin abscess	+	+		+	<i>B fragilis</i> <i>B thetaiotaomicron</i>	—
7	High vaginal swab	ND	+		+	<i>Bacteroides</i> sp	—
8	Wound swab	ND	+		+	<i>Bacteroides</i> sp	—
9	Maxillary sinus	+	+		+	<i>Bacteroides</i> sp	<i>Peptococcus</i> <i>Staph epidermidis</i> <i>Pneumococcus</i> <i>Proteus</i> <i>Enterococcus</i> <i>B asaccharolyticus</i> <i>B ureolyticus</i> <i>Ps anaerobius</i> <i>P prevotii</i> <i>C clostridiiforme</i> <i>Veillonella</i> <i>Peptostreptococcus</i> <i>Propionobacterium</i> <i>B asaccharolyticus</i> <i>F varium</i> <i>Strep milleri</i> <i>Ps anaerobius</i> <i>Peptococcus</i> <i>Enterococcus</i>
10	Sacral abscess	+	+		+	<i>B fragilis</i>	<i>Microaerophilic strep</i> <i>Coliform</i>
11	Axillary abscess	+	+		+	<i>B fragilis</i>	<i>Peptococcus</i> <i>Peptococcus</i> <i>Microaerophilic strep</i> <i>Peptostreptococcus</i> <i>Corynebacterium</i> <i>B ruminicola ss brevis</i> <i>Veillonella</i> <i>P prevotii</i> <i>B asaccharolyticus</i> <i>P acnes</i> <i>Peptostreptococcus</i> <i>F necrophorum</i> <i>Bacteroides</i> (not <i>fragilis</i> group) <i>Coliform</i>
12	Peritoneal fluid (acute appendix)	+	+		+	<i>B fragilis</i> <i>B ovatus</i>	—
13	Pelvic abscess	+	+		—	<i>B fragilis</i>	—
14	Groin wound pus	+	+		—	<i>Bacteroides</i> sp	—
15	Wound pus	ND	+		—	<i>B fragilis</i>	—
16	Frontal lobe abscess	+	—		—	<i>Bacteroides</i> sp	—
17	Frontal sinus	+	+		—	<i>Bacteroides</i> sp	—
18	Buttock abscess	+	+		—	<i>Bacteroides</i> sp	—
19	Ascitic fluid	+	+		—	<i>B fragilis</i> <i>B ovatus</i>	—
20	Perianal abscess	ND	+		—	<i>B ovatus</i>	—
21	Perianal abscess	+	+		—	<i>B fragilis</i>	—
22	Abdominal wound	ND	+		—	<i>B fragilis</i>	—
23	Anal fistula	ND	+		—	<i>Bacteroides</i> sp	<i>Peptostreptococcus</i>
24	Peritoneal fluid	ND	+		—	—	—
25	Pus Ca stomach	+	+		—	—	<i>Peptostreptococcus</i> <i>Ps anaerobius</i> <i>F nucleatum</i> <i>Staph aureus</i> <i>B ureolyticus</i> <i>B asaccharolyticus</i> <i>B oralis</i> <i>P prevotii</i> <i>B asaccharolyticus</i> <i>Coliforms</i> × 3
26	Axillary abscess	ND	+		—	—	<i>P prevotii</i> (2 types)
27	Axillary abscess	ND	+		—	—	<i>Microaerophilic strep</i> <i>Coliform</i> <i>Coliforms</i> × 2, <i>Proteus</i>
28	Faecal fistula	ND	+		—	—	—
29	Abdominal wall abscess	—	—		+	<i>B thetaiotaomicron</i>	—
30	Vulval abscess	ND	—		—	—	—
31	Abdominal wound	ND	—		—	<i>B fragilis</i>	—
32	Anal fistula	ND	—		—	<i>Bacteroides</i> sp	—

+ positive. — negative. ND = not done.

Table 3 Analysis of results of culture and IFA on 67 clinical specimens

Antiserum	No of specimens	IFA	Culture		Sensitivity %	Specificity %
			Positive	Negative		
Barts'	67	Positive	23	5	88	88
		Negative	3	36		
Kit	67	Positive	13	1	50	98
		Negative	13	40		

respectively. The predictive value of a negative IFA test (PV-neg) was 92% for Barts' antisera and 75% for the kit indicating that a negative IFA test with the Barts' antisera is a strong indicator of the absence of the *B fragilis* group of organisms from the specimen.

The results of the GLC on 18 clinical specimens were correlated with IFA and culture results (Table 2). Eight specimens were positive by all three tests and culture, a further eight specimens were positive by GLC and culture and gave positive IFA with Barts' antisera, but gave negative results with the kit IFA test.

Discussion

If this technique is to be useful in a routine clinical bacteriology laboratory it is essential that it gives a reliable result within a few hours. The single stage staining procedure employed by the kit IFA significantly simplifies and shortens the test. However, this may be in part responsible for the low sensitivity (50%) found in this study. The use of the rhodamine-conjugated globulin prestain is intended to eliminate the theoretical problem of non-specific staining by staphylococci, streptococci etc which can bind the Fc segment of immunoglobulins.^{15 16} However this has not proved to be a problem in our experience; non-specific fluorescence differs in character and conformation.

The slides provided in the kit were found to be difficult to use. The inscribed circle within which the film is made disappeared from view after the mounting fluid was added, hindering the location of the specimen under the microscope. The use of Teflon-coated slides made the position of the film obvious and facilitated focusing on the specimen plane. The Teflon coating also prevented the reagents spreading off the film during incubation. The use of $\times 100$ oil immersion objective did not improve the sensitivity in this series but made reading of the slides more time-consuming.

The sensitivities were found to be less than in previous studies on the same antisera.⁹⁻¹¹ The reasons for this are not obvious. The interpretation of fluorescence intensity is subjective but all readings were made by the same observer throughout the

study and in the majority of cases this was checked independently by at least one other. Many of the isolates were identified only to the genus level. The low proportion of *B fragilis* or *B fragilis* group of isolates reported as *Bacteroides* sp may account for some of the false-negatives by IFA test. Observer error, sampling error in preparation of the film, and low number of organisms in the original specimen are other possible causes. The *B fragilis* group of organisms possesses a multiplicity of antigens as demonstrated by the presence of various O-serotypes.¹⁷⁻¹⁹ It is possible therefore that the presence of false-negative results is related to the lack of antigen used in raising the antisera. The Barts' antiserum, however, appears to cover more strains isolated from the two hospitals studied.

Positive reactions by immunofluorescence unsupported by a positive culture may be due to lack of viable organisms following antibiotic therapy or specimen transport difficulties. Isolates may go unrecognised on culture due to overgrowth by other organisms or failure to pick out all morphologically dissimilar colonies. Again, sampling errors may occur during specimen taking and inoculation of media. Cross-reactions with unrelated species would also give false-positive immunofluorescence, but no bacterial species was constantly grown from these specimens which was not also isolated from IFA-negative material. There were no cross-reactions with aerobic Gram-negative organisms isolated from these specimens.

Better correlation occurred with the results of GLC and Barts' IFA test than with the kit. Sixteen out of 18 specimens were positive by GLC and Barts' IFA test and on culture, whereas only eight of those were positive with the kit. GLC, although a useful adjunct in the rapid diagnosis of anaerobic infections, is only adaptable to specimens containing pus or fluid; also in mixed cultures, differentiation of species is not always possible. IFA can be applied to swabs as well as pus specimens and offers a sensitive and rapid test in less than two hours.

Rapid methods of bacterial detection are as yet no substitute for cultural methods. The use of immunofluorescence holds much promise but the choice of antiserum is of the utmost importance; geographical variations in prevalence of serotypes may exist

between hospitals in the UK¹⁹ and it is possible that international variation may be even greater. Therefore, a suitable combination of antisera must be selected to be of such potency and have such cross-reactions as to cover adequately the range of antigenic determinants found in the local strains of *Bacteroides*.

In this study, the kit antisera was found to be more specific and had a higher PV-pos (93%) than the Barts' antisera (PV-pos 82%), but this increased specificity was due to the presence of greater numbers of false-negative results. Hence, although it is much more likely to culture *B. fragilis* group of organisms from a specimen if the kit IFA is positive, it is also much more likely to miss a positive culture when the kit IFA is negative. On the other hand, the Barts' IFA test has a greater sensitivity, and because of the excellent predictive value of a negative test with the Barts' IFA test (PV-neg 92%) compared with the kit IFA (PV-neg 75%), it makes a negative result with the Barts' IFA test a good index of a negative culture. These findings, therefore, suggest that the Barts' IFA test is a reliable test to include in a routine clinical laboratory. A rational antimicrobial approach could be based on these results.

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