The Fluoretec system for rapid diagnosis of bacteroides infections by direct immunofluorescence of clinical specimens

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SUMMARY Fluoretec, a commercial kit for the rapid diagnosis by immunofluorescence of infections caused by *Bacteroides* spp was compared with a standard culture method. A total of 1010 specimens were tested for the presence of *B fragilis* group by Fluoretec F and *B melaninogenicus-oralis* and asaccharolytic groups by Fluoretec M. Fluoretec F was positive in 123/152 specimens culturing *B fragilis* group strains. Seventeen specimens were positive by Fluoretec F but negative on culture. Fluoretec M was positive in 21 of 22 specimens from which *B melaninogenicus* was cultured. The Fluoretec system was convenient in use, results being obtained within one hour of receipt of the specimen.

The use of immunofluorescence for the identification of *Bacteroides* spp was first described by Griffin¹ and further developed by Lombard and Dowell² and by Stauffer *et al.*³ More recently a commercial kit, Fluoretec, has been produced (Pfizer Diagnostics).

Bacteria of the *Bacteroides* spp are important anaerobic pathogens in many clinical conditions.⁴ *Bacteroides fragilis* is predominant, accounting for more than 50% of bowel-associated anaerobic infections⁵ and 70% of *Bacteroides* spp isolated from the blood stream.⁶ *Bacteroides* spp of the *melaninogenicus-oralis* group are associated with infections of the female genital tract and mouth.⁵ The cultural requirements of anaerobes are more stringent and time-consuming than those of many organisms, causing an inevitable delay in the results. Early detection of an anaerobic infection ensures the rapid institution of effective chemotherapy.

Fluoretec (Pfizer Diagnostics) is a commercial kit containing polyvalent rabbit antisera to the *B fragilis* group (Fluoretec F) and to the *B melaninogenicus-oralis* group and asaccharolytic groups (Fluoretec M). These antisera are conjugated with fluorescein isothiocyanate to permit direct immunofluorescence of clinical specimens. Fluoretec F contains antibodies to *B fragilis*, *B vulgatus*, *B distasonis*, *B ovatus*, and *B thetaiotaomicron*. Fluoretec M contains antibodies to *B melaninogenicus*, *B intermedius* and *B asaccharolyticus*. For brevity these will subsequently be

referred to as *B fragilis* group and *B melaninogenicus* group.

The kit contains formalin-fixed positive controls of the *B fragilis* group and the *B melaninogenicus* group. It also includes a Rhodamine-B conjugated human and rabbit gamma globulin (Prestain). This is designed to react with organisms containing protein A, principally *Staphylococcus aureus*, thus blocking the non-specific reaction of protein A with the Fc portion of the fluorescein conjugated anti-bacteroides antisera. Glass microscope slides with two ground glass circles are provided in the package.

In this study the reliability and ease of use of Fluoretec were evaluated. Results obtained by immunofluorescence were compared with the results of standard laboratory cultures.

Material and methods

SPECIMENS

A total of 1010 clinical specimens, all received in the routine diagnostic laboratory were tested for the presence of *Bacteroides* spp. Specimens were selected from the following clinical conditions:

Abdominal and genitourinary surgery, lung abscesses, cerebral abscesses, chronic soft tissue infections, otolaryngological and dental sepsis.

Each specimen was tested immediately by the Fluoretec method and was also examined by conventional culture techniques as described below.

FLUORETEC

Upon receipt in the laboratory, thin smears of each specimen were made in the wells of a double well glass slide. Duplicate smears were prepared and stored. The smears were dried in air and then gently heat-fixed. A drop of Prestain was applied to each well and the slides were incubated for 15 min at room temperature in a moist chamber. The Prestain was removed by gentle blotting with blotting paper. A 20 μ l volume of the *B fragilis* group conjugate was applied to the first well and 20 μ l of *B melaninogenicus* group conjugate was placed in the second well. The slide was incubated for a further 15 min at room temperature. After rinsing in distilled water for 15-20 s, the films were air dried. A large cover slip was placed over the smears and mounted in 90% buffered glycerol. The slides were examined at a magnification of $\times 63$ with a Leitz Dialux fluorescence microscope. Incident-light fluorescence with a 50 W ultra-high pressure mercury lamp and FITC interference filter was used. Results were recorded on a scale of 0, +, ++, and +++ where +++ represented numerous fluorescing bacteria and + represented scanty fluorescence. The fluorescence should be a bright apple-green outlining the organism. *B fragilis* group is bacillary, *B melaninogenicus* group is coccoid.

BACTERIOLOGY

A smear of the specimen was prepared and stained by Gram's method. The number and nature of organisms present were noted. The specimens were plated on to two blood agar plates, 2% (μ g/ml) neomycin blood agar, McConkey's agar and inoculated into a cooked meat medium. In addition each specimen was plated on to prereduced Schaedler's medium (Oxoid) and Schaedler's medium with 2% (μ g/ml) neomycin. One blood agar, the neomycin blood agar and the two Schaedler's plates were placed in an anaerobic jar (Whitley 10 plate anaerobic jar) fitted with a 4 g cold catalyst sachet. The jar was evacuated to 760 mm Hg and filled with a gas mixture of 10% hydrogen, 10% carbon dioxide and 80% nitrogen. A test for a secondary vacuum of 20 mm Hg was performed after 10 min.

Incubation of aerobic cultures was for 48 h. Anaerobic cultures were inspected after 48 h incubation. The cooked meat medium was subcultured after 48 h incubation on to 2 blood agar plates, one of which was incubated aerobically, and the other was incubated anaerobically for a further 48 h. Anaerobic Gram-negative bacilli were assigned to the genus *Bacteroides* by their antibiotic disc resistance patterns⁷ using the Mastring identification test (Mast Laboratories, Liverpool).

Bacteroides strains were identified to the species

level using API 20-A strips (API Laboratory Products, Farnborough, Hants).

If a specimen was positive by fluorescence but no *Bacteroides* spp were isolated, all organisms that were isolated were tested for false-positive fluorescence. Conversely if a *Bacteroides* sp was isolated but no fluorescence had been noted in the original specimen, the duplicate smear was stained and examined.

ANALYSIS OF RESULTS

Results for the two reagents, Fluoretec F and Fluoretec M, were analysed separately. Only those species of bacteroides included in the Fluoretec reagents have been included in the analysis. Sensitivity and specificity of the Fluoretec method was measured by comparison with the completed cultural results. Thus culture was deemed to give the "correct" result. No allowance was made for possible defects in the cultural system.

Results

A total of 1010 clinical specimens were examined between December 1977 and August 1979, 755 specimens were received as dry swabs, 253 specimens were pus and the nature of two specimens was not recorded. The anatomical sites from which the specimens were collected are shown in Table 1.

Fluoretec F

Fluoretec F was positive in 123 of the 152 specimens in which *B fragilis* group strains were detected by culture (Table 2). Cultural isolates from specimens recorded as negative by Fluoretec included 21 strains of *B fragilis*, 6 strains of *B vulgatus* and 2

Table 1 Anatomical sources of 1010 clinical specimens

Source of specimen	No
Gastrointestinal tract	730
Genitourinary tract	188
Thorax	34
Otolaryngology and dental	35
Cerebral abscess	2
Soft tissue infections	21

Table 2 Direct immunofluorescence using Fluoretec F (*B fragilis* group)

Fluorescence	<i>B fragilis</i> group culture	
	Positive	Negative
Positive	123	17
Negative	29	841

Table 3 Species of *Bacteroides fragilis* group obtained from 1010 clinical specimens

Species	Fluoretec F	
	No of specimens fluorescence +ve	No of specimens culture +ve
<i>B fragilis</i>	91	112
<i>B vulgatus</i>	20	26
<i>B distasonis</i>	4	6
<i>B ovatus</i>	1	1
<i>B thetaiotaomicron</i>	7	7
	123	152

One strain of *B fragilis* and one strain of *B vulgatus* were non-fluorescent.

Table 4 Direct immunofluorescence using Fluoretec M (*B melaninogenicus* group)

Fluorescence	<i>B melaninogenicus</i> group culture	
	Positive	Negative
Positive	21	21
Negative	1	967

Table 5 Species of *Bacteroides melaninogenicus* group obtained from 1010 clinical specimens

Species	Fluoretec M	
	No of specimens fluorescence +ve	No of specimens culture +ve
<i>B melaninogenicus</i>	13	14
<i>B intermedius</i>	1	1
<i>B oralis</i>	1	1
<i>B asaccharolyticus</i>	6	6
	21	22

strains of *B distasonis* (Table 3). All but two of these strains (one *B fragilis* and one *B vulgatus*) gave positive results when tested with Fluoretec F.

There were 17 specimens positive by Fluoretec F but negative on culture. All isolates from these specimens were tested for non-specific fluorescence. Fluorescence was observed in organisms isolated from eight of the 17 specimens (5 *Staph aureus*, 3 *Escherichia coli* and 1 *Streptococcus* sp).

Thus the sensitivity and specificity of Fluoretec F as judged by cultural results are 80.9% and 98% respectively.

Fluoretec M

Fluoretec M gave positive results in 21 of the 22 specimens from which organisms of the *B melaninogenicus* group were isolated (Tables 4 and 5). The isolate from the single specimen which was culture-positive but negative by Fluoretec M gave positive fluorescence on retesting with Fluoretec M. There were 21 specimens positive with Fluoretec M from

which no member of the *B melaninogenicus* group was isolated. *B fragilis* was isolated from four of these and a further eight gave growth of organisms which were shown to fluoresce non-specifically with Fluoretec M (3 *Staph aureus*, 1 *E coli* and 4 *Streptococcus* spp). As judged by comparison with the cultural results Fluoretec M had a sensitivity of 95.5% and a specificity of 97.9%.

Effect of repeating Fluoretec tests

When a *Bacteroides* sp was isolated, but no fluorescence had been observed in the original slide of the specimen the duplicate smear was stained and examined. In the case of repeat tests with Fluoretec F, nine extra specimens were found to be positive for *B fragilis*. Repeat tests with Fluoretec M produced no extra positives. It must be emphasised that in order to obviate any tendency toward possible observer bias these repeat results do not form part of the analysis described above.

Discussion

The current popularity of gas liquid chromatography (GLC) for the rapid detection of infection by anaerobes testifies to the usefulness of such immediate techniques. However GLC requires the use of expensive equipment maintained by skilled staff. By contrast Fluoretec makes use of a technique which is already in operation in most microbiology laboratories. In our hands it proved convenient, results being obtainable in under an hour.

The evaluation of a new laboratory test is often complicated by the absence of a reliable external standard against which to measure its accuracy. Ideally a clinical test should predict the presence of a specific disease pattern in the patient. However assessments related to clinical measurement are, of course, impracticable in the short term. For this reason the researcher has to make do with comparisons with existing laboratory methodology while accepting that the so called standard is subject to considerable variation.

In our study we have compared Fluoretec with an anaerobic culture system which we believe to be the best available to the average diagnostic laboratory. In 112 specimens where culture was positive for *Bacteroides fragilis* 21 were negative by Fluoretec. Since 20 of these isolates were positive by fluorescence it may be assumed that these specimens contained too few organisms to be detected by Fluoretec. In this context it is of interest to note that while 30% of pus samples were positive by Fluoretec only 15% of dry swabs gave this result.

Some care is necessary in the interpretation of the fluorescence microscopy. Positive organisms should

have a clear fluorescent outline and morphological similarity to the control organism. A number of organisms, notably *Staph aureus*, gave positive fluorescence despite the use of Rhodamine B conjugate and human gamma globulin (Prestain).

In a similar study Kaspar *et al.*⁸ described the use of indirect immunofluorescence to detect *Bacteroides* spp in clinical material. A *B fragilis* capsular antiserum was more sensitive than Fluoretec detecting all 12 *B fragilis* strains with a 90.3% specificity. *B fragilis* is the only species of the genus bacteroides to possess a polysaccharide capsule.⁹ A pooled whole organism antiserum to the *B fragilis* group was more sensitive (100%) than Fluoretec F (80.9%) but far less specific (64.3%) than Fluoretec F (98%). They did not look for organisms of the *B melaninogenicus* group.

Despite its obvious limitations we feel that the present study provides evidence of the effectiveness of the Fluoretec system. The concept of immediate detection and speciation of bacterial pathogens is an attractive one but the specific immunofluorescence technique, useful though it is in virology, has so far had very limited success in bacteriology. Fluoretec may well represent an advance in this field. Sensitivity of the test is good by bacteriological standards, particularly in view of the fact that no preincubation is needed. The numbers of false-positives are acceptably low. The reagents cover the species of bacteroides of greatest clinical interest⁵ with the exception of *B corrodens*. Fluoretec is therefore a useful addition to the tests available for the detection of infections caused by *Bacteroides* spp particularly for laboratories not possessing equipment for gas-liquid chromatography.

Fluoretec kits are available from Laboratory Impex Limited, Lion Road, Twickenham, Middlesex.

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