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# Indirect Fluorescent Antibody Procedure for the Rapid Detection and Identification of *Bacteroides* and *Fusobacterium* in Clinical Specimens

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An indirect fluorescent antibody (IFA) technique was evaluated as a procedure for rapid detection and identification of members of the *Bacteroidaceae*. Antisera were prepared against 31 members of this family, including species of *Bacteroides* and *Fusobacterium* commonly isolated from human infections. The antisera had demonstrated species and/or subspecies specificity. Thirty clinical specimens were studied. Of 13 specimens yielding *Bacteroidaceae*, for which antisera were available, 12 were presumptively diagnosed by IFA to contain subspecies of *B. fragilis* and/or *Fusobacterium* species. Of 17 specimens yielding negative culture results, two were positive by IFA on direct smear. Frequently the in vivo morphology of cells detected in direct smears by this procedure closely mimicked that of cellular debris, tissue cells, and leukocytes. Polyvalent antisera pools facilitated use of the IFA procedure as a practical tool for rapid diagnosis of infections involving the *Bacteroidaceae*.

As laboratories adopt the use of more adequate methods for culturing specimens, the significance of the *Bacteroidaceae* as major factors in spontaneous and iatrogenic infections is being realized (17, 18). In our own experience over the past decade, 49% of all specimens cultured yielded anaerobes. *Bacteroidaceae* were present in approximately 57% of the anaerobe-positive specimens. These anaerobes are frequently pleomorphic in direct smears of clinical material as well as in primary culture (6, 22, 23, 24). Pleomorphism accentuated by antibiotic therapy (5) may result in the presence of organisms similar to the leukocyte and tissue cell debris found in most purulent lesions. This may complicate or delay preliminary laboratory reports based on stained direct smears of clinical material. Because of the time required to cultivate and identify anaerobic organisms, laboratory results are frequently obtained too late to afford specific help to the clinician or patient and the data become of academic interest only. The need for more rapid methods of detection and identification of anaerobes is especially apparent in the rapidly fulminating and sometimes fatal infections with which the *Bacteroidaceae* may be associated.

Direct fluorescent antibody procedures for identification of members of the *Bacteroidaceae* have been reported (4, 9, 10). These papers suggest that fluorescent antibody methods may be a feasible approach to rapid clinical diagnosis. The present report describes an indirect fluorescent antibody (IFA) procedure for the detection and identification of these organisms in direct smears of clinical material, regardless of their in vivo morphology.

## MATERIALS AND METHODS

**Antigens and antisera.** Identification of organisms used throughout this study was based upon gas-liquid chromatography and biochemical reactions recommended by the Virginia Polytechnic Institute and the Center for Disease Control (3, 11). Bacterial strains used for preparation of antisera are listed in Table 1. Included are the following strains received from the American Type Culture Collection: *Fusobacterium polymorphum* (*F. nucleatum*) 10953; *Sphaerophorus freundii* (*F. mortiferum*) 9817; *S. varius* (*F. varium*) 8501; *Bacteroides symbiosis* (*F. symbiosum*) 12829; and *B. ovatus* (*B. fragilis* subsp. *ovatus*) 8483. Antigens were prepared from 2- to 5-day agar-free thioglycolate broth cultures. Cells were harvested by centrifugation, washed twice, and resuspended in 0.01 M phosphate-buffered saline, pH 7.2, containing 0.4% formaldehyde, to a density equivalent to a McFarland nephelometer tube no. 9 (16). Antisera were prepared in Dutch Belted and New Zealand White rabbits by giving six intravenous injections of antigen of increasing doses from 0.1 to 1.0 ml at 3- to 5-day intervals. Blood was obtained by cardiac puncture 3 days after the last injection of antigen.

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TABLE 1. *Bacterial strains used for preparation of antisera*

| Antiserum | No. of strains |
|---|---|
| *Bacteroides* | |
| *B. fragilis* subsp. *fragilis* | 7 |
| *B. fragilis* subsp. *thetaiotaomicron* | 7 |
| *B. fragilis* subsp. *vulgatus* | 4 |
| *B. fragilis* subsp. *distasonis* | 1 |
| *B. fragilis* subsp. *ovatus* | 1 |
| *B. melaninogenicus* subsp. *asaccharolyticus* | 2 |
| *B. melaninogenicus* subsp. *intermedius* | 1 |
| *Fusobacterium* | |
| *F. nucleatum* | 3 |
| *F. mortiferum* | 2 |
| *F. varium* | 1 |
| *F. symbiosum* | 1 |
| *F. species* | 1 |

**Fluorescent antibody procedure.** Slides of bacterial suspensions or fresh clinical material were air dried, fixed in absolute methanol for at least 15 min, and reacted with normal sera, antiserum, or polyvalent antisera pools in a humid chamber. After 30 to 40 min at room temperature, these were washed in two exchanges of 0.01 M phosphate-buffered saline, pH 7.2 to 7.4, for 10 min each and blotted dry. Slides were then reacted with a 1:4 dilution of conjugated goat anti-rabbit globulin-globulin (Sylvana) for 30 to 40 min at room temperature, washed (as described above), blotted dry, and mounted with buffered glycerol (Difco no. 2329).

Before use, antisera were titrated to determine the highest dilution giving optimum (4+) fluorescence with homologous antigens. Titers ranged from 1:4 to 1:64 when this procedure was used. Throughout all tests, antisera were used at dilutions corresponding to their respective fluorescent titers. To reduce possible nonspecific fluorescence, antisera, normal sera, and fluorescein conjugate were adsorbed by the addition of 0.1 g of mouse tissue powder (Difco no. 2317) to 1.0 ml of serum or conjugate, followed by gentle mixing at 6 C for 90 min. Suspensions were clarified by centrifugation at 10,000 × *g* for 30 min at 4 C.

**Polyvalent antisera pools.** The polyvalent antisera pools listed in Table 2 were prepared by combining individual antisera, each in a final dilution corresponding to previously determined IFA titers.

**Microscopy.** A Leitz Wetzlar Ortholux microscope, with an Osram HBO 200-W mercury vapor lamp, was used. Filters included a heat-adsorbing BG 38 and a blue excitation filter BG12.

**Experimental infections.** Self-limiting subcutaneous lesions were produced in 16- to 20-g female ICR mice by injecting thioglycolate broth cultures of *F. mortiferum*, *F. varium*, *F. symbiosum*, *F. nucleatum*, or *B. fragilis* into the same site on each of 4 consecutive days. Pure-culture lesions were induced by using 0.5 ml of the appropriate broth culture listed in Table 4 per injection. Mixed-culture lesions, listed in Table 5, were induced by using 1.0 ml of the appropriate combination of broth cultures mixed in equal volumes per injection. Mice were sacrificed 24 to 48 h after the final injection. Similarly, pure-culture lesions were induced by using a single strain of *F. varium* and two strains of *F. mortiferum*. However, on days 4 and 5, these mice each received 15 U of penicillin G intravenously for the purpose of in vivo L-phase induction. These mice were sacrificed on day 6. All experimental lesions produced were dissected and portions were removed for culture and preparation of direct smears.

**Clinical specimens.** Thirty specimens from hospitalized patients included various types of abscesses, body fluids, and purulent exudates. Specimens were cultured immediately upon arrival in our laboratory, and direct smears were made for IFA staining. Media used for primary isolation of the anaerobic organisms present in these specimens included sheep blood agar, sheep blood agar with kanamycin (0.1 mg/ml) and/or menadione (0.5 μg/ml), rabbit blood agar, and phenethyl alcohol blood agar plates (BBL). In addition, specimens were inoculated into chopped meat and thioglycolate broths (BBL 135-C). Cultures were incubated at 37 C in GasPak anaerobic jars (BBL) for 5 to 7 days, followed by appropriate selection and identification of isolated colonies.

## RESULTS

**Specificity of antisera.** Antisera, when reacted with homologous and heterologous bacte-

TABLE 2. *Polyvalent antisera pools used for detection and identification of organisms in direct smears of clinical material*

| Antiserum pool | No. of strains |
|---|---|
| Polyvalent pool A: *B. fragilis* subsp. *fragilis* | 5 |
| Polyvalent pool B: *B. fragilis* subsp. *thetaiotaomicron* | 5 |
| Polyvalent pool C: | |
| *B. fragilis* subsp. *fragilis* | 2 |
| subsp. *thetaiotaomicron* | 2 |
| subsp. *vulgatus* | 2 |
| subsp. *distasonis* | 1 |
| subsp. *ovatus* | 1 |
| Polyvalent pool D: | |
| *B. melaninogenicus* subsp. *asaccharolyticus* | 2 |
| subsp. *intermedius* | 1 |
| Polyvalent pool E: | |
| *F. nucleatum* | 3 |
| *F. mortiferum* | 2 |
| *F. varium* | 1 |
| *F. symbiosum* | 1 |
| *F. species* | 1 |

rial cells, were found to be quite specific. When tested against 38 strains of *Bacteroides* and *Fusobacterium*, antisera demonstrated either strain specificity, specificity for groups within subspecies, or occasional specificity between strains representing different subspecies.

Fluorescence obtained with antisera prepared against three strains of *F. nucleatum*, two strains of *F. mortiferum*, and one strain each of *F. varium*, *F. symbiosum*, and *F. species* was strain specific. When testing these eight strains, antisera reacted only with homologous cells and not with heterologous cells of the same or different species. Although these antisera were strain specific, too few of these and other *Fusobacterium* species were tested for us to determine whether this was characteristic for any or all of the *Fusobacterium* species.

Antigens from 27 *B. fragilis* were studied by using antisera prepared against the 20 strains of *B. fragilis* listed in Table 1. When reacted with homologous and heterologous cells, group specificity and at times strain specificity were observed within the subspecies *fragilis*, *thetaiotaomicron*, and *vulgatus*. Although antisera generally demonstrated this degree of specificity, occasional reactions were obtained between strains representing different subspecies. Antisera prepared against three strains of the subspecies *fragilis* demonstrated weak (1+) fluorescence with other subspecies of *B. fragilis*. One of the three antisera reacted with a single strain of the subspecies *vulgatus*, one reacted with a single strain of the subspecies *thetaiotaomicron*, and one reacted with two strains of the subspecies *thetaiotaomicron*. Antisera prepared against one strain of the subspecies *thetaiotaomicron* reacted (2+) with the single strain of *B. fragilis* subsp. *ovatus* available. Antisera prepared against two strains of the subspecies *vulgatus* demonstrated fluorescence with other subspecies of *B. fragilis*. One of these antisera reacted (1+) with a single strain of the subspecies *fragilis* and one reacted (2+) with a single strain of the subspecies *thetaiotaomicron*. Antisera prepared against the single strain of *B. fragilis* subsp. *distasonis* demonstrated fluorescence (2+) with the single strain of the subspecies *ovatus*. Antisera for this strain of *B. fragilis* subsp. *ovatus* demonstrated fluorescence (1+) with a single member of the subspecies *thetaiotaomicron*.

Antisera prepared against two strains of *B. melaninogenicus* subsp. *asaccharolyticus* and one strain of the subspecies *intermedius* reacted strongly only with homologous cells and not with heterologous cells of the same or different subspecies. Studies involving *B. melaninogenicus* included only the three strains for which antisera had been prepared.

Antisera were tested against other gram-negative as well as gram-positive aerobes and anaerobes. Included were species of *Clostridium*, *Eubacterium*, *Peptostreptococcus*, *Propionibacterium*, *Proteus*, *Escherichia*, *Klebsiella*, *Citrobacter*, *Pseudomonas*, *Streptococcus*, and, *Staphylococcus*. Antisera demonstrated occasional fluorescence with strains of *Escherichia*, *Klebsiella*, *Streptococcus*, *Staphylococcus*, and *Propionibacterium*. In all but one case, this fluorescence was also observed when using pooled normal control sera. Adsorption of the involved antisera and normal sera with appropriate formalinized bacterial cells eliminated this fluorescence without a significant reduction of the specific titers.

**Experimental infections.** The IFA procedure was applicable for the detection and identification of *Bacteroidaceae* in direct smears of experimentally produced lesions of mice. For both pure- and mixed-culture lesions, significant fluorescence was observed only with those preparations incubated with antisera specific for the organisms used to induce the respective experimental abscesses (Tables 3 and 4). In addition, fluorescence was demonstrated with cells mimicking leukocyte or tissue cell in direct smears of lesions obtained from similarly infected mice after intravenous administration of penicillin. Again, fluorescence was observed only when smears were incubated with antisera specific for the organisms used to induce the respective experimental abscesses. In all instances, culture results yielded only those organisms originally injected. The injected organism was recovered from each of the pure-culture lesions and each of the lesions from mice receiving penicillin. They were also recovered from each of the mixed-culture lesions, with the exception of *Fusobacterium* species (mouse no. 1), *F. mortiferum* (mouse no. 2), and *F. symbiosum* (mouse no. 3).

**Clinical specimens.** A total of 30 specimens has been examined comparing direct-smear IFA results with culture results. Results of eight specimens reacted with each of the individual antisera are listed in Table 5. Seven of eight isolates identified as *B. fragilis*, a single isolate identified as *F. nucleatum*, and a single isolate identified as *Fusobacterium* species were detected in direct smears by IFA. *B. fragilis* subsp. *ovatus* antiserum reacted with organisms in direct smears of specimen number three and with the subspecies *thetaiotaomicron* isolated from this specimen. *B. fragilis* subsp. *thetaiotaomicron* antiserum reacted with orga-

TABLE 3. *Fluorescence[a] of bacterial cells in direct smears of experimentally induced pure-culture abscesses of mice, when reacted with homologous and heterologous antisera*

| Organism with which experimental abscesses were produced | Mouse no. | Antiserum | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | *F. mortiferum* (A) | *F mortiferum* (B) | *F. varium* | *F. symbiosum* | *F. nucleatum* | *Fusobacterium* sp. | *B. fragilis* |
| *F. mortiferum* (strain A) .. | 1 | 2+ | − | − | − | − | ± | − |
| *F. mortiferum* (strain B) .. | 2 | − | 3+ | − | − | − | ± | − |
| *F. varium* ..... | 3 | − | − | 2+ | − | − | − | − |
| *F. symbiosum* ..... | 4 | − | − | − | 2+ | − | ± | − |
| *F. nucleatum* ..... | 5 | − | − | − | − | 2+ | ± | − |
| *F. species* ..... | 6 | − | − | − | − | ± | 2+ | ± |
| *B. fragilis* ..... | 7 | − | − | − | − | − | − | 4+ |

[a] 2+ through 4+, distinct to intense fluorescence; ±, insignificant fluorescence; −, no fluorescence; normal serum controls were negative.

TABLE 4. *Fluorescence[a] of bacterial cells in direct smears of experimentally induced mixed-culture abscesses of mice, when reacted with homologous and heterologous antisera*

| Organism with which experimental abscesses were produced | Mouse no. | Antiserum | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | *F. necrophorum* | *F. varium* | *F. mortiferum* | *F. symbiosum* | *F. nucleatum* | *Fusobacterium* sp. | *B. fragilis* |
| *F. varium* ..... 1<br>*Fusobacterium* sp. | 1 | − | 2+ | − | − | − | 2+ | − |
| *F. mortiferum* ..... 2<br>*F. nucleatum* | 2 | − | − | 2+ | − | 2+ | − | − |
| *F. varium* ..... 3<br>*F. symbiosum*<br>*Fusobacterium* sp. | 3 | − | 2+ | − | 2+ | − | 2+ | − |
| *F. mortiferum* ..... 4<br>*F. symbiosum*<br>*F. nucleatum* | 4 | − | − | 2+ | − | 2+ | − | − |

[a] 2+ through 4+, distinct to intense fluorescence; ±, insignificant fluorescence; −, no fluorescence; normal serum controls were negative.

nisms in direct smears and with the *B. fragilis* NGF (no good subspecies fit) isolated from specimen number four. Otherwise, complete species and subspecies agreement between direct-smear IFA and culture results were obtained. Specimens six and seven were positive by IFA for *F. nucleatum*, although no growth was obtained when the specimens were cultured. Specimen eight was negative by IFA, although culture results yielded *B. fragilis* subsp. *fragilis*. An additional seven specimens yielded negative IFA results. No organisms, for which antisera were available, were cultured from these seven specimens.

Results of seven specimens reacted with each of the polyvalent antisera pools are listed in Table 6. Six of seven isolates identified as *B. fragilis* and a single isolate identified as *F. nucleatum* were detected in direct smears by IFA. Both pool A and pool B reacted with organisms in direct smears of specimen number three and with the single *B. fragilis* subsp. *fragilis* isolated; however, pool B demonstrated only weak (1+) fluorescence with this organism. Specimen number four was positive by IFA for *B. fragilis* subsp. *fragilis* and specimens five and six were positive by IFA for *Fusobacterium* species, although culture results did not demonstrate the presence of these organisms. Otherwise, complete species and subspecies agreement between direct-smear IFA and culture results were obtained by using

TABLE 5. *Comparison of culture results with direct-smear IFA results, utilizing specific antisera*

| Source | Culture results[a] | IFA results[b] |
|---|---|---|
| 1. Liver abscess | *B. fragilis* subsp. *fragilis*<br>*B. fragilis* subsp. *thetaiotaomicron*<br>*Fusobacterium* sp. | *B. fragilis* subsp. *fragilis*<br>*B. fragilis* subsp. *thetaiotaomicron*<br>*Fusobacterium* sp. |
| 2. Brain abscess | *F. nucleatum* | *F. nucleatum* |
| 3. Cellulitis | *B. fragilis* subsp. *fragilis*<br>*B. fragilis* subsp. *thetaiotaomicron* | *B. fragilis* subsp. *fragilis*<br>*B. fragilis* subsp. *ovatus* |
| 4. Decubitus | *B. fragilis* NGF<br>*B. fragilis*[c] | *B. fragilis* subsp. *thetaiotaomicron* |
| 5. Liver abscess | *B. fragilis* subsp. *fragilis*<br>*B. fragilis* subsp. *thetaiotaomicron* | *B. fragilis* subsp. *fragilis*<br>*B. fragilis* subsp. *thetaiotaomicron*<br>*Fusobacterium* sp. |
| 6. Liver abscess | No growth | *F. nucleatum* |
| 7. Cerebrospinal fluid | No growth | *F. nucleatum* |
| 8. Perirectal abscess | *B. fragilis* subsp. *fragilis* | Negative |

[a] Only those organisms for which antisera were available are listed.
[b] Antisera listed are those which reacted with organisms in direct smears.
[c] Subspecies information not available.

TABLE 6. *Comparison of culture results with direct-smear IFA results, utilizing polyvalent pools of antisera*[a]

| Source | Culture results[b] | IFA results[c] |
|---|---|---|
| 1. Subphrenic abscess | *B. fragilis* subsp. *fragilis* | Pool A |
| 2. Peritoneal fluid | *B. fragilis* subsp. *thetaiotaomicron* | Pool B |
| 3. Abdominal wall pus | *B. fragilis* subsp. *fragilis* | Pools A and B |
| 4. Abdominal wound | *B. fragilis* subsp. *thetaiotaomicron* | Pools A and B |
| 5. Bile | *B. fragilis* subsp. *thetaiotaomicron* | Pools B and E |
| 6. Abdominal wall abscess | *B. fragilis* subsp. *thetaiotaomicron*<br>*B. fragilis* subsp. *distasonis* | Pools B and E |
| 7. Facial abscess | *F. nucleatum*<br>*B. melaninogenicus* subsp. *asaccharolyticus* | Pool E |

[a] Antisera comprising polyvalent pools are listed in Table 2.
[b] Only those organisms for which antisera were available are listed.
[c] Polyvalent pools listed are those which reacted with organisms in direct smears.

polyvalent antisera pools. Culture results of specimens six and seven indicated the presence of *B. fragilis* subsp. *distasonis* and *B. melaninogenicus* subsp. *asaccharolyticus*, respectively. Neither of these anaerobes was detected by IFA in direct smears. An additional eight specimens yielded negative IFA results, and no organisms for which antisera were available were obtained upon culture.

Of the anaerobes isolated from these clinical specimens, only those for which antisera were available are listed in Tables 5 and 6. Culture results of specimens usually yielded a mixture of aerobic and anaerobic organisms. All direct-smear IFA reactions were verified by subsequent IFA reactions obtained with pure cultures of each of the organisms isolated from these specimens.

**Cellular morphology of organisms in clinical material.** The usual bacterial morphology of organisms observed by IFA in direct smears of clinical material was that of variable-sized rods with rounded ends. This conformed to generally accepted morphological descriptions of the *Bacteroidaceae* (6, 8, 25). However, fluorescence observed in direct smears of several specimens demonstrated the presence of quite pleomorphic forms which mimicked cellular debris, tissue cells, and leukocytes. For example (Fig. 1 and 2), fluorescence obtained with antiserum specific for *B. fragilis* subsp. *thetaiotaomicron* might represent L-phase organisms in direct smears of specimens one and five (Table 6). Specificity of these reactions was verified by appropriate controls and by subsequent IFA reactions obtained with pure cultures of the bacterial-phase organisms isolated.

## DISCUSSION

All antisera prepared against members of the *Bacteroidaceae* were quite specific as determined by IFA results obtained using homolo-

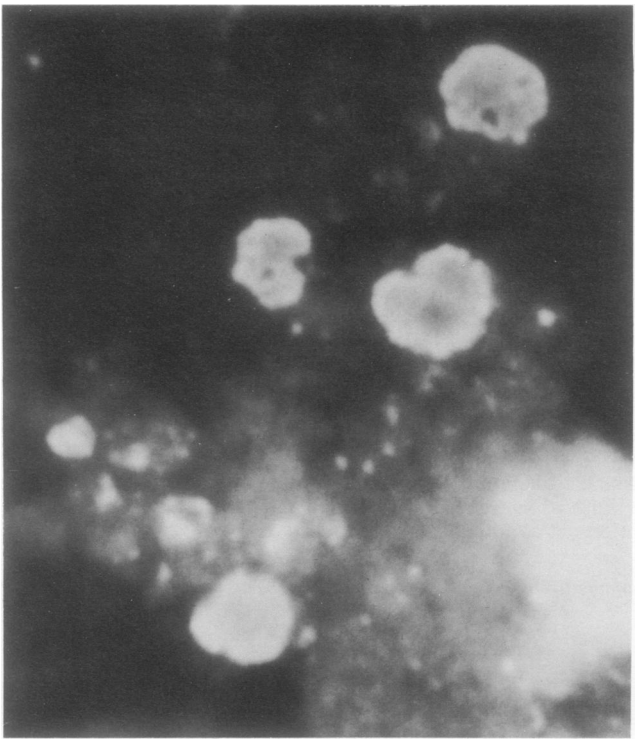

FIG. 1. *In vivo fluorescence of probable L-phase cells, as seen in direct smears of material from a human liver abscess (specimen 1, Table 6), when reacted with B. fragilis antiserum (×1,188).*

gous and heterologous pure-culture suspensions of bacterial cells. This was true for both the *Bacteroides* and the *Fusobacterium* genera. Although results obtained using antisera prepared against *F. nucleatum* and *F. mortiferum* strains were strain specific, the study of additional strains might demonstrate the existence of distinct antigenic groups within these two species. Except for occasional reactions occurring between subspecies, antisera prepared against various *B. fragilis* were either strain specific or specific for possible serological groups within the subspecies (2, 13). Antisera prepared against a very limited number of strains of *B. melaninogenicus* subsp. *asaccharolyticus* and subspecies *intermedius* reacted only with homologous antigens (4, 10).

When utilizing antisera individually, fluorescent antibody results obtained with direct smears of clinical material correlated well with culture results. Due to the highly specific nature of the antisera prepared, detection and identification of the various members of the *Bacteroidaceae* potentially present in clinical material necessitated the preparation and use of numerous antisera. Since many antisera were involved, the IFA procedure was facilitated by the development and use of polyvalent pools of antisera. Utilization of these pools was considerably less time consuming than when antisera were used individually and was also in close agreement with culture results.

Occasional isolation of members of the *Bacteroidaceae* from clinical material, yielding negative direct-smear IFA results, suggested the need for further development of the polyvalent antisera pools utilized. This would require the preparation of additional antisera in order to obtain a more complete coverage of the antigenic types encountered. It would also be desirable to prepare antisera with higher IFA titers in order to facilitate the preparation of polyvalent pools containing numerous individual antisera. Due to the specificity of antisera utilized throughout this study, further development of this procedure may necessarily involve the preparation of polyvalent antisera pools that would effectively screen only for strains of *B. fragilis*. These members of the *Bacteroidaceae* are frequently isolated from clinical material (17, 18, 26) and are the most refractory to routine antibiotic therapy (7, 12, 15, 19, 20).

As indicated by culture results, individual antisera and polyvalent pools occasionally re-

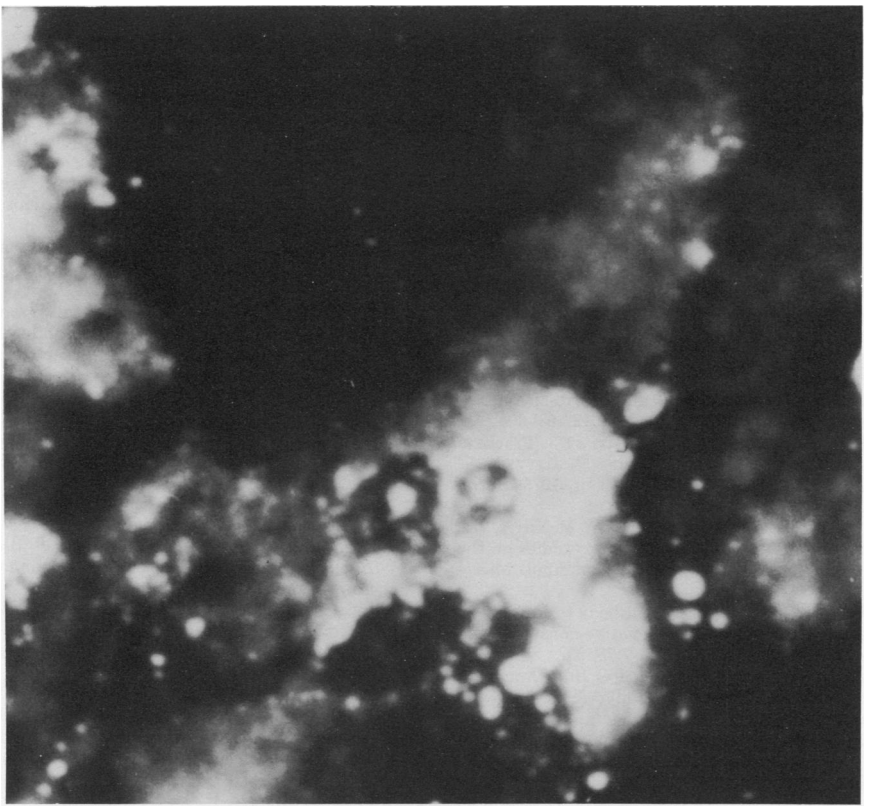

FIG. 2. *In vivo fluorescence of probable L-phase cells, as seen in direct smears of material from a human liver abscess (specimen 5, Table 6), when reacted with B. fragilis antiserum (×1,188).*

acted with organisms on direct smear that were not subsequently isolated. Antibiotic therapy, inappropriate collection methods, and/or transportation delays often adversely affect laboratory culture results. However, positive fluorescence is not dependent upon the presence of viable organisms in the specimen. This suggests the IFA technique to be useful for the detection and identification of organisms present in clinical material, regardless of their viability. Current methods of detection and identification require viable organisms to be present in the clinical material.

Pleomorphism among the *Bacteroidaceae* is not uncommon (6). This tendency for pleomorphism may be potentiated by the effect of antibiotic therapy upon bacterial cell morphology (5). Therefore, extremely pleomorphic organisms may be present, although overlooked, in direct smears of clinical material and in primary cultures (24). This adds an additional dimension of complication and delay to the laboratory diagnosis. Through the use of the IFA procedure, it appears that pleomorphic organisms of this type were detected and identified in direct smears of clinical material. This could be a significant observation in light of recent reports of cell wall-defective bacteria in clinical material (1, 14, 27).

To date, 30 clinical specimens have been tested. Of 13 specimens yielding *Bacteroidaceae*, for which antisera were available, 12 were presumptively diagnosed by IFA to contain subspecies of *B. fragilis* and/or *Fusobacterium* species. Of 17 specimens yielding negative culture results, two were positive by IFA on direct smear. Through the use of polyvalent pools of antisera, an indirect fluorescent antibody procedure would seem to be a practical tool for diagnosis of infections involving members of the family *Bacteroidaceae*.

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