

THE USE OF PAGE AND GAS CHROMATOGRAPHY OF CELLULAR FATTY
FOR THE RAPID IDENTIFICATION OF FUSOBACTERIA AND
CAPNOCYTOPHAGA

by

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(ABSTRACT)

The use of PAGE of soluble cellular proteins and gas chromatography of cellular fatty acids was studied to determine the possible use of both methods for rapid identification of anaerobic bacteria. Species of Fusobacterium and Capnocytophaga were analyzed to determine the identification accuracy of each system.

The electrophoretic patterns of soluble cellular proteins and the cellular fatty acid patterns determined by gas chromatography were found to remain relatively constant within the species examined. This similarity allowed for the development of automated systems using computer programs to analyze the patterns, and compare them to the patterns of known species. Procedures for gas chromatography of cellular fatty acids were developed by Myron Sasser, University of Delaware, and Microbial Identification Systems (Suite 115 Barksdale Professional Center, Newark, Delaware 19711), in cooperation with Hewlett Packard.

From this study it was determined that the accuracy of identification of the PAGE analyses was not high, and

therefore this method would have limited use in a clinical laboratory. Gas chromatography of cellular fatty acids had a relatively high accuracy, which is still being improved.

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INTRODUCTION

Precise identification of most species of anaerobic bacteria is difficult, time consuming, and expensive. Some rapid identification methods currently are being used to make this task easier. But, because these methods are based on relatively few biochemical tests, they often are unreliable. The tests can be difficult to interpret and results may differ among strains within species, leading to the misidentification of the microorganism. There is a major need for more reliable methods.

Polyacrylamide gel electrophoresis (PAGE) of soluble cellular proteins and gas chromatography of cellular fatty acids may prove to be more reliable methods for anaerobic bacterial identification. The electrophoretic cellular protein patterns and the cellular fatty acid patterns obtained with these methods appear to remain relatively constant within a species and to differ between species. With this information and the technology of computers, it may be possible to develop easy, reliable and relatively inexpensive identification procedures.

The gas chromatography of cellular fatty acids for identification of some groups of aerobic bacteria has been developed by Dr. Myron Sasser of the University of Delaware, Hewlett Packard and Microbial Identification Systems,

Incorporated. Chromatographic and polyacrylamide gel electrophoretic identification procedures for fusobacteria and capnocytohaga based on methods developed by W.E.C. Moore, D.E. Hash, L.V. Holdeman and E.P. Cato were further developed and compared in this study.

LITERATURE REVIEW

Polyacrylamide gel electrophoresis. Electrophoresis is the migration of ions in an electric field. This procedure is very useful for the analysis of proteins, nucleic acids and peptides. Discontinuous electrophoresis is often used for protein separations. In this system a mixture of proteins migrates through a short porous section of lower pH (stacking gel) and then into a less porous section of higher pH (resolving gel). As the proteins pass from the stacking gel to the resolving gel the change in pH concentrates each protein into a sharp thin band (8,38).

Bacterial cells may contain 1,000 or more proteins or protein complexes that can be extracted from the cell membranes, walls, or cytoplasm. Soluble proteins that are released from broken cells without chemical treatment are easily separated by electrophoresis and yield simple patterns of up to 30 protein bands that are relatively easy to compare. The resulting distinctive patterns have proven useful for comparisons of species of bacteria in many genera (3,4,5,13,15,17). Comparisons of the patterns may provide a faster and easier means of identifying bacteria at the species level. The procedure provides a rapid and inexpensive means to screen multiple isolates from complex floras because isolates with identical patterns invariably

are members of the same species (29,32,33,34,36). By comparing the patterns of new isolates with the patterns of known strains, PAGE patterns also provide a method to verify identifications made by conventional biochemical methods.

The reliability of PAGE patterns for identification depends upon the repeatability, which is affected by several factors. Protein migration in the gel is primarily a function of molecular size, but since no sodium dodecyl sulfate (29) is used in this system, molecular charge may also have some affect. The growth medium has little affect upon the protein pattern, but certain bands can be enhanced by some substrates, so the use of a single medium has been found to give more repeatable results. Lactic acid, produced by many cultures, causes extreme smearing of the protein bands, therefore it is necessary to use media with low carbohydrate content. Culture age may also affect the pattern. Protein bands are heaviest, usually most numerous and most easily detected from cultures that have relatively good growth and are harvested in late log or early stationary growth phase. The major factor that affects the variability of the protein patterns is the concentration of polyacrylamide used in the gel. A variation in the amount used will change the density of the gels, thus causing the proteins to migrate to different points. Oxygen, atmospheric moisture, and temperature during gel preparation

also affect polyacrylamide gel density and protein migration. To avoid some of these problems the acrylamide solution is degassed before the addition of the ammonium persulfate, a catalyst which catalyzes the cross linking of the acrylamide gel matrix (29). In addition, a protein standard (cell extract) is run on each gel to provide a correction for differences among gels.

Gas-liquid chromatography. Recently, patterns of methylated cellular fatty acids obtained by capillary column gas-liquid chromatography have proven useful for bacterial identification. In this method a fused silica capillary column coated with a nonvolatile liquid in which the volatile mixture is soluble is used to separate the methylated fatty acids from bacterial cells. The mixture to be analyzed is injected into the column and is flash evaporated. The methylated acids are swept through the column by a stream of inert gas (nitrogen) flowing at a constant rate. The column temperature is increased in a predetermined manner to increase the volatility of progressively larger molecules. Each component in the mixture moves through the column at a different rate depending on its boiling point and its partition coefficient between the gas phase and the nonvolatile liquid phase. The individual components emerge at the other end of the column and are detected by a flame ionization detector (44).

Fatty acids are aliphatic carboxylic acids that occur mainly in ester or amide linkages in lipid compounds. Bacterial cellular fatty acids are mainly monocarboxylic acids containing an ionizable carboxyl group and a nonpolar unbranched hydrocarbon chain. They usually contain an even number of carbon atoms, and they may be saturated or contain one or more double bonds (44). Recently, Myron Sasser at the University of Delaware, (Sasser, M., and D.H. Smith. Abstr. Q-76, p.296. Abstracts American Society for Microbiology, Washington, D.C.) (41) developed an automated system for Hewlett Packard that uses the differences in composition of cellular fatty acids of different species as a tool for rapid identification. Cellular fatty acid composition is extremely sensitive to culture substrate, temperature of incubation and age. If these variables are controlled, the fatty acid patterns appear to be relatively stable and to correlate with DNA relatedness, thus showing promise for development of a more rapid, more reliable, and less expensive identification method than conventional biochemical tests.

DESCRIPTION OF BACTERIAL SPECIES ANALYZED

FUSOBACTERIA

Fusobacteria are nonsporeforming rods that are gram - negative, obligately anaerobic, nonmotile chemoorganotrophs.

The genus was described by Knorr in 1922 (20). It now includes bacteria whose major metabolic end product is butyric acid without isobutyric or isovaleric acid. The fusobacteria also may produce acetate, lactate, propionate, succinate, and formate as other metabolic products. The mol% G+C of DNA content ranges from 26-34% for Fusobacterium nucleatum, the type species, and five other described species. One species, Fusobacterium prausnitzii, has a mol% G+C content of 52-57%. Fusobacteria are found as part of the normal flora of the human and animal intestinal tracts and mouth, and some species are pathogenic. They are found in about 5% of all clinical specimens containing anaerobes (12). Pathogenic fusobacteria occur in various purulent or gangrenous infections and in organ infarcts (35). Bartlett and Finegold reported isolates of Fusobacterium species from pulmonary infection resulting from complication from dental disease (2). Fusobacterium species have also been found in eye infections (47), ear infections (24), brain abscess (9), pneumonitis and lung abscesses (9), and liver abscesses (9).

Jantzen and Hofstad (16) found that cellular fatty acid patterns of fusobacteria fell into two groups. One characterized by the presence of 3-OH-14:0 cellular fatty acid includes F. nucleatum, F. necrophorum, F.

gonidiaformans, F. mortiferum, F. varium, F. russii and F. naviforme. The second group without 3-OH-14:0 fatty acid includes F. prausnitzii, F. russii, and F. naviforme. They found that F. nucleatum is the only described species of Fusobacterium that contains 3:OH-16:0 fatty acid.

Hofstad and Skaug (11) also reported that 3-hydroxy-tetradecanoic acid (3-OH-14:0) and n-tetradecanoic acid (14:0) are common lipopolysaccharide constituents of all fusobacteria that they examined, and that lipopolysaccharides from F. nucleatum are endotoxic for rabbits and have o-antigenic specificity.

Fusobacterium nucleatum Knorr 1922, 17 is the type species of Fusobacterium. It contains the diamino acid lanthionine as a major component of the cell wall peptidoglycan (18,28). This species can grow in the presence of up to 6% oxygen. It does not convert lactate to propionate and is commonly isolated from the human gingival margin and sulcus. It also occurs as a pathogen in infections of the upper respiratory tract and pleural cavity. The mol% G+C of the DNA is 27-28%. Love and Johnson (27) showed that there are four DNA homology subgroups of F. nucleatum. Strains within subgroups are related at 86% homology while strains between subgroups are only related at 63-68% homology.

Fusobacterium necrophorum (Flugge 1886, 1-692) Moore and Holdeman 1969 has DNA with a G+C content ranging from 31-34% (35). This species has been isolated from natural cavities of man and other animals and from human and animal clinical specimens, particularly liver abscesses in cattle and foot rot in many domestic animals. Initially, it was thought that this species was a secondary invader requiring a predisposing factor to infect the host, but pure cultures have been found to cause disease. In animals the disease is typified by necrosis of infected tissues, abscess formation, and usually a putrid odor (21).

Fusobacterium perfoetens (Tissier 1905, 109) Moore and Holdeman 1973, 69 originally was isolated by Tissier in 1900 from an infant with diarrhea and was isolated later in 1905 from nursing infants (46). The mol% G+C of DNA of this species ranges from 28-30% (35).

Fusobacterium naviforme (Jungano 1909, 122) Moore and Holdeman 1970, 45 originally was isolated by Jungano from the large intestine of a laboratory rat. It has been found in the human gingival sulcus, clinical specimens, and the bovine rumen. The DNA G+C content has not been determined for this species (35).

Fusobacterium mortiferum (Harris 1901, 519) Moore and Holdeman 1970 has a G+C content of DNA of 26-28%. It has been isolated from blood and other human clinical specimens,

intestinal tract, and feces, and once from feces of an irradiated mouse (35).

Fusobacterium necrogenes (Weinberg, Nativellw, and Prevot 1937, 1-1186) Moore and Holdeman 1970 originally was isolated by Kawamura in 1926 (19) from a necrotic abscess of a chicken. Other strains have been isolated from human feces and the ceca of poultry (1). The DNA G+C content of this species is 28 mol% (35).

Fusobacterium prausnitzii (Hauduroy, Ehringer, Urbain, Guillot, and Magrou 1937, 68) Moore and Holdeman 1970 originally was isolated from human purulent pleurisy by Prausnitz in 1922 (39), and was named "Bacillus mucosus anaerobius". In 1937 Hauduroy et al. proposed that the species be placed in the genus Bacteroides, as Bacteroides prausnitzii (10). This name was accepted as legitimate. In 1970 it was reclassified as F. prausnitzii (31), and a neotype strain was designated in 1974 (7). The G+C of the DNA for the species ranges from 52-57 mol%. F. prausnitzii is a predominant inhabitant of the normal flora of the human intestinal tract (7).

Fusobacterium russii (Hauduroy, Ehringer, Urbain, Guillot, and Magrou) Moore and Holdeman 1970 originally was isolated by Russ in 1905 from a perianal abscess. It has been reported from actinomycotic and other infections of cats, and from human and animal feces. The G+C content of

DNA for the species is 31 mol%. The type strain of the species, isolated from a cat, has PAGE patterns similar to patterns of intestinal isolates, but oral strains with similar phenotypic characteristics have PAGE patterns that are different from those of the type and the intestinal isolates (35), which suggested that these phenotypically similar isolates are members of a distinct species. Most of these phenotypically similar oral isolates are F. alocis or F. sulci (6).

Fusobacterium alocis Cato, Moore, and Moore 1985, 475 and Fusobacterium sulci Cato, Moore, and Moore 1985, 475 are both phenotypically similar to F. russii but give distinct cellular protein patterns on a PAGE analysis. Both species seem to grow best in the presence of 10% CO₂ and 90% N₂. They are found primarily in the human gingival sulcus. The G+C content of the DNA of the type strain of F. alocis is 34%. The G+C content of the DNA of the type strain of F. sulci is 39%. Until the present work, the two strains were distinguishable only by their electrophoretic pattern on PAGE analysis, or by DNA homology (6). Because they are difficult to distinguish and only have recently been described, their significance in human infections is not established, although the close association of F. alocis, and F. sulci with periodontitis has been documented (6).

Fusobacterium gonidiaformans (Tunnickliff and Jackson 1925, 430) Moore and Holdeman 1970 has been isolated from the intestinal and urogenital tracts of humans, and also is associated with various human infections (35).

Fusobacterium varium (Eggerth and Gagnon 1933, 389) Moore and Holdeman 1969 has been isolated from human feces, cecal contents, and purulent infections. This species has a G+C content of DNA of 29 mol% (35).

CAPNOCYTOPHAGA

In 1956 Prévot et al. (40) described two strains of obligately anaerobic, gram-negative rods that they considered to be variants of "Fusiformis nucleatus", and these strains were designated "F. nucleatus var ochraceus". In a later study conducted by Sebald (42), the G+C content of DNA of "F. nucleatus" and "F. nucleatus var. ochraceus" were found to be different, and the base ratio of "F. nucleatus var. ochraceus" was similar to that of organisms then classified in the genus "Ristella". Therefore, Sebald proposed that these species be reclassified as "Ristella ochracea." In 1964, Loesche (25) described six strains from the human oral cavity that were determined to have characteristics similar to the strains described by Prévot and Sebald. These strains described by Loesche were designated B. oralis "var. elongatus". One of these strains was compared with three

strains of "R. ochracea" at VPI&SU Anaerobe Laboratory and they were found to belong to the same species. This species was then transferred to Bacteroides as B. ochraceus (31) (the Approved list of bacterial names (43) attributes this name to Prévot, Joubert, Tardiéux, and de Cadore 1956, 787).

In the 1960's Elizabeth King, Center for Disease Control, characterized a group of bacteria that, in retrospect, had characteristics similar to B. ochraceus and she designated these strains as DF-1. In 1982, the genus Capnocytophaga Leadbetter, Holt, and Socransky 1982, 266 was proposed (37,38) and it was subsequently recognized that B. ochraceus "species" were members of this new genus. Williams et al. (48), examined eight strains of Center for Disease Control group DF-1 and found that seven of them had 62-87% DNA homology with the proposed type strain C. ochracea. One strain examined had 72% DNA homology with the type strain of Capnocytophaga gingivalis. Four strains of Bacteroides ochraceus also were examined and found to have 76-86% DNA homology with the proposed type strain of C. ochracea. They concluded that the strains examined should be classified as Capnocytophaga species. According to the rules of nomenclature (22), the type strain of the basonym species of Bacteroides ochraceus (ATCC 27872) is now the type strain of Capnocytophaga ochracea.

Species of Capnocytophaga are "gliding" gram negative rods with a DNA containing 33-41 mol% G+C. The type species is C. ochracea (Prévot, Joubert, Tardiéux, and de Cadore 1956) Leadbetter, Holt, Socransky 1982, 266. Members of this genus are isolated primarily from human supragingival dental plaque of healthy subjects and from the gingival sulcus of periodontal diseased sites (14,23,45). However, they are not likely to be periodontal disease pathogens because they are found in higher numbers in sites of healthy subjects than in sites from subjects affected with periodontitis (14). Morphologically they are similar to fusobacteria, but they are saccharolytic and do not produce butyric acid (23). They may move by spreading over solid surfaces, but this "gliding" is not exhibited on all media. Blood agar plates manufactured by BBL support the spreading growth of the colonies, whereas other blood agar plates usually do not. An increase in the agar concentration to about 2%-3% (w/v) also supports this spreading phenomenon. They grow anaerobically and in a CO₂ enriched aerobic atmosphere and their metabolic end products are succinic and acetic acids, which distinguish them from Fusobacterium, Bacteroides, or Leptotrichia species. Three significant features of the genus are 1) "gliding motility" on certain media, 2) the ability to carry out fermentative metabolism and 3) aerotolerance or growth in air with 10% CO₂ (23),

and the absence of catalase activity (45). However, London et. al. (26) reported that some strains of C. gingivalis do not require an enriched CO₂ atmosphere, and can metabolize glucose by fermentation or respiration.

Presently, there are three named species of Capnocytophaga: C. ochracea, C. gingivalis Leadbetter, Holt, and Socransky 1982, 266, and C. sputigena Leadbetter, Holt, and Socransky 1982, 266. Members of these species have been isolated from the oral cavity and from systemic disease in a compromised host (23,48). Sepsis with these organisms occurs in patients with hematological malignancies, and who are granulocytopenic, usually as a result of complications from cancer therapy. Newman et al. report that Capnocytophaga species may be isolated from clinical laboratory samples more often than reports would indicate. They found that because they are relatively fastidious and slow growing and require elevated amounts of CO₂ for growth, colonies may not be apparent on 24 hour blood agar plates. With prolonged incubation, the colonies may be overgrown by other bacteria. Therefore, if they are present in a specimen, they may not be detected, or the culture may be discarded before they grow (37).

Materials and Methods

Strains. All strains used in this study were obtained from the culture collection at VPI&SU Anaerobe Laboratory (Table 1).

PAGE. The PAGE procedure previously published by Moore et.al (29) was used with minor modifications.

Glass plates, 138 X 156 mm, were cleaned with detergent and then wiped with acetone-alcohol. Three lucite spacers, (Aquebogue, NY) 5/16 inches wide X 7.5 inches long X 1.2 mm thick, were covered with a thin film of Crisco (hydrogenated vegetable oil), and one end of two of them was dipped into the Crisco. These two spacers were placed on the sides of one plate, and a third was placed along the bottom using a wooden template as a guide. A second notched glass plate was positioned on top of the spacers and the two plates were clamped together with binder clips, two on the bottom and two on each side. To receive the resolving and stacking gels, the "gel sandwich" was placed into a holder.

The resolving gel (8.5% acrylamide) solution contained 11.3 g acrylamide, 0.3 g N,N'-methylenebisacrylamide and 134 ml pH 8.8 tris-chloride buffer. This solution was mixed and filtered through ashless filter paper (Eaton-Dikeman No. 615), and then 1.05 μ l TEMED (N,N,N',N'-Tetramethylethylenediamine BIO-RAD Richmond, California) was added.

Table 1. VPI strain number, labelled identification, and sources (human unless otherwise indicated) of 214 strains of Capnocytophaga and Fusobacterium species examined in this study

VPI no. ^A (other collection no.)	Designation	Source
D132A-8	<u>C. gingivalis</u>	Periodontium
D107D-25	"	"
D129C-16	"	"
D133A-8	"	"
D137B-30	"	"
D137F-1	"	"
D138A-3	"	"
D141B-26	"	"
D142C-27	"	"
D143D-4	"	"
D153A-29	"	"
D97M-9	"	"
D82N-30	"	"
D83N-21	"	"
D96Y-18	"	"
D96W-17A	"	"
D98C-26	"	"
E3Y-7	"	"
E4P-16	"	"
E4R-13	"	"
14457 ^T (ATCC 33624)	"	Periodontium
9775 ^T (ATCC 27872)	<u>C. ochracea</u>	Oral cavity
D120C-19	"	Periodontium
D120D-5B	"	"
D124E-23B	"	"
D130C-29	"	"
D136B-7	"	"
D138C-28	"	"
D139A-13	"	"
D140B-17	"	"
D142C-21	"	"
D143D-23	"	"
D155B-1	"	"
D79S-30	"	"
D81A-22	"	"
D83X-7	"	"
D94B-5	"	"
D96P-11	"	"
D97T-22	"	"
D98D-6	"	"

Table 1. cont.

VPI no. ^A (other collection no.)	Designation	Source
D131B-17	<u>C. sputigena</u>	Periodontium
D101B-29	"	"
D122A-18	"	"
D124E-23B	"	"
D129A-1	"	"
D129C-16	"	"
D139B-2	"	"
D143D-23	"	"
D146B-18A	"	"
D155D-4	"	"
D156D-26	"	"
D80D-5	"	"
D82N-30	"	"
D83M-1	"	"
E4P-16	"	"
E6X-18	"	"
E8V-30	"	"
14458 ^T (ATCC 33612)	"	"
D118G-2	<u>F. alocis</u>	Periodontium
D126C-20	"	"
D17A-26	"	"
D20A-3	"	"
D22B-13	"	"
D39D-26	"	"
D40B-5 ^T (ATCC 35896)	"	"
D45B-4	"	"
D62D-20	"	"
D73D-11	"	"
D118G-2	"	"
D126C-20	"	"
D132F-10	"	"
D39D-26	"	"
0482A ^T (Prévot 2554A)	<u>F. gonidiaformans</u>	Unknown
0436	"	Rectal abscess
2758-B	"	Urinary tract
4381	"	"
4937	"	Abscess
6808	"	Urine
7301B	"	Pilonidal cyst
7470	"	Maxillary sinus
8362D	"	Hip drainage
9150	"	Abdominal
9372	"	Bartholin gland

Table 1. cont.

VPI no. A (other collection no.)	Designation	Source
10488	<u>F. gonidiaformans</u>	Foot abscess
10494	"	Foot abscess
12034	"	Scrotum
11360	"	Pus
4123A-3 ^T (ATCC 9817)	<u>F. mortiferum</u>	Sinus
4249	"	Abscess
5696	<u>F. mortiferum</u>	Urine catheter
4698	"	Unknown
5672	"	Cervix
5699	"	Wound
5700	"	Feces
5721	"	Blood
7000	"	Drainage
7430	"	Appendix
8365	"	Wound
8644A	"	Abdominal wound
9996 (ATCC 25557)	"	Abscess
3206H	<u>F. naviforme</u>	Rectal abscess
4085G	"	Abscess
5658 ^T (ATCC 25832)	"	Head lesion
5566	"	Abscess
6122	"	Cyst
6597A	"	Wound
6700	"	Abscess
7436	"	Cranial fluid
7939	"	Incision
8906A	"	Drainage
2368-1 ^T (ATCC 25556)	<u>F. necrogenes</u>	Duck cecum
0492-1	<u>F. necrophorum</u>	Blood
0299-1	"	Pleurisy
0606-1	"	Human
11672	"	Dental
2386 (Beerens B495)	"	Unknown
2891 ^T (ATCC 25286)	"	Cattle liver
2892 (Fievez 2362)	"	Unknown
2894	"	"
2900	"	"
2901	"	"
2927	"	"
4124	"	Pleural pus
4236	"	Inguinal pus
4387 (Prévot 1610)	"	Unknown

Table 1. cont.

VPI No. ^A (other collection no.)	Designation	Source
4380	<u>F. necrophorum</u>	Blood
4875	"	Rabbit
5127 (Prévot 2439)	"	Unknown
9932	"	Spinal
10987	"	Blood
1779	<u>F. nucleatum</u>	Unknown
5105	"	Lung abscess
4351 (Prévot 1210)	"	"
4355 ^T (ATCC 25586)	"	Cat
2388 (Beerens F398)	"	"
4357	"	"
5137 (Prévot 42280)	"	Unknown
4359	"	Brain abscess
5382 (ATCC 10953)	"	Unknown
5562 (Prévot 3017A)	"	"
5564	"	Dental
5566	"	Pleurisy
6030	"	Pleural fluid
6280	"	Spinal fluid
D146A-4	"	Periodontium
D146A-27	"	"
D146A-22	"	"
D146C-3	"	"
D146B-20	"	"
D14D-4	"	"
E1D1	"	"
11077 ^T (ATCC 29250)	<u>F. perfoetens</u>	Pig feces
13726	"	Periodontium
11672	<u>F. prausnitzii</u>	Dental
2282-1	"	Large intestine
3783-A	"	"
C3-15	"	Feces
C3-19-1	"	"
C3-47-1	"	"
C6-14-1	"	"
C13-20-1A	"	"
13871 ^T (ATCC 33693)	"	Human feces
0306	<u>F. russii</u>	Cat
0307 ^T (ATCC 593A)	"	"
14209	"	"
4045	"	"
4344	"	"
4346	"	"

Table 1. cont.

VPI No. ^A (other collection no.)	Designation	Source
2820	<u>F. russii</u>	Unknown
9833	"	Leg ulcer
8470-Q	"	Tibia
10204	"	Scrotum
10646-1	"	Unknown
10586	"	Unknown
12936-E	"	Animal
11018	"	Submandible
8055	"	Mouth
8590	"	Abdominal
3296-1	"	Feces
0045-2	"	Cow rumen
3522	"	Feces
3356	"	Cheese
D123A-19	<u>F. sulci</u>	Periodontium
D136C-5	"	"
D140B-30	"	"
D142B-20	"	"
D154B-1	"	"
D25A-5-1	"	"
D26C-13	"	"
D41A-16	"	"
D45A-29A ^T (ATCC 35585)	"	"
D83A-25	"	"
D99B-8	"	"
E4Y-17	"	"
0497	<u>F. varium</u>	Cat feces
0499-A	"	Cat feces
0473 (ATCC 9817)	"	Fallopian tube
0500 (Prévot 1669BB)	"	Unknown
0501 ^T (ATCC 8501)	"	Feces
2377-1B	"	Wound
2377-1A	"	"
2387-B (Beerens B130)	"	Unknown
4135-1	"	Unknown
4138	"	Cow intestine
4232 (Prévot 2333)	"	Unknown
4234 (Prévot 2587A)	"	"
4384-1 (Prévot B17Ia)	"	"
4386-1B (Prévot CSA)	"	"
5036	"	Peritoneum
5688-1	"	Feces

T = type strain; ATCC, American Type Culture Collection

A ATCC = American type culture collection. Prévot strains were obtained from the collection at the Service des Anaerobies, Pasteur Institute in Paris. Beerens strains were obtained from H. Beerens Pasteur Institute in Lille, France.

The mixture was placed in the freezer to cool for five minutes, after which, 30 ml of the solution was drawn into a 50 ml syringe and degassed by covering the tip of the syringe while pulling down on the plunger to create a vacuum, while tapping in against the countertop. Three-tenths ml of 10% ammonium persulfate was added, and the mixture was degassed again and injected through a hypodermic needle into the "gel sandwich". After five minutes the resolving gels were overlain with distilled water and then stored at 4°C in sealed plastic bags containing enough Tris-chloride buffer pH 8.8 to cover the bottom of the gels.

When the gels were to be used, the surface of the resolving gels was blotted dry, and the stacking gels were poured. The stacking gels contained 2.83 g acrylamide, 0.08 g N,N'-methylenebisacrylamide, and 1 ml of 0.25% bromphenol blue solution (dye marker) in 60 ml of pH 7.0 Tris-chloride buffer. For each gel, 3 ml of this solution was drawn into a 50 ml syringe and 2.5 μ l of TEMED was added. The solution was degassed, 0.025 ml of 10% ammonium persulfate was added, and the solution was degassed again and injected over the resolving gel. A 20-tooth comb, 1.2mm thick, was placed into the stacking gel immediately after pouring and the gel was allowed to solidify at room temperature for at least 20 minutes. The comb and bottom spacer were gently

removed under running water and the gels were placed in the electrophoresis unit. The top and bottom chambers of the unit were filled with 70% electrode buffer (5.4 g Tris, 27.0 g glycine, and 1800 ml H₂O) until the top and bottom of the gels were covered. Air bubbles were purged from the bottom of the sandwich by a stream of buffer from a syringe with a curved needle.

The bacterial samples to be analyzed on PAGE were grown in Brain heart infusion broth plus 0.1% calcium carbonate (Appendix 1) for 24-48 hours or until good growth was obtained. The cultures were Gram's stained to check purity and the purity was verified by the protein pattern obtained after electrophoresis. Each culture was then centrifuged at 8,000 g for 10 min. and the supernatant was discarded. The cell pellets could be stored at -70°C. For analysis, the cell pellets if frozen were thawed, and 0.10 ml of 0.15 M Tris-Cl pH 7.0 buffer + EDTA (1.02 g/l) + 2.5% 1 M urea was added to the pellet. The cells were then broken with 0.18 mm glass beads in each tube, followed by brief mixing on a vortex mixer and vigorous agitation in a mechanical shaker for 2 intervals of 2 minutes each. The tubes were then heated in a 55°C water bath for five minutes to precipitate cellular components that may cause smearing of the pattern. The samples were then centrifuged as before, and powdered sucrose equal to one-third the volume of the supernatant was

added. Forty microliters of each sucrose-saturated supernatant was then loaded into a well in the gel. Electrophoresis at 150 V was continued for about 3 hours, until the stacking dye marker reached the bottom edge of the gel. The gels were placed in 12% aqueous trichloroacetic acid (v/v) for at least 30 min., stained with 1.5 g Coomassie Blue (Serva Blau R 35051 Coomassie Brilliant Blue-R C.I. 42660 86%) dissolved in 450 ml ethanol, 200 ml glacial acetic acid, and 900 ml water, for 12 minutes and destained with 10% (v/v) glacial acetic acid for 20 to 30 minutes and with approximately 150 ml of 10% glacial acetic acid with 0.5ml methanol overnight. The stained gels were photographed and were scanned on a LKB Soft laser scanning densitometer Model no. SL-504TL for analysis by computer programs. The densitometry measurement data from a reference lane and all sample lanes on each gel, were fed into a Digital Minc 11 computer.

The reference lane of Streptococcus faecalis (VPI 8374, ATCC 19433) had three distinct protein bands whose peak values were stored in the computer as three peaks. These peaks were used to adjust for differences in protein migration among different gels. The first 20 and the last 50 points of 2,000 generated for each scan were discarded to allow for variations in placement of the gels on the scanner. Each 10 remaining points in sequence were averaged and the

averaged data were stored for each scan. Sample scans were then matched against a library of known strains that were stored in the computer. The program for matching the scans, written by Patrick Robinson, VPI&SU Anaerobe Laboratory, aligned the "unknown" scan with each known pattern to be compared, by aligning the highest peak in the first half of the unknown scan with the highest peak of the reference scan that fell within two 10-point units of the highest unknown peak. The scans were then matched point by point and the Pearson Product-Moment Correlation was calculated. These correlation values were stored for each reference strain comparison. The values were sorted and the highest matching values were printed. Determinations were made for each known strain run as an "unknown" to determine the accuracy of the system.

GAS CHROMATOGRAPHY. Bacterial strains to be analyzed by gas chromatography were cultured in 10 ml of glucose broth media (Appendix 1) at 37⁰C until good growth was obtained. The cultures were Gram's stained to check for purity and the purity was verified by the protein pattern obtained after electrophoresis. The cultures were then centrifuged at 6,000 g for 10 min and the supernatant was discarded. One ml of Reagent 1 (45 g NaOH, 150 ml methanol, 150 ml distilled water) was added to each cell pellet and

they were mixed on a vortex mixer and placed in boiling water for 30 min to saponify the cellular fatty acids. The samples were then cooled to room temperature and 2 ml of Reagent 2 (325 ml 6.00 N HCl, 275 ml methanol) was added. The tubes were heated in an 80⁰C water bath for 10 min, cooled immediately in a pan of cold water, and 1.25 ml of Reagent 3 (200 ml hexane, 200 ml ether) was added to extract the fatty acids. The tubes were placed in an end-over -end laboratory rotator for 10 min to extract the methylated acids, and then the aqueous phase was removed. To wash the hexane-ether extract, 3 ml of Reagent 4 (10.8 g NaOH 900 ml distilled water) was added and the tubes were rotated again for 5 minutes. The solvent-sample phase was removed and transferred to a small vial, which was tightly capped with a serum stopper and placed on the HP 5898A automatic sample injector for analysis by temperature programmed FID capillary chromatography.

After many samples were run on the HP 5898A, a library of reference patterns for each species of Fusobacterium and Capnocytophaga was created. The first step in creation of the library was the use of a cluster analysis program written by Patrick Robinson. This program provided a dendogram that portrayed relative similarities between samples and clusters of samples. This dendogram was used to find sample profiles that may have been from bad

preparations or misidentified cultures. It was also used to determine if subgroups could be formed within groups of samples. After the cluster analysis was run and any suspect analyses were removed, the library creation program used for the HP 5898A was run. This program provides a mean and standard deviation of % peak area for each significant component peak in each sample. If the deviation from the mean for major peaks from a given sample was especially high, the sample data for that entry was not used for the library. All strains were compared against the resulting library of patterns to determine the accuracy of the Hewlett-Packard identification system.

RESULTS

PAGE Analysis. Protein electrophoretic patterns of type strains of Fusobacterium and Capnocytophaga species are shown in Figure 1. The patterns show the variation in cellular protein patterns from one species to another and the similarity of different strains of the same species on the same gel (lanes 8,18; 9,19; 10,20). Figure 2 is a picture of a PAGE gel containing four different species of Fusobacterium. The first lane is the gel reference lane of Streptococcus faecalis VPI no. 8374. Lanes 2-5 are from different strains of F. nucleatum; lanes 6-10, different strains of F. necrophorum; lanes 11-16, different strains of F. varium; and lanes 17-20, different strains of F. mortiferum. In this example gel the similarity of protein patterns from different strains of the same species is evident, as are the differences in the patterns between species. The slight differences among strains within species usually are constant in repeated analyses.

An example of the printout obtained from computer analysis of the PAGE gels is shown in Figure 3. The last column is the correlation value, ranging from 0.00000 to 1.00000, that was obtained by the computer analysis. The first line represents the "unknown" entry.

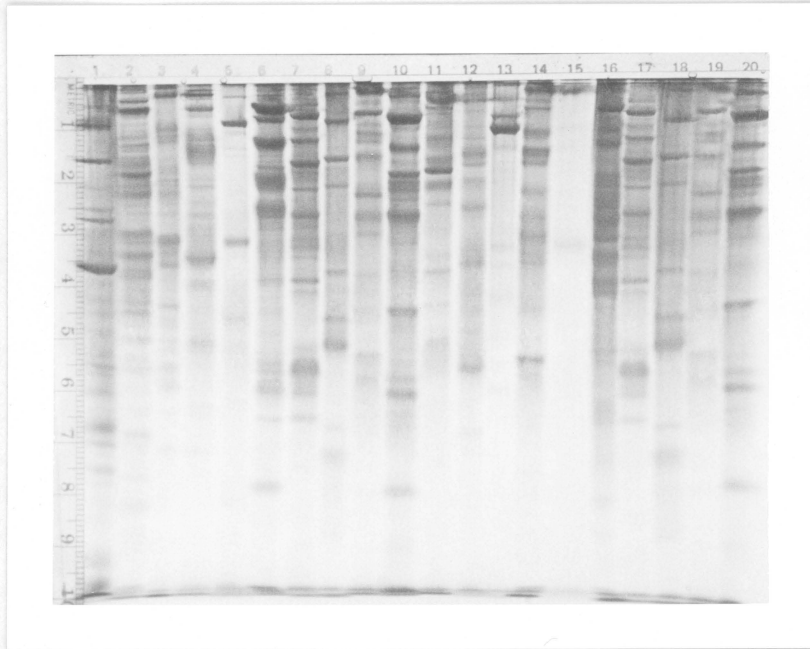


Figure 1. PAGE gel of type strains. Lane 1 = Streptococcus faecalis VPI 8374. Lane 2 = C. gingivalis VPI 14457. Lane 3 = C. ochracea VPI 9775. Lane 4 = C. sputigena VPI 14458. Lane 5 = F. alocis VPI D40B-5. Lane 6 = F. gonidiaformans VPI 0482A. Lane 7 = F. mortiferum VPI 4123A. Lane 8 = F. naviforme VPI 5658. Lane 9 = F. necrogenes VPI 5658. Lane 10 = F. necrophorum VPI 2891. Lane 11 = F. nucleatum VPI 4355. Lane 12 = F. prausnitzii VPI C13-51-1. Lane 13 = F. perfoetens VPI 11077. Lane 14 = F. russii VPI 0307-3. Lane 15 = F. sulci VPI D45A-29A. Lane 16 = F. varium VPI 0501. Lane 17 = F. mortiferum VPI 4123A. Lane 18 = F. naviforme VPI 5658. Lane 19 = F. necrogenes VPI 2368-1. Lane 20 = F. necrophorum VPI 2891.

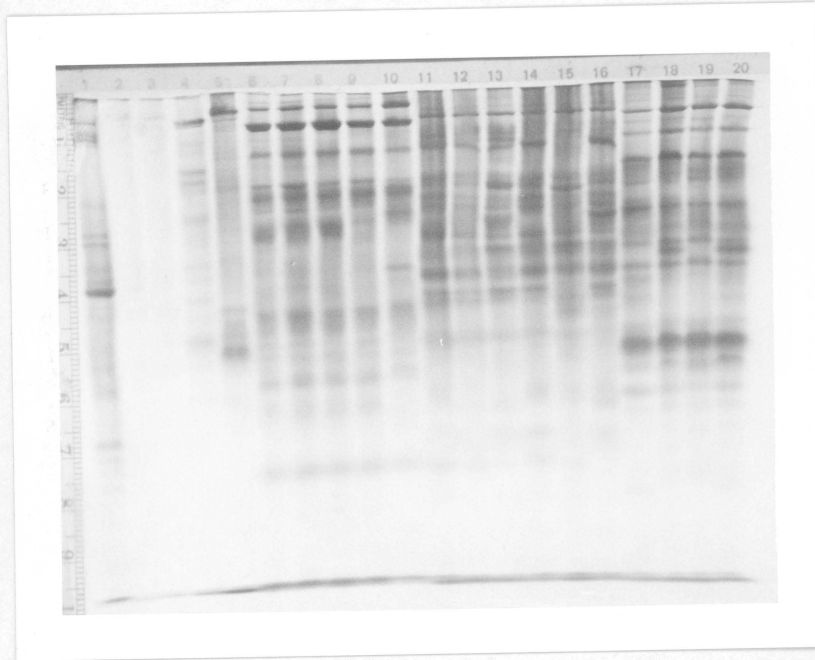


Figure 2. PAGE gel. Lane 1 = Streptococcus faecalis VPI 8374. Lane 2 = F. nucleatum VPI 1779. Lane 3 = F. nucleatum VPI 2818. Lane 4 = F. nucleatum VPI 4357. Lane 5 = F. nucleatum VPI 5566. Lane 6 = F. necrophorum VPI 2900. Lane 7 = F. necrophorum VPI 2386. Lane 8 = F. necrophorum VPI 2901. Lane 9 = F. necrophorum VPI 2927. Lane 10 = F. necrophorum VPI 4236. Lane 11 = F. varium VPI 0499-A. Lane 12 = F. varium VPI 0500. Lane 13 = F. varium VPI 0501. Lane 14 = F. varium VPI 4232-1. Lane 15 = F. varium VPI 4234. Lane 16 = F. varium VPI 0497. Lane 17 = F. mortiferum VPI 0473. Lane 18 = F. mortiferum VPI 5672. Lane 19 = F. mortiferum VPI 4123-A. Lane 20 = F. mortiferum VPI 5696.

NR CAP. OCH D138C-28	13	II	050187	
NR CAP. OCH D138C-28	13	II	050187	1.00000
NR CAP. OCH D155B-1	16	II	050187	0.94830
NR CAP. OCH D83X-7	17	II	050187	0.93922
NR CAP. OCH D136B-7	15	II	050187	0.88786
NR F. RUSSI 0307	9	II	070386	0.88768
NR CAP. GIN D83N-21	9	II	050187	0.87724
NR F. NECRO 2894	11	II	050187	0.80704
NR F. VARIU 5036	13	I	050187	0.80082
NR F. ALOCI D22B-13	15	I	061687	0.78301
NR F. M-6 10398	18	II	050687	0.77634
NR CAP. OCH D139A-13	14	III	050187	
NR CAP. OCH D139A-13	14	III	050187	1.00000
NR CAP. SPU D122A-18	14	IV	050187	0.89869
NR CAP. OCH D124E-23B	9	III	050687	0.89273
NR CAP. SPU D131B-17	3	III	050187	0.87839
NR CAP. OCH D131A21A	18	III	071585	0.86341
NR CAP. OCH D120C-19	11	III	050187	0.84546
NR E.CORR D146J11	7	III	052986	0.83886
NR CAP.SPU D142A14	15	I	012386	0.83478
NR B.CAP 11858B2	13	II	081386	0.83423
NR E.CORR D146J12	9	III	052986	0.83208

Figure 3. PAGE analysis printout. The first column gives the Gram's stain reaction (N = negative), and the morphology (R = rod). The second column gives the genus and species designation of the sample run, followed by the VPI number. The next three columns give the lane of the gel, the gel number and the date the gel was run. The last column gives the correlation value determined by the analysis with the computer programs. The following lines are the best reference pattern matches in descending order. B. CAP, Bacteroides capsularis; CAP. GIN, Capnocytophaga gingivalis; CAP. OCH, Capnocytophaga ochracea; CAP. SPU, Capnocytophaga sputigena; E. CORR, Eikenella corrodens; F. ALOCI, Fusobacterium alocis; F. M-6, Fusobacterium species "m-6"; F. NECRO, Fusobacterium necrophorum; F. RUSSII, Fusobacterium russii;

Table 2 shows the results of the analysis of the accuracy of the computer identification of the electrophoretic patterns.

Analysis of Gas Chromatography of Cellular Fatty Acids.

The variation in the composition of cellular fatty acids between species is shown in Figs. 4-9. Figure 4 shows the methylated cellular fatty acid patterns obtained for the type strains of Capnocytophaga run on the HP 5898A. Figures 5-8 are the fatty acid patterns for the type strains of Fusobacterium. Figure 9 contains patterns from two strains of F. nucleatum. The similarity of patterns of different strains of the same species is evident.

A numerical analysis (Fig. 10) is obtained with each pattern, and it is these data that are stored in the Hewlett-Packard computer for statistical principal component analysis according to the proprietary Hewlett-Packard identification computer program. The "similarity index" of the best match among reference species in the library is related to the standard deviation from the mean library index reference pattern. A value of 0.5 or above is considered to be a "good" match.

An initial test of individual strains analyzed as "unknowns" and compared against the library reference

Table 2. Analysis of accuracy of the computer programs used for PAGE analysis, showing the species analyzed, the no. of strains, the total no. of analyses for each species, the number correctly identified, and the % correct identification.

Species (no. of strains examined)	No. analyzed	No. correctly identified (1st choice)	% correctly identified (1st choice)
<u>Capnocytophaga</u>			
<u>gingivalis</u> (13)	13	4	30.7
<u>ochracea</u> (16)	17	5	29.4
<u>sputigena</u> (14)	14	4	28.5
<u>Fusobacterium</u>			
<u>alocis</u> (9)	11	7	63.6
<u>gonidiaformans</u> (7)	10	7	70.0
<u>mortiferum</u> (11)	14	11	78.6
<u>naviforme</u> (8)	10	8	80.0
<u>necrogenes</u> (1)	7	4	57.1
<u>necrophorum</u> (14)	15	10	66.7
<u>nucleatum</u> (17)	17	10	58.8
<u>perfoetens</u> (2)	10	6	60.0
<u>prausnitzii</u> (8)	7	2	28.6
<u>russii</u> (7)	10	4	40.0
<u>sulci</u> (8)	10	8	80.0
<u>varium</u> (14)	17	10	58.8

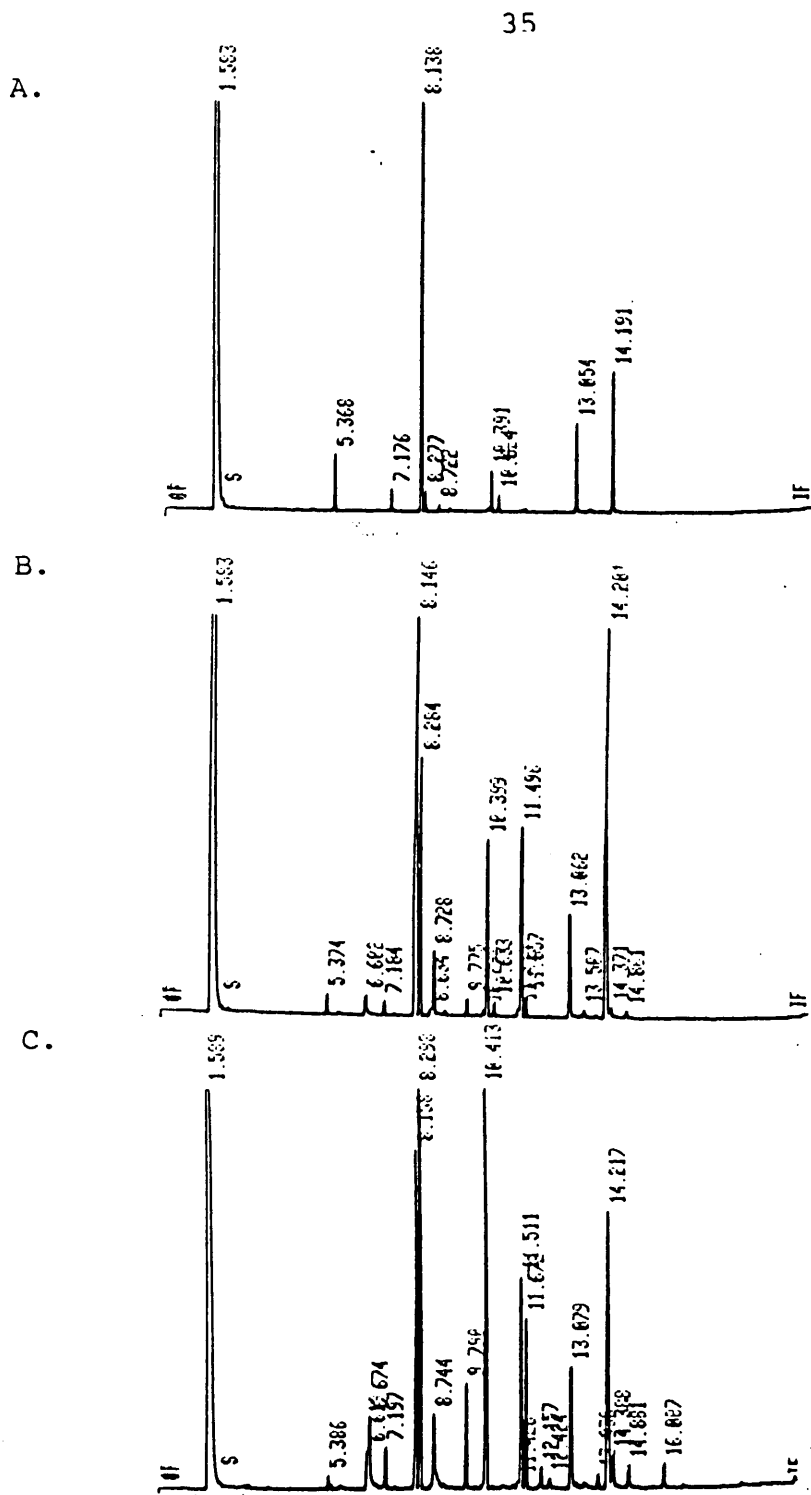


Figure 4. Fatty acid patterns of type strains of *Capnocytophaga* species. A = *C. gingivalis* VPI 14457^T. B = *C. ochracea* VPI 9775^T. C = *C. sputigena* VPI 14458^T.

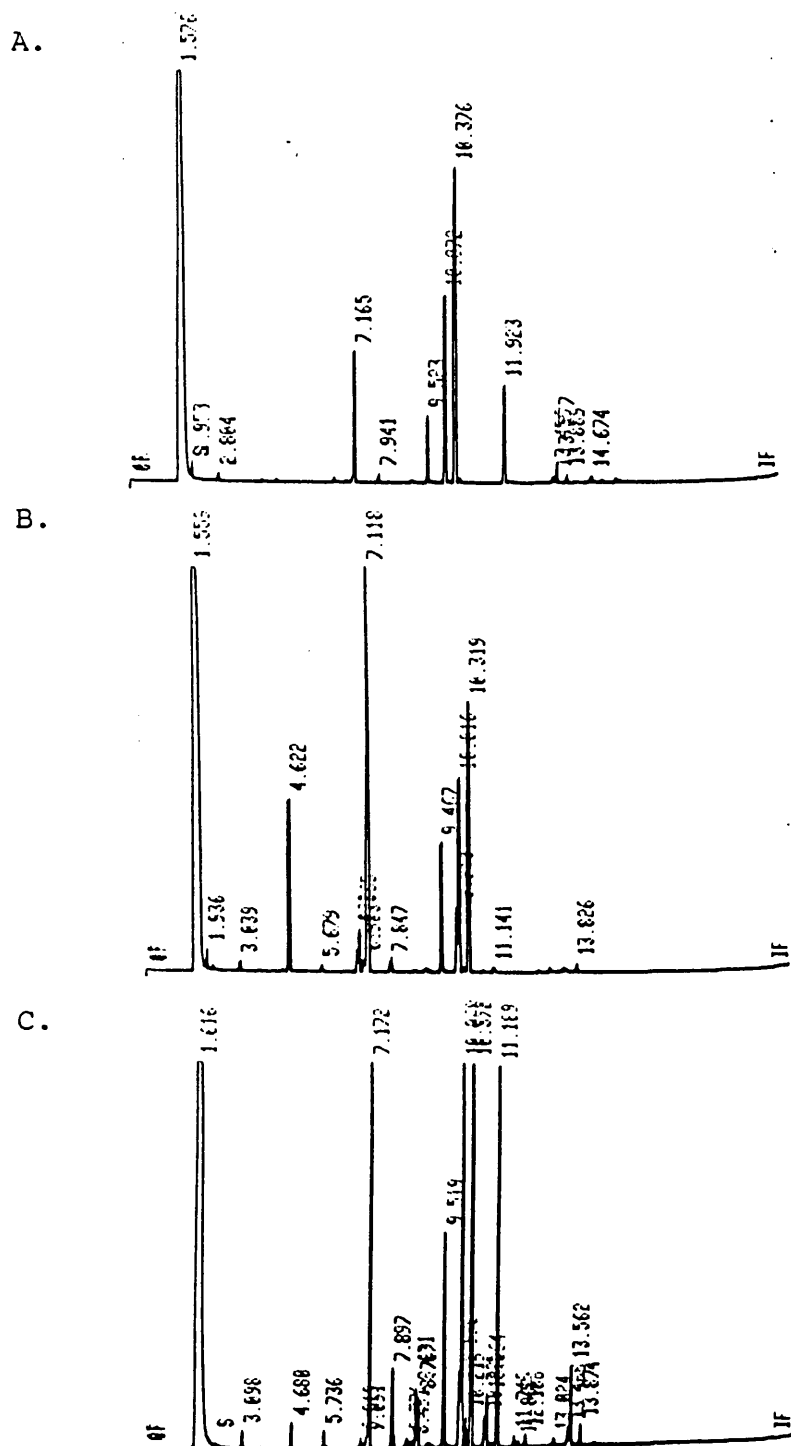


Figure 5. Fatty acid patterns of type strain of *Fusobacterium* species. A = *F. alocis* VPI D40B-5^T. B = *F. gonidiaformans* VPI 0482A^T. C = *F. mortiferum* VPI 4123A^T.

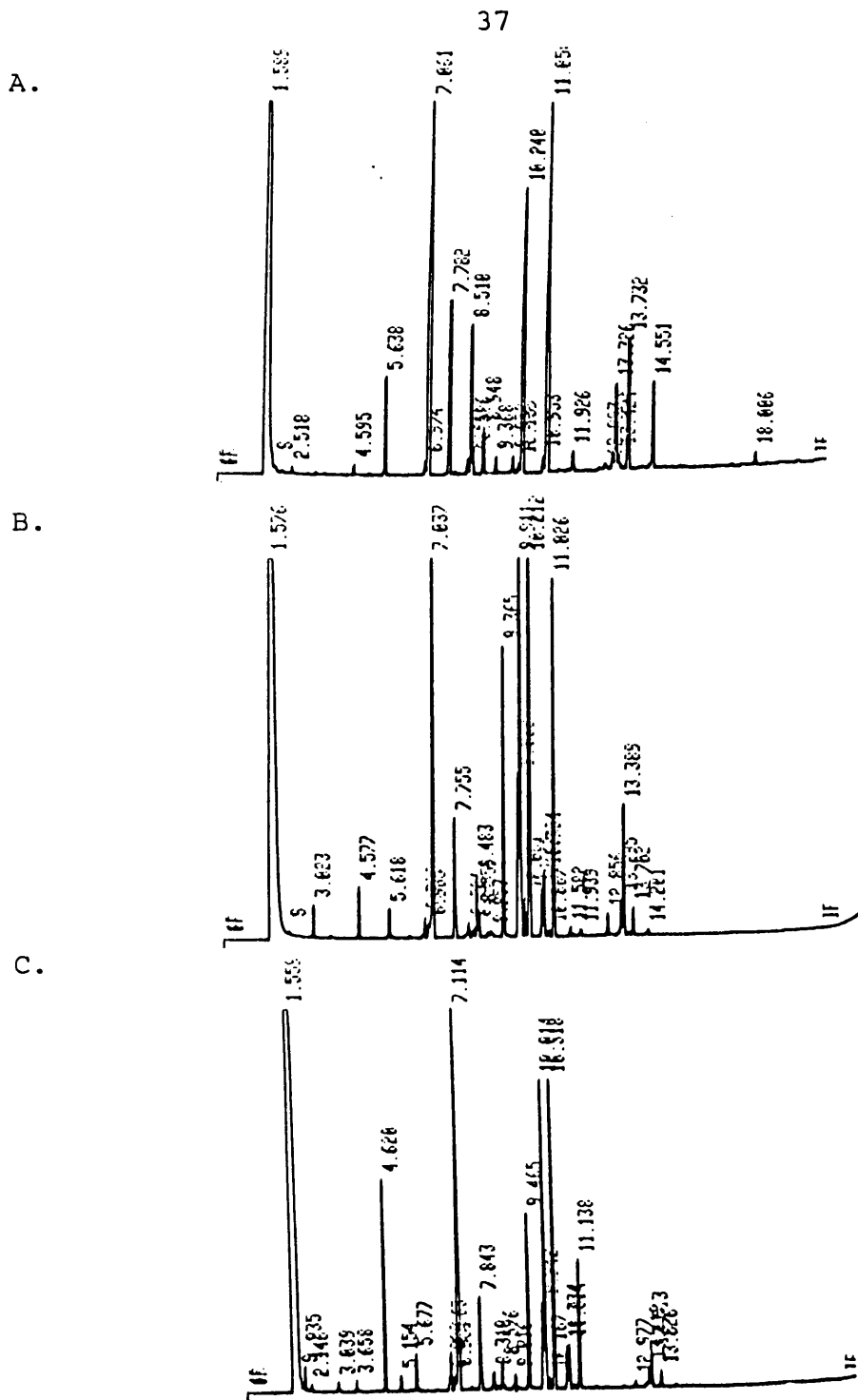


Figure 6. Fatty acid patterns of type strains of *Fusobacterium* species. A = *F. naviforme* VPI 5658. B = *F. necrogenes* VPI 2368^T. C = *F. necrophorum* VPI 2891^T.

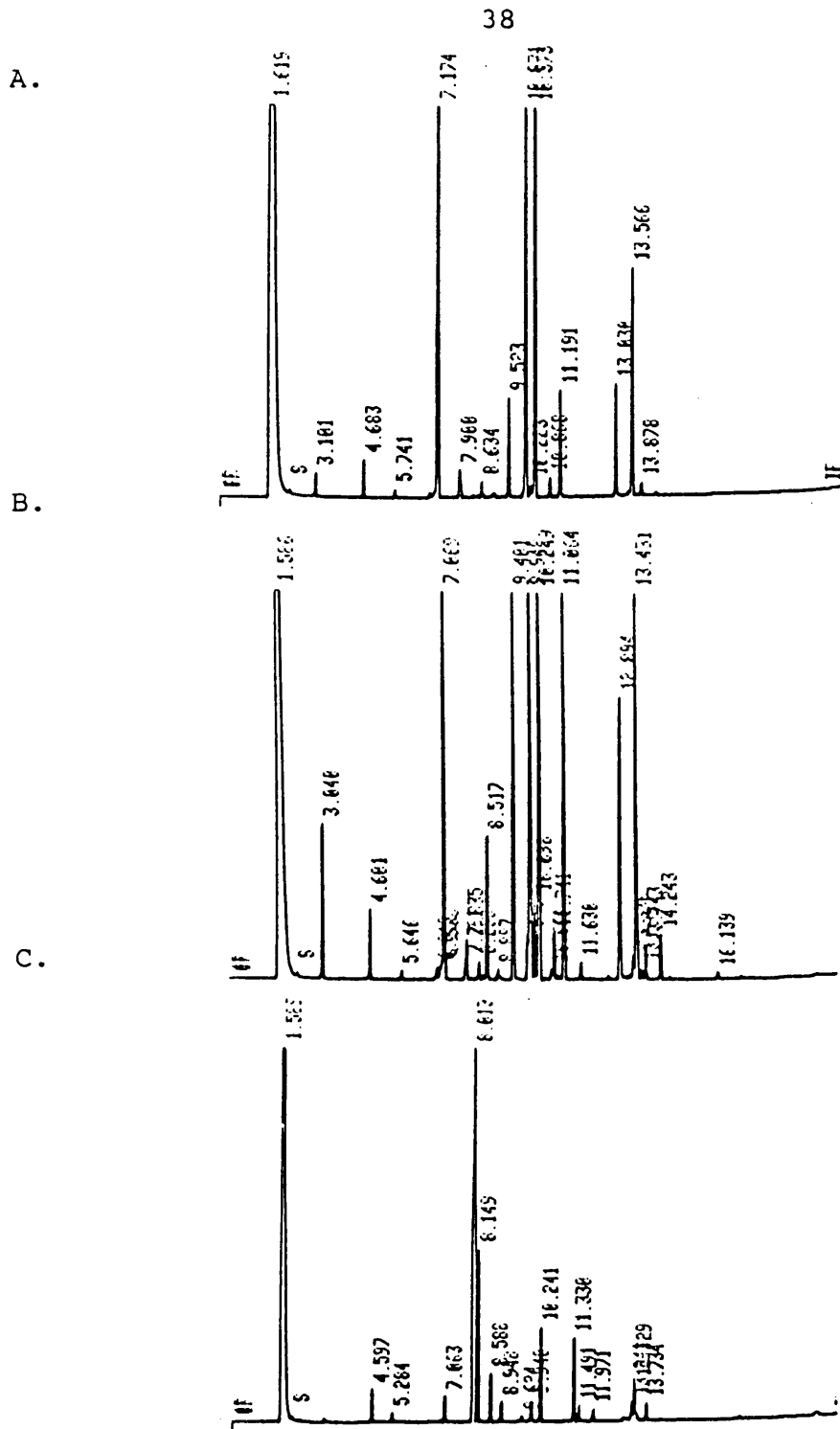


Figure 7. Fatty acid patterns of type strains of *Fusobacterium* species. A = *F. nucleatum* VPI 4355^T. B = *F. perfoetens* VPI 11077^T. C = *F. prausnitzii* VPI C13-51-1^T.

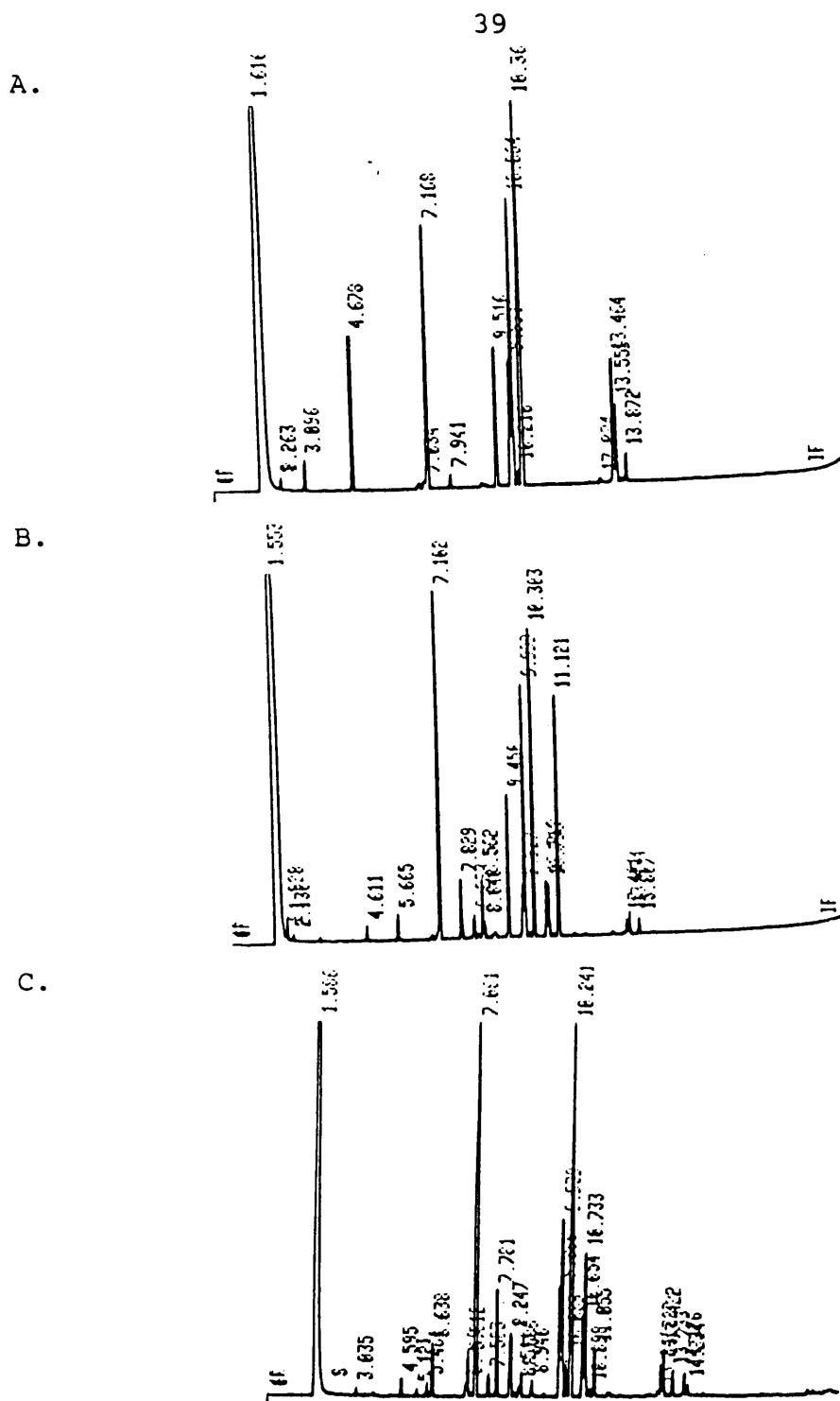


Figure 8. Fatty acid patterns of type strains of *Fusobacterium* species. A = *F. russii* VPI 0307^T. B = *F. sulci* VPI D45A-29A^T. C = *F. varium* VPI 0501^T.



Figure 9. Fatty acid patterns of two strains of *Fusobacterium nucleatum*.

File: C:\MSDC\F06619476
 bottles: 59
 ID: 5941
 Name: B-FUSO-NUCLE (D1466-20 JL 10-23M)

*** SAMPLE (ANALOGUE) ***

Date of report: 20-Nov-86 05:09:09
 Date of run: 20-Nov-86 05:05:09

RT	Area	Ar/Ht	Response	ECL	Name	Area %	Comment 1	Comment 2
1.502	4055000	0.081	...	6.993	SOLVENT PEAK	
3.045	2658	0.035	1.144	10.061	10:0	0.69	ECL deviates 0.001	Reference 0.010
4.060	4525	0.035	1.045	11.998	12:0	1.33	ECL deviates -0.002	Reference 0.004
5.720	2173	0.035	1.011	12.929	13:1 AT 12-13	0.94	ECL deviates -0.002	
6.925	2185	0.038	0.905	13.814	13:0 ISD 20h	0.62	ECL deviates 0.000	
7.137	100720	0.039	0.980	14.000	14:0	26.46	ECL deviates 0.000	Reference 0.004
7.476	13266	0.050	0.969	14.473	Sum In Feature 2	3.76	ECL deviates 0.003	13:0 30h/15:1 1/h
8.127	894	0.041	0.900	14.622	15:0 ISD	0.23	ECL deviates 0.001	Reference 0.005
8.307	1913	0.047	...	14.780	
8.844	6251	0.042	...	14.950	
9.017	730	0.042	...	15.178	
9.559	10294	0.044	0.951	15.469	Sum In Feature 3	4.26	ECL deviates -0.001	14:0 30h/10:1 ISD 1
10.016	1730	0.036	0.947	15.775	16:1 b	0.46	ECL deviates 0.001	
10.007	57197	0.040	0.947	15.816	16:1 CIS 9	16.41	ECL deviates 0.001	
10.242	1422	0.045	0.946	15.909	16:1 c	0.39	ECL deviates 0.001	
10.374	72796	0.045	0.945	16.000	16:0	20.46	ECL deviates 0.000	Reference 0.003
10.697	6658	0.045	...	16.265	
11.216	21456	0.044	0.940	16.473	Sum In Feature 5	8.66	ECL deviates -0.003	17:1 ISD 1/ANTEL 6
13.056	15737	0.048	0.933	17.520	16:0 30h	4.63	ECL deviates -0.000	
16.500	1165	0.044	0.932	17.770	18:1 CIS 9	0.32	ECL deviates 0.001	
18.594	25822	0.049	0.932	17.823	Sum In Feature 7	7.05	ECL deviates 0.001	18:1 CIS 11/t 9/t 6
18.908	2255	0.048	0.932	18.000	18:0	0.62	ECL deviates -0.000	Reference 0.002
14.511	2656	0.047	...	18.264	
*****	13266	SUNNED FEATURE 2	3.76	15:1 ISD 1/13:0 30h	13:0 30h/15:1 1 1/h
*****	15:1 ISD 1/13:0 30h	
*****	10294	SUNNED FEATURE 3	4.26	16:1 ISD 1/14:0 30h	14:0 30h/16:1 ISD 1
*****	31466	SUNNED FEATURE 5	8.66	17:1 ISD 1/ANTEL 6	17:1 ANTEISD 6/1 1
*****	25822	SUNNED FEATURE 7	7.05	18:1 CIS 11/t 9/t 6	18:1 THINS 9/t/c/11
*****	18:1 THINS 6/14/c/11	

Percent Ar	Total Area	Named Area	% named	Total Amt	Nbr Ref	Ref	ECL Shift	ECL Deviation
9.250000	375265	336647	90.04	341597	6	0.005	0.001	

NAME0 (Rev 1.2) Fusobacterium 0.339
 F. nucleatus 0.339
 NAME0 (Rev 1.2) ** NO MATCH **

Figure 10. Sample printout of analysis of sample by HP 5898A RT, retention time; Area, area of the peak; Ar/Ht, Area/Height ratio of the peak; ECL, Equivalent Chain Length; Name, Name of the fatty acid peak; Area %, % of total area named after a quantitative correction; Comment, any comments about the fatty acid peak; Solvent Ar, amount of area for hexane/ether peak; Total Area, Total area count of peaks that elute between C9:0 and C20:0; Named Area, (total area - unnamed area) to give total named area; % Named, % of total area named; Most Likely Matches, list of the most likely matches for the sample run; Similarity, Similarity index between the sample and the most likely matches.

patterns (based on these same strains) indicated that identification accuracy ranged from 17 to 100% (mean 83.1%) depending upon the species (Table 3). These initial analyses have served as a basis for further improvements in the library to produce greater accuracy.

Table 3. Results of CFA analysis showing the species analyzed, the no. of analyses, the no. correctly identified, and the % correct identification.

Species	No. analyzed	No. correctly identified (1st choice)	% correctly identified (1st choice)
<u>Capnocytophaga</u>			
<u>gingivalis</u>	28	27	96.4
<u>ochracea</u>	24	23	95.8
<u>sputigena</u>	16	16	100.0
<u>Fusobacterium</u>			
<u>alocis</u>	17	8	47.0
<u>gonidiaformans</u>	19	15	78.9
<u>mortiferum</u>	19	18	94.7
<u>naviforme</u>	23	15	65.2
<u>necrogenes</u>	19	19	100.0
<u>necrophorum</u>	25	24	96.0
<u>nucleatum</u>	25	25	100.0
<u>perfoetens</u>	22	22	100.0
<u>prausnitzii</u>	17	11	64.7
<u>russii</u>	52	9	17.3
<u>sulci</u>	22	21	95.4
<u>varium</u>	21	20	95.2

Discussion

The PAGE analysis showed low percentages of first choice correct identifications (mean 55.4%). There are many factors that could have caused the low results. With the PAGE procedure the variation in the density of the gels is a problem. Corrections for this variation based on the reference pattern reduce, but do not eliminate, the problem. Further, isozymes and possibly culture age and conditions may effect the relative position of some protein bands. A variation in the protein patterns of strains within species could be a useful epidemiological tool, but if the variation is too great the strain could be identified as a member of another species. In work done subsequent to the work presented here, it was found that seven of the strains that were analyzed were labeled incorrectly (Table 4), or did not belong to a named species of bacteria. Because each strain is compared to each other strain in the library, instead of an average protein pattern for each species, these incorrectly labeled strains could significantly lower the percentage of correct identification.

Table 4. Strains of Fusobacterium and Capnocytophaga that were reclassified

VPI strain no.	Original species designation	New species designation
D128D-22	<u>C. ochracea</u>	<u>C. gingivalis</u>
D22B-13	<u>F. alocis</u>	<u>F. sulci</u>
D126C-20	"	"
D45B-4	"	"
8055	<u>F. russii</u>	<u>F. alocis</u>
12936-E	"	"
11018	"	"

The results from the analyses of PAGE showed a higher % correct identification than that with the CFA analyses for F. alocis, F. russii, and F. naviforme. This could have been due to the fact that many species with unknown identification were moved into F. russii for the CFA analysis, and not for the PAGE analysis. Also, not all of the same strains were analyzed on each system. The PAGE analyses had fewer strains whose species designation was questionable, therefore giving better accuracy in some instances.

At this time, because of these problems and the lack of further automation, the PAGE identification procedure does not seem to be a candidate for routine use in a clinical laboratory. Although, the protein patterns created by strains within a species have proven to be a useful tool for a tentative species identification when examined by eye, automated identification using this procedure would still require more development.

The percentages of first choice correct identifications for the cellular fatty acid analysis ranged from 17.3% to 100%. Subsequent evaluation of some of the strains showed that they were incorrectly labeled (Table 4). Because most of the species analyzed do not ferment, the identification is based on the results of the soluble cellular protein patterns obtained by PAGE, the cellular fatty acid patterns

obtained by gas chromatography, and some biochemical tests. The changes that were made in the species shown in Table 4 were also based on those results. Also, some of the analyses were not good due to insufficient growth, or a procedural problem such as insufficient saponification, methylation, or extraction of the fatty acids during the sample preparation. Another problem was contamination of the extract from the rubber stoppers that cap the vials. This contamination resulted in some peaks that were not cellular components.

From work done subsequent to the initial analyses presented here, it has been found that when more strains and analyses are included in the library, the accuracy of the identification generally improves. Table 5 presents recent data for all strains of Fusobacterium and Capnocytophaga that have now been analyzed by the HP 5898A, and the number of questionable analyses included in the data base for each species. During this work, it has been found that the library contains some strains that do not belong to any named species of Fusobacterium or Capnocytophaga. This was the reason for some of the initial low % correct identifications, especially F. russii and F. alocis. When these questionable strains (or questionable analytical runs) are deleted from the CFA library entries, all (100%) of the strains of each species were correctly identified.

Table 5. Data for all strains of Fusobacterium and Capnocytophaga analyzed by the HP 5898A

Species designation	No. of strains analyzed	Total no. of analyses	No. of questioned strains
<u>C. gingivalis</u>	49	63	3
<u>C. ochracea</u>	49	63	2
<u>C. sputigena</u>	20	26	0
<u>F. alocis</u>	12	25	8
<u>F. gonidiaformans</u>	15	19	4
<u>F. mortiferum</u>	14	21	4
<u>F. naviforme</u>	14	28	12
<u>F. necrogenes</u>	1	19	0
<u>F. necrophorum</u>	20	27	3
<u>F. nucleatum</u>	32	35	1
<u>F. perfoetens</u>	2	22	0
<u>F. prausnitzii</u>	15	21	8
<u>F. russii</u>	21	48	41
<u>F. sulci</u>	14	25	2
<u>F. varium</u>	20	23	2

Some of the questioned strains might be members of Fusobacterium periodonticum Slots, Potts, and Mashimo 1984, 270 a recently described species that was not included in this data base (because no strain was available when this work was initiated). It is possible, but unlikely, that some of these strains that do not match the existing library are members of another recently described species, Fusobacterium simiae Slots, and Potts 1982, 193, from monkeys, because most bacterial species are host specific, and most of the strains analyzed were of human origin. Development of a library reference pattern for each of these two species may be the easiest way to determine whether or not any of the questioned strains are members of these species.

With further work in characterizing these strains, and also with more strains of the known species analyzed, the accuracy of the analysis has improved, and may be expected to improve further. The library for anaerobic bacteria is still being developed, but already it promises to provide a simple and rapid method for anaerobic bacterial identification.

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Appendix 1. Formulas for media used.

BHI-C - Brain Heart Infusion Broth supplemented with yeast extract, vitamin K₁, hemin, and 0.1% calcium carbonate.

Brain Heart Infusion Broth (dehydrated)	3.7 g
Yeast extract	0.5 g
Distilled water	100.0 ml
Resazurin solution	0.4 ml
Boil, cool, add:	
Cysteine HCL·H ₂ O	0.05 g
Hemin solution	1.0 ml
Vitamin K ₁	0.02 ml
Calcium carbonate	0.1g

PYG broth

Peptone	0.5 g
Trypticase	0.5 g
Yeast extract	1.0 g
Resazurin solution	0.4 ml
Salts solution	4.0 ml
Glucose	1.0 g
Distilled water	100.0 ml
Boil, cool, add:	
Cysteine HCL·H ₂ O	0.5 g
Hemin solution	1.0 ml
Vitamin K ₁	0.02 ml

Hemin solution: Dissolve 50 mg hemin in 1 ml 1 N NaOH; make to 100 ml with distilled water. Autoclave at 121°C for 15 min.

Vitamin K₁: Dissolve 0.15 ml of Vitamin K₁ in 30 ml of 95% ethanol. Do not sterilize since it is added before autoclaving.

Appendix 2. Numerical analysis printouts from HP 5898A for type strains of species of Fusobacterium and Capnocytophaga

 ID: 8153 B-CAMPN-GINGI (14457* AR 10-24H F) Date of run: 04-JUN-07 12:20:16
 Bottle: 30 SAMPLE [ANAMEROBE]

RT	Area	Ar/Ht	Respon	ECL	Name	%	Comment 1	Comment 2
1.609	43793000	0.087	. . .	6.984	SOLVENT PEAK		(min rt	
5.347	8270	0.036	1.016	12.614	13:0 ISO FAME	4.44	ECL deviates 0.000	Reference -0.008
6.547	2153	0.045	0.989	13.566	Sum In Feature 3	1.13	ECL deviates -0.002	UN 13.570
7.134	2039	0.039	0.979	14.000	14:0 FAME	1.06	ECL deviates 0.000	Reference -0.009
8.086	135160	0.042	0.967	14.623	15:0 ISO FAME	69.11	ECL deviates 0.000	Reference -0.009
8.224	2655	0.042	0.965	14.713	15:0 ANTEISO FAME	1.36	ECL deviates -0.001	Reference -0.010
8.664	1175	0.042	0.961	15.001	15:0 FAME	0.60	ECL deviates 0.001	Reference -0.008
10.321	4433	0.045	0.949	15.999	16:0 FAME	2.22	ECL deviates -0.001	Reference -0.010
10.555	5500	0.048	0.948	16.135	15:0 ISO 3OH FAME	1.79	ECL deviates 0.000	
11.329	1496	0.055	. . .	16.582			
11.413	905	0.045	0.944	16.631	17:0 ISO FAME	0.45	ECL deviates 0.001	Reference -0.009
12.339	917	0.055	0.941	17.162	Sum In Feature 9	0.46	ECL deviates 0.005	UN 17.157 DMA
12.972	7845	0.050	0.941	17.521	16:0 3OH FAME	3.90	ECL deviates -0.001	
13.819	1093	0.057	0.940	18.000	18:0 FAME	0.54	ECL deviates -0.000	Reference -0.011
14.104	26028	0.049	0.940	18.162	Sum In Feature 11	12.94	ECL deviates -0.001	17:0 ISO 3OH FAME
*****	2153	SUMMED FEATURE 3	1.13	15:0 ISO ALDE ??	UN 13.570
*****	917	SUMMED FEATURE 9	0.46	16:0 ISO 3OH FAME	UN 17.157 DMA
*****	26028	SUMMED FEATURE 11	12.94	17:0 ISO 3OH FAME	18:2 DMA

Solvent Ar	Total Area	Named Area	% Named	Total Amt	Nbr Ref	ECL Deviation	Ref ECL Shift
43793000	197749	196253	99.24	189118	8	0.002	0.009

ID: 8335 B-CAPN-UCHAR (9775* DW 10-18H F) Date of run: 23-JUN-07 07:07:16
 Bottle: 31 SAMPLE (ANNEAUDE)

RT	Area	Hr/Ht	Respon	ECL	Name	%	Comment 1	Comment 2
1.509	42631000	0.085	. . .	6.989	SOLVENT PEAK	< min rt	
5.296	2563	0.035	1.020	12.614	13:0 ISO FAME	0.60	ECL deviates -0.000	Reference 0.000
5.664	1068	0.050	1.009	12.942	Sum In Feature 1	0.25	ECL deviates -0.004	11:1 2OH FAME
6.494	8476	0.055	0.991	13.569	Sum In Feature 3	1.91	ECL deviates 0.001	UN 13.570
7.075	1530	0.046	0.981	14.000	14:0 FAME	0.34	ECL deviates 0.000	Reference -0.001
8.023	139610	0.042	0.968	14.624	15:0 ISO FAME	40.90	ECL deviates 0.001	Reference -0.001
8.159	45278	0.042	0.966	14.713	15:0 ANTEISO FAME	9.96	ECL deviates -0.001	Reference -0.003
8.598	16572	0.043	0.961	15.001	15:0 FAME	3.58	ECL deviates 0.001	Reference -0.001
8.959	1201	0.051	. . .	15.219
9.634	7237	0.043	0.952	15.626	16:0 ISO FAME	1.57	ECL deviates -0.001	Reference -0.002
9.951	897	0.048	0.950	15.818	16:1 CIS 9 FAME	0.19	ECL deviates -0.000	
10.151	844	0.047	. . .	15.939
10.252	36589	0.045	0.948	15.999	16:0 FAME	8.33	ECL deviates -0.001	Reference -0.002
10.485	1521	0.050	0.946	16.134	15:0 ISO 3OH FAME	0.33	ECL deviates -0.001	
11.259	1644	0.053	. . .	16.582
11.341	42513	0.046	0.942	16.630	17:0 ISO FAME	9.12	ECL deviates -0.000	Reference -0.002
11.501	3462	0.045	0.942	16.722	17:0 ANTEISO FAME	0.74	ECL deviates -0.001	
11.982	1011	0.049	0.940	17.001	17:0 FAME	0.22	ECL deviates 0.001	Reference -0.001
12.261	1059	0.059	0.939	17.159	Sum In Feature 9	0.23	ECL deviates 0.002	UN 17.157 DMA
12.898	20643	0.054	0.938	17.519	16:0 3OH FAME	4.41	ECL deviates -0.003	
13.253	760	0.045	0.938	17.720	18:2 CIS 9,12 FAME	0.16	ECL deviates -0.003	
13.340	2335	0.071	0.938	17.770	18:1 CIS 9 FAME	0.50	ECL deviates -0.001	
14.829	74569	0.052	0.938	18.162	Sum In Feature 11	15.93	ECL deviates -0.001	17:0 ISO 3OH FAME
14.688	3462	0.059	0.939	18.537	17:0 3OH ? FAME	0.74	ECL deviates -0.002	
*****	1068	SUMMED FEATURE 1	0.25	13:1 CIS 12 FAME	14:0 ALDE
*****
*****	8476	SUMMED FEATURE 3	1.91	15:0 ISO ALDE ??	UN 13.570
*****	1059	SUMMED FEATURE 9	0.23	16:0 ISO 3OH FAME	UN 17.157 DMA
*****	74569	SUMMED FEATURE 11	15.93	17:0 ISO 3OH FAME	18:2 DMA

Solvent Ar	Total Area	Named Area	% Named	Total Amt	Nbr Ref	ECL Deviation	Ref ECL Shift
42631000	462844	450955	99.16	439171	9	0.001	0.002

 ID: 8003 B-CAPN-SPUTI (14458* JL 10 48H .5) Date of run: 27-MAY-87 12:02:45
 Bottle: 09 SAMPLE (ANAKRÜBE) Date edited: 22-JUL-87 14:39:27

RT	Area	Ar/Ht	Respon	ECL	Name	%	Comment 1	Comment 2
1.603	43000000	0.087	. . .	6.984	SOLVENT PEAK	< min rt	
5.331	7335	0.036	1.021	12.614	13:0 ISO FAME	1.36	ECL deviates 0.000	Reference -0.005
5.700	2508	0.050	1.011	12.940	Sum In Feature 1	0.47	ECL deviates 0.004	14:0 ALDE
6.535	11648	0.060	0.993	13.569	Sum In Feature 3	2.10	ECL deviates 0.001	UN 13.570
7.116	8149	0.041	0.982	13.999	14:0 FAME	1.45	ECL deviates -0.001	Reference -0.005
8.071	361550	0.042	0.968	14.625	15:0 ISO FAME	63.59	ECL deviates 0.002	Reference -0.003
8.205	36991	0.042	0.960	14.713	15:0 ANEISO FAME	6.49	ECL deviates -0.001	Reference -0.006
8.645	20691	0.043	0.961	15.001	15:0 FAME	3.61	ECL deviates 0.001	Reference -0.004
8.829	807	0.046	0.959	15.112	15:0 ISO DMA	0.14	ECL deviates -0.000	Reference -0.005
9.634	2118	0.043	0.951	15.627	16:0 ISO FAME	0.37	ECL deviates 0.000	Reference -0.005
10.001	842	0.046	0.949	15.818	16:1 CIS 9 FAME	0.15	ECL deviates 0.000	
10.301	18691	0.045	0.946	15.999	16:0 FAME	3.21	ECL deviates -0.001	Reference -0.006
10.534	8008	0.048	0.945	16.134	15:0 ISO 3UH FAME	1.37	ECL deviates -0.001	
11.178	1603	0.055	0.941	16.506	15:0 3UH FAME	0.27	ECL deviates 0.000	
11.393	8637	0.045	0.940	16.630	17:0 ISO FAME	1.48	ECL deviates 0.000	Reference -0.005
12.949	50620	0.049	0.935	17.519	16:0 3UH FAME	5.20	ECL deviates -0.003	
13.304	578	0.042	0.934	17.721	18:2 CIS 9,12 FAME	0.10	ECL deviates -0.002	
13.592	1087	0.045	0.934	17.771	18:1 CIS 9 FAME	0.16	ECL deviates -0.000	
13.797	1094	0.058	0.933	18.000	18:0 FAME	0.19	ECL deviates 0.000	Reference -0.006
14.082	46912	0.050	0.933	18.162	Sum In Feature 11	7.89	ECL deviates -0.001	17:0 ISO 3UH FAME
14.751	2207	0.081	0.933	18.543	17:0 3UH ? FAME	0.37	ECL deviates 0.004	
*****	2508	SUMMED FEATURE 1	0.47	13:1 CIS 12 FAME	14:0 ALDE
*****	11:1 2UH FAME	
*****	11648	SUMMED FEATURE 3	2.10	15:0 ISO ALDE 77	UN 13.570
*****	46912	SUMMED FEATURE 11	7.89	17:0 ISO 3UH FAME	18:2 DMA

Solvent Ar	Total Area	Named Area	% Named	Total Amt	Nbr Ref	ECL Deviation	Ref ECL Shift
43000000	571736	571736	100.00	550440	10	0.002	0.005

 ID: 8703 B-FUSD-ALUCI (D40B-5* DW 20-74H-.5R C F) Date of run: 26-JUL-87 16:38:08
 Bottle: 4 SAMPLE (ANAEKDBE)

RT	Area	Ar/Ht	Respon	ECL	Name	%	Comment 1	Comment 2
1.576	34228000	0.068	. . .	7.023	SOLVENT PEAK	< min rt	
2.490	459	0.025	. . .	8.927	< min rt	
3.003	2036	0.029	1.154	9.995	10:0 FAME	3.32	ECL deviates -0.005	
3.310	724	0.029	. . .	10.461		
3.608	1027	0.033	. . .	10.910		
4.550	4989	0.034	1.054	11.999	12:0 FAME	5.34	ECL deviates -0.001	Reference -0.012
5.073	1315	0.035	1.036	12.470	11:0 DMA	1.30	ECL deviates 0.001	Reference -0.011
6.753	904	0.043	. . .	13.813		
7.002	9526	0.038	0.986	13.999	14:0 FAME	9.54	ECL deviates -0.001	Reference -0.012
7.720	700	0.038	0.974	14.473	14:0 DMA	0.69	ECL deviates 0.001	Reference -0.010
8.444	4685	0.042	0.963	14.950	16:0 ALDE	4.58	ECL deviates -0.001	Reference -0.012
8.881	3523	0.050	. . .	15.219		
9.801	1842	0.045	0.949	15.776	16:1 CIS 7 FAME . .	1.77	ECL deviates 0.002	
9.868	6509	0.047	0.948	15.817	16:1 CIS 9 FAME . .	6.27	ECL deviates -0.001	
10.170	19540	0.044	0.945	16.000	16:0 FAME	18.76	ECL deviates -0.000	Reference -0.012
10.661	1543	0.043	0.941	16.285	16:1 CIS 9 DMA . . .	1.48	ECL deviates -0.000	
10.983	10432	0.044	0.939	16.471	16:0 DMA	17.58	ECL deviates 0.000	Reference -0.011
11.545	1428	0.072	0.936	16.797	Sum In Feature 8 . .	1.36	ECL deviates 0.003	17:1 CIS 9 FAME
12.909	1482	0.049	. . .	17.576		
13.163	3063	0.047	0.929	17.720	18:2 CIS 9,12 FAME .	2.89	ECL deviates -0.003	
13.249	7616	0.051	0.928	17.769	18:1 CIS 9 FAME . .	7.18	ECL deviates -0.002	
13.543	5490	0.051	0.928	17.822	Sum In Feature 10 . .	5.18	ECL deviates -0.002	18:1c11/19/16 FAME
13.656	4419	0.050	0.927	18.000	18:0 FAME	4.16	ECL deviates 0.000	Reference -0.011
14.046	1720	0.054	0.927	18.222	18:1 CIS 9 DMA . . .	1.62	ECL deviates -0.002	
14.156	3592	0.050	0.927	18.284	18:1 CIS 11 DMA . .	3.38	ECL deviates -0.001	
14.474	3739	0.053	0.926	18.465	18:0 DMA	3.52	ECL deviates -0.001	
*****	1428	SUMMED FEATURE 8 . .	1.36	17:1 CIS 9 FAME	17:2 FAME @ 16.801
*****	5490	SUMMED FEATURE 10 . .	5.18	18:1c11/19/16 FAME	UN 17.834
Solvent Ar	Total Area	Named Area	% Named	Total Amt	Nbr Ref	ECL Deviation	Ref ECL Shift	
3-2228000	110644	102984	93.08	98462	8	0.002	0.011	

 ID: 5721 B-FUSD-GONID (U482A-1* JL 1U-22H-.5R) Date of run: 08-JAN-87 10:59:05
 Bottle: 3 SAMPLE (ANWERDGE) Date edited: 03-AUG-87 16:49:52

RT	Area	Hr/Ht	Respon	ECL	Name	%	Comment 1	Comment 2
1.506	37402000	0.074	. . .	7.004	SOLVENT PEAK	< min rt	
2.228	593	0.024	. . .	8.299	< min rt	
3.072	2259	0.029	1.191	10.001	10:0 FAME	1.44	ECL deviates 0.001	Reference -0.002
3.700	1612	0.029	. . .	10.916		
4.609	8990	0.033	1.083	11.999	12:0 FAME	5.25	ECL deviates -0.001	Reference -0.004
6.663	2340	0.057	. . .	13.767		
6.926	3995	0.038	. . .	13.813		
7.045	1639	0.039	1.002	13.900	14:1 CIS 9 FAME	0.91	ECL deviates -0.002	
7.179	53564	0.040	0.999	13.998	14:0 FAME	28.84	ECL deviates -0.002	Reference -0.005
7.913	772	0.034	0.983	14.474	14:0 DMA	0.41	ECL deviates 0.002	Reference -0.001
7.957	1927	0.043	. . .	14.503		
9.540	16962	0.044	0.952	15.487	Sum In Feature 5	8.70	ECL deviates -0.002	14:0 30H FAME
10.019	11792	0.044	0.945	15.774	16:1 CIS 7 FAME	6.00	ECL deviates -0.000	
10.092	36145	0.045	0.944	15.818	16:1 CIS 9 FAME	18.38	ECL deviates -0.000	
10.243	2497	0.045	0.941	15.908	16:1 CIS 11 FAME	1.27	ECL deviates -0.001	
10.396	50464	0.044	0.939	15.999	16:0 FAME	25.54	ECL deviates -0.001	Reference -0.005
11.219	1212	0.052	0.929	16.472	16:0 DMA	0.61	ECL deviates 0.001	Reference -0.003
13.059	940	0.049	0.910	17.520	16:0 30H FAME	0.46	ECL deviates -0.002	
13.504	1159	0.046	0.907	17.770	18:1 CIS 9 FAME	0.57	ECL deviates -0.001	
13.590	2221	0.049	0.907	17.823	Sum In Feature 10	1.09	ECL deviates -0.001	18:1c11/t9/t6 FAME
13.910	1113	0.051	0.904	17.999	18:0 FAME	0.54	ECL deviates -0.001	Reference -0.005
*****	16962	SUMMED FEATURE 5	8.70	15:0 DMA	14:0 30H FAME
*****	2221	SUMMED FEATURE 10	1.09	18:1c11/t9/t6 FAME	UN 17.834

Solvent Ar	Total Area	Named Area	% Named	Total Amt	Nbr Ref	ECL Deviation	Ref ECL Shift
37402000	201633	191759	95.10	185558	7	0.001	0.004

ID: 6049 B-FUSO-MORTI (4123A-3) JL 10-24H
 Bottle: 26 SAMPLE (ANAEKQUE)

Date of run: 30-JAN-87 09:53:42
 Date edited: 01-MAR-88 00:00:00

RI	Area	Ar/Mt	Respon	ECL	Name	%	Comment 1	Comment 2
1.569	32626000	0.077	. . .	7.000	SOLVENT PEAK	< min rt	
3.053	734	0.028	1.156	10.000	10:0 FAME	0.61	ECL deviates 0.000	Reference 0.001
3.673	471	0.029	. . .	10.919		
4.624	2341	0.033	1.050	12.000	12:0 FAME	1.77	ECL deviates 0.000	Reference 0.000
5.674	2700	0.036	1.014	12.930	Sum In Feature 1 . . .	1.97	ECL deviates -0.000	13:1 CIS 12 FAME
7.104	35652	0.041	0.982	14.000	14:0 FAME	25.23	ECL deviates -0.000	Reference -0.001
7.828	7937	0.047	0.970	14.473	14:0 DMA	5.55	ECL deviates 0.001	Reference -0.000
8.294	1201	0.063	0.964	14.778	Sum In Feature 4 . . .	0.83	ECL deviates -0.002	15:2 FAME
8.558	3924	0.042	0.960	14.950	16:0 ALDE	2.72	ECL deviates -0.001	Reference -0.002
9.444	12523	0.045	0.951	15.488	Sum In Feature 5 . . .	8.58	ECL deviates -0.001	14:0 3OH FAME
9.920	1732	0.040	0.947	15.774	16:1 CIS 7 FAME . . .	1.18	ECL deviates 0.000	
9.991	23103	0.045	0.946	15.817	16:1 CIS 9 FAME . . .	15.75	ECL deviates -0.001	
10.143	1033	0.043	0.945	15.909	16:1 CIS 11 FAME . . .	0.70	ECL deviates -0.000	
10.294	27115	0.044	0.944	15.999	16:0 FAME	18.44	ECL deviates -0.001	Reference -0.002
10.708	1409	0.045	0.941	16.239	Sum In Feature 6 . . .	0.96	ECL deviates -0.001	16:1 CIS 7 DMA
10.787	2127	0.045	0.940	16.285	16:1 CIS 9 DMA	1.44	ECL deviates -0.000	
11.110	17358	0.044	0.939	16.471	16:0 DMA	11.74	ECL deviates 0.000	Reference -0.002
13.389	899	0.048	0.930	17.772	18:1 CIS 9 FAME . . .	0.60	ECL deviates 0.001	
13.481	2070	0.048	0.930	17.824	Sum In Feature 10 . . .	1.39	ECL deviates -0.000	18:1c11/19/16 FAME
13.793	771	0.049	0.930	18.000	18:0 FAME	0.52	ECL deviates 0.000	Reference -0.002
*****	2700	SUMMED FEATURE 1 . . .	1.97	13:1 CIS 12 FAME	14:0 ALDE
*****	11:1 2OH FAME	
*****	1201	SUMMED FEATURE 4 . . .	0.83	UN 14.762 15:2 ? FA	15:2 FAME
*****	15:1 CIS 7	
*****	12523	SUMMED FEATURE 5 . . .	8.58	15:0 DMA	14:0 3OH FAME
*****	1409	SUMMED FEATURE 6 . . .	0.96	15:0 ANTEL 3OH FAME	16:1 CIS 7 DMA
*****	2070	SUMMED FEATURE 10 . . .	1.39	18:1c11/19/16 FAME	UN 17.834
Solvent Ar	Total Area	Named Area	% Named	Total Amt	Nbr Ref	ECL Deviation	Ref ECL Shift	
38628000	145100	144629	99.68	138736	8	0.001	0.002	

ID: c067 B-FUSO-NAOIF (8906A* JL 10-20H)
 Bottle: 23 SAMPLE (ANNAEROGE)

Date of run: 19-MAR-87 02:40:32
 Date edited: 05-AUG-87 16:02:20

RT	Area	Ar/Ht	Respon	ECL	Name	%	Comment 1	Comment 2
1.619	41212000	0.082	. . .	6.985	SOLVENT PEAK	< min rt	
2.574	1464	0.034	. . .	8.927	< min rt	
3.102	2704	0.034	1.153	10.000	10:0 FAME	5.74	ECL deviates -0.000	Reference 0.003
3.418	1772	0.034	. . .	10.464		
4.686	2582	0.035	1.048	12.000	12:0 FAME	4.98	ECL deviates 0.000	Reference 0.002
5.742	1928	0.039	1.013	12.950	Sum In Feature 1 . . .	3.60	ECL deviates -0.000	13:1 CIS 12 FAME
6.927	1222	0.037	. . .	13.814		
7.178	24001	0.039	0.982	13.999	14:0 FAME	43.40	ECL deviates -0.001	Reference -0.001
7.904	4688	0.040	0.971	14.472	14:0 DMA	8.38	ECL deviates 0.000	Reference 0.000
8.378	1322	0.044	0.965	14.781	Sum In Feature 4 . . .	2.35	ECL deviates 0.001	15:2 FAME
8.638	685	0.040	0.961	14.951	16:0 ALDE	1.21	ECL deviates -0.000	Reference -0.001
10.025	2792	0.048	0.948	15.818	16:1 CIS 9 FAME . . .	4.87	ECL deviates 0.000	
10.328	7851	0.046	0.946	16.000	16:0 FAME	13.67	ECL deviates 0.000	Reference -0.001
10.822	3695	0.045	0.943	16.285	16:1 CIS 9 DMA	6.41	ECL deviates -0.000	
11.195	3102	0.045	0.941	16.471	16:0 DMA	5.37	ECL deviates 0.000	Reference -0.001
*****	1928	SUMMED FEATURE 1 . . .	3.60	13:1 CIS 12 FAME	14:0 ALDE
*****	11:1 20H FAME	
*****	1322	SUMMED FEATURE 4 . . .	2.35	UN 14.762 15:2 ? FA	15:2 FAME
*****	15:1 CIS 7	

Solvent Ar	Total Area	Named Area	% Named	Total Amt	Nbr Ref	ECL Deviation	Ref ECL Shift
41212000	58344	55350	94.87	54301	7	0.001	0.001

 ID: 7245 B-FUSO-RECNG (2368-1 JL 10-21H) Date of run: 01-APR-87 20:53:48
 Bottle: 8 SAMPLE (ANAEKUBE)

RT	Area	Ar/Ht	Respon	ECL	Name	%	Comment 1	Comment 2
1.576	40129000	0.080	. . .	6.991	SOLVENT PEAK	< min rt	
3.023	1969	0.030	1.147	10.000	10:0 FAME	0.72	ECL deviates 0.000	Reference 0.001
4.578	2486	0.034	1.045	12.000	12:0 FAME	0.83	ECL deviates 0.000	Reference 0.001
5.618	2574	0.039	1.011	12.929	Sum In Feature 1	0.83	ECL deviates -0.001	13:1 CIS 12 FAME
6.788	1916	0.045	. . .	13.813		
6.905	1155	0.037	0.983	13.900	14:1 CIS 9 FAME	0.36	ECL deviates -0.002	
7.057	50984	0.040	0.981	13.999	14:0 FAME	17.82	ECL deviates -0.001	Reference -0.001
7.757	10612	0.048	0.971	14.473	14:0 DMA	3.28	ECL deviates 0.001	Reference 0.000
8.221	2024	0.064	0.965	14.778	Sum In Feature 4	0.62	ECL deviates -0.002	15:2 FAME
8.366	1603	0.062	. . .	14.886		
8.484	4800	0.043	0.962	14.951	16:0 ALDE	1.47	ECL deviates -0.000	Reference -0.001
8.559	1640	0.042	0.961	15.000	15:0 FAME	0.50	ECL deviates 0.000	Reference -0.001
8.852	685	0.044	. . .	15.177		
9.366	22557	0.045	0.953	15.488	Sum In Feature 5	6.85	ECL deviates -0.001	14:0 3OH FAME
9.839	12482	0.043	0.949	15.774	16:1 CIS 7 FAME	3.78	ECL deviates 0.000	
9.911	61697	0.044	0.949	15.817	16:1 CIS 9 FAME	18.60	ECL deviates -0.001	
10.061	3220	0.042	0.948	15.908	16:1 CIS 11 FAME	0.97	ECL deviates -0.001	
10.213	80251	0.045	0.947	16.000	16:0 FAME	26.64	ECL deviates 0.000	Reference -0.001
10.625	3344	0.044	0.944	16.239	Sum In Feature 6	1.01	ECL deviates -0.001	16:1 CIS 7 DMA
10.705	5029	0.044	0.944	16.285	16:1 CIS 9 DMA	1.51	ECL deviates 0.000	
11.027	27109	0.045	0.942	16.472	16:0 DMA	8.14	ECL deviates 0.001	Reference -0.001
11.583	818	0.050	0.940	16.794	Sum In Feature 8	0.25	ECL deviates 0.000	17:1 CIS 9 FAME
12.856	1859	0.047	0.936	17.520	16:0 3OH FAME	0.55	ECL deviates -0.002	
13.296	3113	0.048	0.936	17.770	18:1 CIS 9 FAME	0.93	ECL deviates -0.001	
13.390	11798	0.048	0.936	17.823	Sum In Feature 10	3.52	ECL deviates -0.001	18:1c11/t9/t6 FAME
13.701	2544	0.048	0.935	18.000	18:0 FAME	0.76	ECL deviates -0.000	Reference -0.003
*****	2574	SUMMED FEATURE 1	0.83	13:1 CIS 12 FAME	14:0 ALDE
*****	11:1 2OH FAME	
*****	2024	SUMMED FEATURE 4	0.62	UN 14.762 15:2 ? FA	15:2 FAME
*****	15:1 CIS 7	
*****	22557	SUMMED FEATURE 5	6.85	15:0 DMA	14:0 3OH FAME
*****	3344	SUMMED FEATURE 6	1.01	15:0 ANTEI 3OH FAME	16:1 CIS 7 DMA
*****	818	SUMMED FEATURE 8	0.25	17:1 CIS 9 FAME	17:2 FAME @ 16.801
*****	11798	SUMMED FEATURE 10	3.52	18:1c11/t9/t6 FAME	UN 17.834

Solvent Ar	Total Area	Named Area	% Named	Total Amt	Nbr Ref	ECL Deviation	Ref ECL Shift
40129000	332243	328041	98.74	313725	9	0.001	0.001

ID: 6892 B-FUSO-NECRU (2891)* JL 10-20M Date of run: 19-Mar-87 05:10:33
 Bottle: 29 SAMPLE (ANAEROBE) Date edited: 03-AUG-87 17:04:43

RT	Area	Ar/Ht	Respon	ECL	Name	%	Comment 1	Comment 2
1.619	41303000	0.082	. . .	6.985	SOLVENT PEAK	< min rt	
3.103	1790	0.032	1.153	10.000	10:0 FAME	0.42	ECL deviates 0.000	Reference 0.004
3.725	521	0.034	. . .	10.914		
4.686	30232	0.034	1.048	11.999	12:0 FAME	6.49	ECL deviates -0.001	Reference 0.002
4.798	1561	0.035	. . .	12.098		
5.221	1197	0.038	1.030	12.470	11:0 DMA	0.25	ECL deviates 0.001	Reference 0.003
5.744	2354	0.037	1.013	12.930	Sum In Feature 1 . .	0.49	ECL deviates 0.000	13:1 CIS 12 FAME
5.824	1260	0.037	1.011	13.000	13:0 FAME	0.26	ECL deviates 0.000	Reference 0.002
6.864	2578	0.038	. . .	13.766		
6.929	5474	0.039	. . .	13.814		
7.044	2682	0.041	0.984	13.899	14:1 CIS 9 FAME . .	0.54	ECL deviates -0.003	
7.180	87760	0.041	0.982	13.999	14:0 FAME	17.65	ECL deviates -0.001	Reference 0.001
7.831	960	0.041	. . .	14.423		
7.906	16795	0.051	0.971	14.472	14:0 DMA	3.34	ECL deviates 0.000	Reference 0.002
8.379	3669	0.081	0.965	14.780	Sum In Feature 4 . .	0.72	ECL deviates 0.000	15:2 FAME
8.497	1387	0.063	0.963	14.857	15:1 CIS 9/t 8 FAME	0.37	ECL deviates 0.000	
8.640	5046	0.043	0.961	14.950	16:0 ALDE	0.99	ECL deviates -0.001	Reference 0.001
8.716	2994	0.043	0.960	15.000	15:0 FAME	0.59	ECL deviates -0.000	Reference 0.001
9.010	1209	0.046	. . .	15.176		
9.529	40015	0.046	0.952	15.488	Sum In Feature 5 . .	7.81	ECL deviates -0.001	14:0 30H FAME
10.004	22620	0.044	0.949	15.774	16:1 CIS 7 FAME . .	4.39	ECL deviates -0.000	
10.078	67729	0.045	0.948	15.818	16:1 CIS 9 FAME . .	13.15	ECL deviates 0.000	
10.228	3771	0.045	0.947	15.908	16:1 CIS 11 FAME . .	0.73	ECL deviates -0.001	
10.381	122230	0.045	0.946	16.000	16:0 FAME	23.68	ECL deviates 0.000	Reference 0.001
10.796	7180	0.046	0.943	16.239	Sum In Feature 6 . .	1.39	ECL deviates -0.001	16:1 CIS 7 DMA
10.874	8284	0.045	0.943	16.284	16:1 CIS 9 DMA . . .	1.60	ECL deviates -0.001	
11.201	45757	0.050	0.941	16.473	16:0 DMA	8.82	ECL deviates 0.002	Reference 0.003
11.753	2216	0.071	0.938	16.791	Sum In Feature 8 . .	0.43	ECL deviates -0.003	17:1 CIS 9 FAME
12.118	1626	0.061	0.937	17.001	17:0 FAME	0.31	ECL deviates 0.001	Reference 0.002
12.983	3413	0.109	. . .	17.489		
13.384	2652	0.050	0.933	17.716	18:2 CIS 9,12 FAME .	0.51	ECL deviates -0.007	
13.479	3735	0.047	0.933	17.769	18:1 CIS 9 FAME . .	0.71	ECL deviates -0.002	
13.573	12946	0.049	0.933	17.823	Sum In Feature 10 . .	2.47	ECL deviates -0.001	18:1c11/t9/t6 FAME
13.865	3783	0.048	0.933	17.999	18:0 FAME	0.72	ECL deviates -0.001	Reference 0.000
14.384	1216	0.061	0.932	18.282	18:1 CIS 11 DMA . .	0.23	ECL deviates -0.003	
15.140	4850	0.051	0.932	18.710	22:0 NMC	0.93	ECL deviates -0.002	
16.876	6446	0.049	. . .	19.702		
18.579	7245	0.050	. . .	20.680		
*****	2354	SUMMED FEATURE 1 . .	0.49	13:1 CIS 12 FAME	14:0 ALDE
*****	11:1 20H FAME	
*****	3669	SUMMED FEATURE 4 . .	0.72	UN 14.762 15:2 ? FA	15:2 FAME
*****	15:1 CIS 7	
*****	40015	SUMMED FEATURE 5 . .	7.81	15:0 DMA	14:0 30H FAME
*****	7180	SUMMED FEATURE 6 . .	1.39	15:0 FAME 30H FAME	16:1 CIS 7 DMA
*****	2216	SUMMED FEATURE 8 . .	0.43	17:1 CIS 9 FAME	17:2 FAME @ 16.801
*****	12946	SUMMED FEATURE 10 . .	2.47	18:1c11/t9/t6 FAME	UN 17.834

Solvent Ar	Total Area	Named Area	% Named	Total Amt	Nbr Ref	ECL Deviation	Ref ECL Shift
41303000	530438	568276	95.82	488242	12	0.002	0.002

ID: 4250 B-FUSO-NUCLE (4355 JL 2-18H) Date of run: 04-OCT-86 07:20:55
 Bottle: 23 SAMPLE () Date edited: 04-AUG-87 13:48:55

RT	Area	Ar/Ht	Respon	ECL	Name	%	Comment 1	Comment 2
1.549	45485000	0.090	. . .	6.995	SOLVENT PEAK	< min rt	
1.919	611	0.024	. . .	7.750	< min rt	
2.189	497	0.026	. . .	8.302	< min rt	
2.824	807	0.046	1.150	9.602	10:0 ISO FAME	0.44	ECL deviates -0.001	Reference 0.006
3.020	2216	0.031	1.132	10.001	10:0 FAME	1.20	ECL deviates 0.001	Reference 0.007
4.601	3650	0.032	1.054	12.000	12:0 FAME	1.84	ECL deviates 0.000	Reference 0.005
5.656	1039	0.035	1.025	12.929	Sum In Feature 1	0.51	ECL deviates -0.001	13:1 CIS 12 FAME
7.073	51065	0.039	0.997	13.999	14:0 FAME	24.28	ECL deviates -0.001	Reference 0.003
7.821	3207	0.042	0.986	14.473	14:0 DMA	1.51	ECL deviates 0.001	Reference 0.006
8.290	828	0.041	0.979	14.779	Sum In Feature 4	0.39	ECL deviates -0.001	15:2 FAME
8.553	3247	0.041	0.976	14.950	16:0 ALDE	1.51	ECL deviates -0.001	Reference 0.003
9.441	10886	0.046	0.965	15.487	Sum In Feature 5	5.01	ECL deviates -0.002	14:0 3OH FAME
9.990	41195	0.045	0.960	15.817	16:1 CIS 9 FAME	18.86	ECL deviates -0.001	
10.143	990	0.044	0.958	15.908	16:1 CIS 11 FAME	0.45	ECL deviates -0.001	
10.294	43623	0.044	0.956	15.999	16:0 FAME	19.91	ECL deviates -0.001	Reference 0.003
10.789	3079	0.046	0.952	16.285	16:1 CIS 9 DMA	1.40	ECL deviates -0.000	
11.114	16994	0.045	0.949	16.472	16:0 DMA	7.69	ECL deviates 0.001	Reference 0.005
12.948	12378	0.048	0.935	17.519	16:0 3OH FAME	5.52	ECL deviates -0.003	
13.485	17847	0.047	0.931	17.822	Sum In Feature 10	7.93	ECL deviates -0.002	18:1c11/t9/t6 FAME
13.799	1518	0.049	0.929	18.000	18:0 FAME	0.67	ECL deviates -0.000	Reference 0.004
14.299	1217	0.049	0.926	18.284	18:1 CIS 11 DMA	0.54	ECL deviates -0.001	
15.053	780	0.043	0.922	18.711	22:0 NHC	0.34	ECL deviates -0.001	
16.789	1343	0.048	. . .	19.704		
18.491	1642	0.049	. . .	20.683	> max rt	
.....	1039	SUMMED FEATURE 1	0.51	13:1 CIS 12 FAME	14:0 ALDE
.....	11:1 2OH FAME	
.....	828	SUMMED FEATURE 4	0.39	UN 14.762 15:2 7 FA	15:2 FAME
.....	15:1 CIS 7	
.....	10886	SUMMED FEATURE 5	5.01	15:0 DMA	14:0 3OH FAME
.....	17847	SUMMED FEATURE 10	7.93	18:1c11/t9/t6 FAME	UN 17.834
Solvent Ar	Total Area	Named Area	% Named	Total Amt	Nbr Ref	ECL Deviation	Ref ECL Shift	
45485000	217909	216566	99.38	209605	y	0.001	0.005	

ID: 7012 B-FUSO-PERFO (11077* JL 10-2UH-.5R) Date of run: 24-MAR-87 02:08:40
 Bottle: 26 SAMPLE (AVERAGE) Date edited: 04-AUG-87 08:26:04

RT	Area	Ar/Ht	Respon	ECL	Name	%	Comment 1	Comment 2
1.583	40993000	0.082	. . .	6.988	SOLVENT PEAK	(min rt	
3.041	13571	0.029	1.142	10.000	10:0 FAME	3.37	ECL deviates 0.000	Reference 0.001
4.602	28194	0.035	1.045	12.000	12:0 FAME	4.68	ECL deviates 0.000	Reference 0.000
5.148	1920	0.037	. . .	12.486		
5.646	1196	0.035	1.012	12.929	Sum In Feature 1 . . .	0.19	ECL deviates -0.001	13:1 CIS 12 FAME
6.337	7331	0.038	0.997	13.454	Sum In Feature 2 . . .	1.16	ECL deviates -0.002	12:0 3OH FAME
6.758	1478	0.037	. . .	13.767		
6.821	3870	0.039	. . .	13.814		
6.938	965	0.037	0.985	13.901	14:1 CIS 9 FAME . . .	0.15	ECL deviates -0.001	
7.070	75784	0.039	0.983	13.999	14:0 FAME	11.84	ECL deviates -0.001	Reference -0.002
7.291	7129	0.048	0.972	14.472	14:0 DMA	1.10	ECL deviates 0.000	Reference -0.001
8.231	2168	0.079	0.966	14.761	Sum In Feature 4 . . .	0.33	ECL deviates -0.001	UN 14.762 15:2 ? FA
8.520	3985	0.042	0.963	14.950	16:0 ALGE	0.61	ECL deviates -0.001	Reference -0.002
8.957	1421	0.058	. . .	15.218		
9.059	2234	0.048	. . .	15.280		
9.404	23754	0.044	0.954	15.488	Sum In Feature 5 . . .	3.60	ECL deviates -0.001	14:0 3OH FAME
9.878	43643	0.045	0.951	15.774	16:1 CIS 7 FAME . . .	6.60	ECL deviates 0.000	
9.951	93157	0.045	0.950	15.819	16:1 CIS 9 FAME . . .	14.07	ECL deviates 0.001	
10.101	13141	0.044	0.949	15.909	16:1 CIS 11 FAME . . .	1.98	ECL deviates 0.000	
10.256	274860	0.045	0.948	16.003	16:0 FAME	41.42	ECL deviates 0.003	Reference 0.001
10.664	5251	0.045	0.945	16.239	Sum In Feature 6 . . .	0.79	ECL deviates -0.001	16:1 CIS 7 DMA
10.743	4265	0.045	0.945	16.284	16:1 CIS 9 DMA	0.64	ECL deviates -0.001	
10.908	1279	0.044	. . .	16.300		
11.066	22179	0.045	0.943	16.471	16:0 DMA	3.33	ECL deviates 0.000	Reference -0.002
12.898	1320	0.049	0.937	17.519	16:0 3OH FAME	0.20	ECL deviates -0.003	
13.338	7322	0.049	0.936	17.769	18:1 CIS 9 FAME	1.09	ECL deviates -0.002	
13.433	8298	0.049	0.936	17.822	Sum In Feature 10 . . .	1.23	ECL deviates -0.002	18:1c11/19/16 FAME
13.602	2319	0.049	0.936	17.918	18:1 CIS 13 FAME . . .	0.34	ECL deviates -0.001	
13.745	8531	0.047	0.936	17.999	18:0 FAME	1.27	ECL deviates -0.001	Reference -0.003
*****	1196	SUMMED FEATURE 1 . . .	0.19	13:1 CIS 12 FAME	14:0 ALGE
*****		
*****	7331	SUMMED FEATURE 2 . . .	1.16	12:0 3OH FAME	13:0 DMA
*****	2168	SUMMED FEATURE 4 . . .	0.33	UN 14.762 15:2 ? FA	15:2 FAME
*****		
*****	23754	SUMMED FEATURE 5 . . .	3.60	15:1 CIS 7	
*****	5251	SUMMED FEATURE 6 . . .	0.79	15:0 DMA	14:0 3OH FAME
*****	8298	SUMMED FEATURE 10 . . .	1.23	18:1c11/19/16 FAME	16:1 CIS 7 DMA
*****		UN 17.834

Solvent Ar	Total Area	Named Area	% Named	Total Amt	Nbr Ref	ECL Deviation	Ref ECL Shift
40993000	66565	65363	98.17	629105	8	0.001	0.002

ID: 5740 B-FUSO-PRAS (13871 JL 10-22H-5R)
 Bottle: 22 SAMPLE (ANMERUOE)

Date of run: 09-JAN-87 00:54:11
 Date edited: 04-AUG-87 08:30:19

RT	Area	Ar/Ht	Respon	ECL	Name	%	Comment 1	Comment 2
1.586	36031000	0.073	. . .	7.009	SOLVENT PEAK	< min rt	
2.225	499	0.024	. . .	8.298	< min rt	
7.178	3156	0.039	0.998	14.000	14:0 FAME	5.57	ECL deviates 0.000	Reference 0.000
8.648	891	0.045	0.966	14.951	16:0 ALDE	1.52	ECL deviates 0.000	Reference 0.000
9.088	919	0.050	. . .	15.218		
10.017	2430	0.040	0.942	15.774	16:1 CIS 7 FAME . .	4.05	ECL deviates -0.000	
10.089	2741	0.045	0.941	15.817	16:1 CIS 9 FAME . .	4.56	ECL deviates -0.001	
10.241	1307	0.050	0.939	15.908	16:1 CIS 11 FAME . .	2.17	ECL deviates -0.001	
10.394	22369	0.045	0.937	15.999	16:0 FAME	37.07	ECL deviates -0.001	Reference 0.000
11.216	3684	0.044	0.926	16.472	16:0 DMA	6.04	ECL deviates 0.001	Reference 0.001
11.786	1337	0.047	0.920	16.799	Sum In Feature 8 . .	2.18	ECL deviates -0.002	17:2 FAME @ 16.801
13.500	1552	0.049	0.905	17.770	18:1 CIS 9 FAME . .	2.48	ECL deviates -0.001	
13.595	13078	0.048	0.904	17.824	Sum In Feature 10 . .	20.92	ECL deviates -0.000	18:1c11/t9/t6 FAME
13.907	1589	0.045	0.902	18.000	18:0 FAME	2.54	ECL deviates 0.000	Reference -0.002
14.411	6848	0.049	0.900	18.286	18:1 CIS 11 DMA . .	10.90	ECL deviates 0.001	
*****	1337	SUMMED FEATURE 8 . .	2.18	17:1 CIS 9 FAME	17:2 FAME @ 16.801
*****	13078	SUMMED FEATURE 10 . .	20.92	18:1c11/t9/t6 FAME	UN 17.834

Solvent Ar	Total Area	Named Area	% Named	Total Amt	Nbr Ref	ECL Deviation	Ref ECL Shift
36031000	61901	60982	98.52	50532	5	0.001	0.001

ID: 0079 B-FUSD-RUSS1 (0307-3* JL 10-2UH) Date of run: 18-MAR-87 22:10:34
 Bottle: 15 SAMPLE (AVERAGE) Date edited: 03-AUG-87 17:04:51

RT	Area	Ar/Ht	Respon	ECL	Name	%	Comment 1	Comment 2
1.610	41237000	0.082	. . .	6.983	SOLVENT PEAK	< min rt	
3.097	1081	0.032	1.153	10.000	10:0 FAME	1.31	ECL deviates 0.000	Reference 0.001
4.679	4528	0.035	1.048	12.000	12:0 FAME	4.98	ECL deviates -0.000	Reference 0.000
7.170	10250	0.042	0.982	14.000	14:0 FAME	10.56	ECL deviates 0.000	Reference -0.001
7.943	855	0.042	. . .	14.504		
9.510	8364	0.045	0.953	15.489	Sum In Feature 5	8.36	ECL deviates 0.000	14:0 3GM FAME
9.994	8732	0.045	0.949	15.775	16:1 CIS 7 FAME	8.69	ECL deviates 0.001	
10.000	10349	0.045	0.948	15.818	16:1 CIS 9 FAME	18.25	ECL deviates 0.000	
10.210	1302	0.046	0.947	15.909	16:1 CIS 11 FAME	1.29	ECL deviates -0.000	
10.308	31569	0.045	0.946	16.000	16:0 FAME	31.33	ECL deviates 0.000	Reference -0.002
13.460	7659	0.050	0.933	17.771	18:1 CIS 9 FAME	7.50	ECL deviates -0.000	
13.500	6328	0.049	0.933	17.824	Sum In Feature 10	6.19	ECL deviates -0.000	18:1c11/t9/16 FAME
13.872	1573	0.047	0.933	18.000	18:0 FAME	1.54	ECL deviates 0.000	Reference -0.002
*****	8364	SUMMED FEATURE 5	8.36	15:0 DMA	14:0 3GM FAME
*****	6320	SUMMED FEATURE 10	6.19	18:1c11/t9/16 FAME	UN 17.834

Solvent Ar	Total Area	Named Area	% Named	Total Awnt	Nbr Ref	ECL Deviation	Ref ECL Shift
41237000	100590	99735	99.15	95312	5	0.000	0.001

 ID: 5741 B-FUSD-SULCI (D45A-29A* JL 10-22H-.5R C) Date of run: 08-JAN-87 11:58:41
 bottle: 23 STAT SAMPLE (ANEROBE) Date edited: 14-JAN-87 11:28:03

RT	Area	Ar/Ht	Respon	ECL	Name	%	Comment 1	Comment 2
1.398	8735	0.067	. . .	6.625	< min rt	
1.589	35339000	0.070	. . .	7.010	SOLVENT PEAK	< min rt	
1.804	15763	0.023	. . .	7.605	< min rt	
1.960	626	0.028	. . .	7.798	< min rt	
2.226	10044	0.023	. . .	8.294	< min rt	
2.962	7715	0.025	. . .	9.779		
3.072	501	0.026	1.191	10.000	10:0 FAME	0.65	ECL deviates 0.000	Reference -0.002
4.282	3868	0.030	. . .	11.575		
4.669	1339	0.036	1.083	11.999	12:0 FAME	1.58	ECL deviates -0.001	Reference -0.004
5.733	838	0.049	1.041	12.929	Sum In Feature 1 . . .	0.95	ECL deviates -0.001	13:1 CIS 12 FAME
6.261	1883	0.034	. . .	13.327		
7.179	20006	0.039	0.999	13.999	14:0 FAME	21.71	ECL deviates -0.001	Reference -0.005
7.910	1621	0.040	0.983	14.473	14:0 DMA	1.73	ECL deviates 0.001	Reference -0.003
8.363	1216	0.049	0.973	14.779	Sum In Feature 4 . . .	1.28	ECL deviates -0.001	15:2 FAME
8.651	5083	0.049	0.967	14.953	16:0 ALDE	5.34	ECL deviates 0.002	Reference -0.003
10.090	2681	0.045	0.944	15.017	16:1 CIS 9 FAME . . .	2.75	ECL deviates -0.001	
10.394	15151	0.044	0.939	15.998	16:0 FAME	15.46	ECL deviates -0.002	Reference -0.006
10.891	3321	0.044	0.933	16.284	16:1 CIS 9 DMA	3.36	ECL deviates -0.001	
11.218	17715	0.046	0.929	16.472	16:0 DMA	17.87	ECL deviates 0.001	Reference -0.003
11.786	3055	0.056	0.922	16.798	Sum In Feature 8 . . .	3.06	ECL deviates -0.003	17:2 FAME @ 16.801
13.416	1100	0.048	0.908	17.721	18:2 CIS 9,12 FAME . .	1.08	ECL deviates -0.002	
13.502	2503	0.049	0.907	17.769	18:1 CIS 9 FAME . . .	2.47	ECL deviates -0.002	
13.596	4142	0.049	0.907	17.822	Sum In Feature 10 . . .	4.08	ECL deviates -0.002	18:1c11/t9/t6 FAME
13.911	3065	0.067	0.904	18.000	18:0 FAME	3.01	ECL deviates -0.000	Reference -0.005
14.412	12589	0.049	0.902	18.283	18:1 CIS 11 DMA . . .	12.33	ECL deviates -0.002	
14.731	1327	0.048	0.900	18.464	18:0 DMA	1.30	ECL deviates -0.002	
14.958	1178	0.059	. . .	18.592		
16.542	921	0.048	. . .	19.494		
18.426	1158	0.051	. . .	20.574	> max rt	
18.999	1057	0.052	. . .	20.900	> max rt	
*****	838	SUMMED FEATURE 1 . . .	0.95	13:1 CIS 12 FAME	14:0 ALDE
*****	11:1 20H FAME	
*****	1216	SUMMED FEATURE 4 . . .	1.28	UN 14.762 15:2 7 FA	15:2 FAME
*****	15:1 CIS 7	
*****	3055	SUMMED FEATURE 8 . . .	3.06	17:1 CIS 9 FAME	17:2 FAME @ 16.801
*****	4142	SUMMED FEATURE 10 . .	4.08	18:1c11/t9/t6 FAME	UN 17.834

Solvent Ar	Total Area	Named Area	% Named	Total Amt	Nbr Ref	ECL Deviation	Ref ECL Shift
35339000	112818	97253	86.20	92065	8	0.002	0.004

ID: 3601 8-FUSU-VARIO (0501 JL 2-20H) Date of run: 13-SEP-86 00:59:21
 Bottle: 12 SAMPLE 1 Date edited: 04-AUG-87 09:59:20

RT	Area	Ar/Ht	Respon	ECL	Name	%	Comment 1	Comment 2
1.583	44844000	0.009	. . .	7.000	SOLVENT PEAK	< min rt	
4.686	611	0.032	1.069	12.000	12:0 FAME	0.55	ECL deviates 0.000	Reference -0.002
5.754	1383	0.035	1.031	12.928	Sum In Feature 1 . . .	1.20	ECL deviates -0.002	13:1 CIS 12 FAME
6.952	952	0.049	. . .	13.814		
7.206	22390	0.040	0.995	13.999	14:0 FAME	18.76	ECL deviates -0.001	Reference -0.004
7.939	5294	0.046	0.981	14.473	14:0 DMA	4.38	ECL deviates 0.001	Reference -0.002
8.406	2852	0.073	0.973	14.775	Sum In Feature 4 . . .	2.34	ECL deviates -0.005	15:2 FAME
8.677	4724	0.042	0.969	14.950	16:0 ALDE	3.86	ECL deviates -0.001	Reference -0.004
9.572	10019	0.044	0.956	15.409	Sum In Feature 5 . . .	8.08	ECL deviates -0.000	14:0 30H FAME
10.051	3112	0.042	0.950	15.774	16:1 CIS 7 FAME . . .	2.49	ECL deviates 0.000	
10.124	22038	0.045	0.949	15.818	16:1 CIS 9 FAME . . .	17.63	ECL deviates 0.000	
10.428	20959	0.043	0.945	15.999	16:0 FAME	16.70	ECL deviates -0.001	Reference -0.004
10.846	5011	0.045	0.940	16.239	Sum In Feature 6 . . .	3.97	ECL deviates -0.001	16:1 CIS 7 DMA
10.924	5179	0.044	0.939	16.284	16:1 CIS 9 DMA	4.10	ECL deviates -0.001	
11.251	18095	0.045	0.936	16.471	16:0 DMA	14.28	ECL deviates 0.000	Reference -0.003
13.633	2134	0.048	0.915	17.824	Sum In Feature 10 . . .	1.65	ECL deviates -0.000	18:1c11/19/16 FAME
*****	1383	SUMMED FEATURE 1 . . .	1.20	13:1 CIS 12 FAME	14:0 ALDE
*****	11:1 20H FAME	
*****	2852	SUMMED FEATURE 4 . . .	2.34	UN 14.762 15:2 ? FA	15:2 FAME
*****	15:1 CIS 7	
*****	10019	SUMMED FEATURE 5 . . .	8.08	15:0 DMA	14:0 30H FAME
*****	5011	SUMMED FEATURE 6 . . .	3.97	15:0 ANTEI 30H FAME	16:1 CIS 7 DMA
*****	2134	SUMMED FEATURE 10 . . .	1.65	18:1c11/19/16 FAME	UN 17.834

Solvent Ar	Total Area	Named Area	% Named	Total Amt	Nbr Ref	ECL Deviation	Ref ECL Shift
44844000	124713	123761	99.24	118567	6	0.002	0.003

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