New Quantitative, Qualitative, and Confirmatory Media for Rapid Analysis of Food for *Clostridium perfringens*

SYED A. SHAHIDI AND ALPHONZA R. FERGUSON

New York City Department of Health, New York, New York 10013

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A selective and differential medium, Shahidi-Ferguson Perfringens agar (SFP agar), and a confirmatory medium, lactose-motility agar (LM agar), were developed for the enumeration and identification of *Clostridium perfringens* in foods. These media provide a rapid, specific, and direct diagnosis of *C. perfringens*. SFP agar contains sodium metabisulfite and ferric ammonium citrate to demonstrate H₂S production and egg yolk to demonstrate lecithinase production by *C. perfringens*. On SFP agar, *C. perfringens* produces black colonies, 2 to 3 mm in diameter, surrounded by zones of opaque precipitate. The typical colonies are confirmed on LM agar. Enumeration and identification are completed within 48 hr. All of the ingredients of SFP agar are stable to heat and storage conditions. SFP agar also contains two antibiotics, kanamycin and polymyxin B, which are inhibitory to many bacteria commonly occurring in foods. A comparative study of SFP agar and noninhibitory media showed that SFP agar did not inhibit any of the 16 strains of *C. perfringens* tested. Recovery of *C. perfringens* added to foods averaged 90.6% for SFP agar as compared with 69.8% for sulfite polymyxin-sulfadiazine (SPS) agar (BBL) and 60.2% for SPS agar (Difco). The colonies on the SFP agar were much larger and were consistently black. Of 464 food samples tested, *C. perfringens* was found in 27 samples with SFP agar and in 5 samples with SPS agar (Difco), with a recovery ratio considerably higher on SFP agar. SFP agar is a more specific presumptive medium for the enumeration of *C. perfringens* and in conjunction with LM agar should save considerable time, effort, and materials toward the final identification of the species.

It has been reported that especially large numbers (comprising more than several million) of actively growing *Clostridium perfringens* are required to produce typical *C. perfringens* foodborne illness in laboratory animals and human volunteers (4–6). Therefore, quantitation of *C. perfringens* in foods directly or indirectly involved in food poisoning is of paramount importance in the assessment of disease. Unfortunately, suitable media are not currently available to the microbiologist for qualitative and quantitative analysis of food for *C. perfringens*. The use of McClung-Toabe egg yolk plates (4) and blood-agar plates (6) has declined considerably because they were semiquantitative, time-consuming, and not selective. Angelotti et al. (1) modified Mossel's medium (9) and developed sulfite polymyxin-sulfadiazine (SPS) agar for the isolation and enumeration of *C. perfringens* in food; to date, this is the most commonly used medium commercially available for the isolation of these organisms. During the 3-year period in which commercially prepared SPS agar was used by our laboratory, the recovery of *C. perfringens* from implicated food was negligible, even though such indications as incubation period, food involved, symptoms, and history of the foodborne disease in patients suggested *C. perfringens* food poisoning. Our preliminary investigation with pure cultures revealed that commercially available SPS medium was quite inhibitory to various strains of *C. perfringens* and very often organisms failed to produce the characteristic black colonies due to unstable ingredients of the medium. In addition, the authors and others (3) found it difficult consistently to demonstrate nitrate reduction and sporulation of *C. perfringens*. These reactions are used as confirmatory tests in conjunction with SPS agar (1). Marshall et al. (8) reported a new

1 A preliminary account of this work was presented at the 1969 Annual Meeting of the American Society for Microbiology at Miami Beach, Fla., 4-9 May 1969.
rapid technique for enumeration of C. perfringens with tryptone-sulfite-neomycin (TSN) agar utilizing an incubation temperature of 46 C. However, to our knowledge no other laboratory has demonstrated the suitability of this medium for the study of foodborne outbreaks.

With these considerations in mind, a study was undertaken to develop a satisfactory nutritive and selective medium, with stable ingredients, which would provide rapid quantitative and qualitative analysis of C. perfringens in foods. This medium, Shahidi-Ferguson Perfringens (SFP) agar, is the subject of this report. This paper also introduces lactose-motility agar (LM agar) as a single confirmatory test for C. perfringens in conjunction with SFP agar.

MATERIALS AND METHODS

Media: SFP agar. One liter of SFP agar contains: Tryptose (Difco), 15 g; yeast extract (Difco), 5 g; ferric ammonium citrate (N.F. Brown Pearls, Mallincrodt), 1 g; sodium metabisulfite (Na2S2O5), 1 g; polymyxin B sulfate (Antimicrobial Vial P, Difco), 30,000 units; kanamycin sulfate (Bristol Labs., New York), 0.012 g; agar, 20 g; water, 900 ml; and egg yolk emulsion (50% in saline), 100 ml (7). All of the ingredients with the exception of egg yolk emulsion were dissolved in water. After thorough mixing, the pH was adjusted to 7.6. The basal medium was autoclaved in 900-ml portions at 121 C for 10 min. After cooling to about 50 C, 100 ml of the 50% egg yolk emulsion was added to each 900 ml of the basal medium, thoroughly mixed, and added aseptically in 10- to 12-ml amounts to petri dishes (20 by 100 mm). The agar was allowed to solidify, incubated overnight at 35 C, and examined the following day for dryness and sterility. The antibiotics can be added to the basal medium before or after autoclaving, depending upon the laboratory preference.

SFP overlay agar. The ingredients and preparation of the SFP overlay agar were similar as the SFP agar except that the egg yolk emulsion was omitted and the ingredients were dissolved in 1,000 ml of distilled water instead of 900 ml. The medium was dispensed into small bottles in 100-ml portions with the caps loosely placed and autoclaved at 121 C for 10 min. Ten milliliters of this medium was used to overlay the incubated but dry plates. As a control in these experiments, SFP agar and SFP overlay agar were prepared without antibiotics. At all other times, except when indicated otherwise, SFP agar and SFP overlay agar contained antibiotics.

LM agar. For confirmation of C. perfringens, SFP agar was prepared with: tryptose, 15 g; yeast extract, 10 g; lactose, 10 g; sodium phosphate (di-basic), 5 g; phenol red, 0.05 g; agar, 3 g; and distilled water, 1,000 ml. The ingredients were dissolved in the water, the pH was adjusted to 7.5, and the lactose and phenol red were added. Ten milliliters of the medium was dispensed into tubes (150 by 15 mm). The cotton-plugged tubes containing the medium were autoclaved at 118 C for 15 min.

Before use, the medium was boiled for 10 min and cooled immediately. A single colony was picked with a needle, and a stab was made three-fourths the distance into the medium with a needle. The tubes were incu-

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**TABLE 1. Comparison of the recovery ratios of 16 strains of Clostridium perfringens on blood-agar, SFP agar without antibiotics, and SFP agar**

| C. perfringens type A strainsa | Blood-agar | SFP agar without antibiotics | SFP agar |
|-----------------------------|-------------|-------------------------------|----------|
| No. | Strain | Serotype | Count per ml | Recovery (%) | Count per ml | Recovery (%) | Count per ml | Recovery (%) |
|-----|-------|---------|--------------|-------------|--------------|-------------|--------------|-------------|
| 1   | NCTC 8797 | 1       | 12 × 10² | 100         | 12 × 10² | 100.0       | 12 × 10² | 100.0       |
| 2   | NCTC 8238 | 2       | 28 × 10³ | 100         | 17 × 10² | 94.4        | 17 × 10³ | 94.4        |
| 3   | NCTC 8239 | 3       | 18 × 10³ | 100         | 14 × 10² | 94.4        | 14 × 10³ | 94.4        |
| 4   | NCTC 8247 | 4       | 13 × 10³ | 100         | 11 × 10² | 94.4        | 11 × 10³ | 94.4        |
| 5   | NCTC 8678 | 5       | 11 × 10² | 100         | 11 × 10² | 94.4        | 11 × 10² | 94.4        |
| 6   | NCTC 8679 | 6       | 15 × 10² | 100         | 15 × 10² | 94.4        | 15 × 10² | 94.4        |
| 7   | NCTC 8449 | 7       | 65 × 10² | 100         | 67 × 10² | 94.4        | 67 × 10² | 94.4        |
| 8   | NCTC 8235 | 8       | 11 × 10³ | 100         | 17 × 10² | 94.4        | 17 × 10³ | 94.4        |
| 9   | NCTC 8798 | 9       | 18 × 10³ | 100         | 14 × 10² | 94.4        | 14 × 10³ | 94.4        |
| 10  | NCTC 9799 | 10      | 17 × 10³ | 100         | 16 × 10² | 94.4        | 16 × 10³ | 94.4        |
| 11  | NCTC 9851 | 11      | 4 × 10³  | 100         | 4 × 10³  | 94.4        | 4 × 10³  | 94.4        |
| 12  | NCTC 10239 | 12    | 54 × 10³ | 100         | 53 × 10² | 98.6        | 53 × 10³ | 98.6        |
| 13  | NCTC 10240 | 13     | 9 × 10³  | 100         | 9 × 10³  | 98.6        | 9 × 10³  | 98.6        |
| 14  | SEC S34   | From turkey | 63 × 10³ | 100         | 62 × 10² | 99.9        | 62 × 10³ | 99.9        |
| 15  | SEC S40   | From turkey gravy | 110 × 10³ | 100         | 110 × 10² | 99.9        | 110 × 10³ | 99.9        |
| 16  | SEC S45   | From dried beef | 60 × 10³ | 100         | 59 × 10³ | 98.3        | 59 × 10³ | 98.3        |

a Strains 1 to 13 from B. C. Hobbs, Central Public Health Laboratory, London, England; strains 14 to 16 from H. E. Hall, R. A. Taft Sanitary Engineering Center (SEC), Cincinnati, Ohio.

b Overall average recovery: blood-agar, 100 %; SFP agar without antibiotics, 99.5 %; SFP agar, 98.5 %.
Table 2. Comparative plate counts of pure cultures of various bacteria on blood-agar and SFP agar

| Species                        | Blood-agar | SFP agar |
|-------------------------------|------------|----------|
| *Achromobacter anitratus*     | 78 × 10^7  | 0        |
| *Bacillus subtilis*           | 14 × 10^7  | 0        |
| *Pseudomonas aeruginosa*      | 18 × 10^7  | 0        |
| *Salmonella choleraesuis*     | 89 × 10^7  | 0        |
| *S. pullorum*                 | 69 × 10^7  | 0        |
| *Shigella boydii*             | 63 × 10^7  | 0        |
| *S. dysenteriae*              | 47 × 10^7  | 0        |
| *S. flexneri*                 | 97 × 10^7  | 0        |
| *S. sonnei*                   | 49 × 10^7  | 0        |
| *Staphylococcus aureus*       | 63 × 10^7  | 0        |
| *Aerobacter aerogenes*        | 20 × 10^8  | 10 × 10^4|
| *Escherichia coli*            | 14 × 10^8  | 10 × 10^4|
| *S. enteritidis*              | 59 × 10^8  | 18 × 10^4|
| *S. Newport*                  | 22 × 10^8  | 31 × 10^8|
| *B. cereus*                   | 13 × 10^8  | 40 × 10^8|
| *Proteus morganii*            | 15 × 10^8  | 12 × 10^8|
| *P. vulgaris*                 | 29 × 10^8  | 28 × 10^8|
| *S. eptoccocus faecalis*      | 20 × 10^8  | 19 × 10^8|

* Blood-agar plates incubated aerobically and SFP agar plates anaerobically.

Bated at 35 C for 24 hr. Lactose fermentation was indicated by gas bubbles and a change in the color of the medium from red to yellow.

Other media. Blood-agar (Difco), SPS agar (Difco and BBL), cooked meat medium (Difco), fluid thioglycollate medium (Difco), plate count agar (Difco), Trypticase soy agar (BBL), and Trypticase soy broth (BBL) were obtained commercially and prepared according to the instructions printed on the label except where otherwise indicated in the text. The sporulating broth and motility-nitrate medium were prepared as described by Angelotti et al. (1).

Cultures. Sixteen strains of *C. perfringens* listed in Table 1 were used in this study. These cultures were propagated and maintained in cooked meat medium at room temperature. Other species (Table 2) used in this study were purified and maintained on Trypticase soy agar slants.

Anaerobiosis. The anaero-jar (Case Laboratories, Inc.) was used for anaerobic conditions as follows. The plates were placed upright in the jar; the jar then was evacuated to obtain a reading of 23 inches (ca. 63.5 cm) of vacuum on the gauge installed on the cover and then filled with nitrogen containing 10% carbon dioxide until the gauge returned to the zero position (1). The evacuation and filling process was repeated again before the jar was placed in the incubator.

Foods. Food samples were obtained from restaurants, supermarkets, and private homes. Food samples directly involved in food poisoning incidents were also examined.

Procedure for bacteriological analysis of food and pure cultures. The method for *C. perfringens* analysis consisted of homogenizing 11 g of food samples with 99 ml of water in Waring Blender cups for 2 min. Appropriate serial dilutions were made. The streak method was used for SFP agar. A 0.1-ml amount of each dilution was pipetted into the center of the agar plate. The inoculum was spread over the entire surface of the agar with a 2-inch (ca. 5.08 cm) diameter inoculating wire loop by using a back and forth motion while turning the plate until the inoculum was completely dry. The same streaking technique was used when pure culture analysis of *C. perfringens* was made on blood- and SFP agar. The pour plates were used with SPS agar. The surfaces of the SFP agar medium were covered with 10 ml of SFP overlay agar and the SPS agar medium with SPS agar. All plates were incubated anaerobically at 35 C for 24 hr.

RESULTS

The following physiological characteristics of *C. perfringens* were used in development of SFP agar and LM agar: (i) reduction of sulfite to sulfide, (ii) lecithinase production, (iii) resistance to action of polymyxin B and low levels of kanamycin, (iv) motility, and (v) fermentation of lactose.

The composition of SFP agar was determined after extensive investigation of numerous ingredients in various combinations, selecting the least inhibitory, most stable, and the best suitable for better growth of various strains of *C. perfringens*. The amounts of the two antibiotics (12 µg of kanamycin per ml and 30 units of polymyxin B per ml) used in SFP agar were also derived through a series of antibiotic assay experiments and were found to be noninhibitory to 16 *C. perfringens* strains studied. The spread plate technique was preferred to the pour plate technique because lecithinase reaction was more conspicuous on the former after 24 hr of incubation. In addition, the pour plate technique presented the cumbersome task of addition of egg yolk emulsion to each basic SFP agar bottle before plating.

On SFP agar, all of the 16 strains of *C. perfringens* developed black colonies 1 to 2 mm in diameter surrounded by distinct white zones of opaque emulsion to precipitate 3 to 4 mm in diameter (Fig. 1). A few strains produced faint zones which always became dense and prominent when the plates were left at room temperature for 2 to 3 hr. The agar thickness influenced the size and intensity of the zone of precipitation. The zones were smaller and faint on thick plates containing more than 20 ml of agar (base and overlay agar combined). The best results were obtained on the plates containing 18 to 20 ml of agar. To obtain the black colonies, it was necessary to cover the plates with SFP overlay agar. Egg yolk emulsion was omitted from the overlay agar because it did not improve
the lecithinase reaction and diminished the visibility of the black colonies.

All of the ingredients, particularly ferric ammonium citrate (iron source), sodium metabisulfite (H₂S source), polymyxin B sulfate, and kanamycin sulfate, were stable after autoclaving and storage. Prepared basal medium or poured plates showed no deterioration for 2 months when stored in plastic bags under refrigeration. Each batch of SFP agar maintained its characteristics and stability, which was demonstrated consistently by good growth and development of typical colonies of *C. perfringens*. Occasionally, autoclaving of the medium for 15 min resulted in the development of turbidity or precipitate; however, the performance and property of the medium were never affected.

After establishing the formula of the SFP agar, we conducted several experiments to evaluate its performance and properties. A comparative study of SFP agar and SPS agar was conducted on pure cultures of *C. perfringens*, on food inoculated with these organisms, and on foods obtained from various food establishments.

**Performance evaluation of SFP agar on C. perfringens and other bacteria.** Comparative plate counts on SFP agar, SFP agar without antibiotics, and blood-agar were made to evaluate the performance of the SFP agar. A 1-ml amount of each stock culture was transferred into fluid thioglycollate medium and incubated at 35°C for 24 hr. Serial dilutions were made and streaked in duplicate on SFP agar, SFP agar without antibiotics, and blood-agar. The last two media served as controls. All plates were incubated anaerobically for 24 hr. The effect of SFP agar on recovery of *C. perfringens* is given in Table 1. The average recovery ratios of 16 strains of *C. perfringens* were 98.5% on SFP agar and 99.5% on SFP agar without antibiotics, as compared to 100% for blood-agar. These results indicated that the percentage recovery on SFP agar was similar to that of SFP agar without antibiotics and blood-agar. The recovery ratio and luxuriant growth of *C. perfringens* on SFP agar without antibiotics as compared to blood demonstrated that SFP agar was nutritionally as good as blood-agar.

A 0.1-ml amount of 24-hr Trypticase soy broth cultures (Table 2) was streaked on blood-agar and SFP agar to determine the inhibitory effect of SFP agar on pure cultures of organisms frequently found in foods. The SFP agar was incubated anaerobically and blood-agar, aerobically for 24 hr. Comparative growth response of various bacteria found in food on blood and SFP agar is shown in Table 2. The presence of the two antibiotics in the SFP agar inhibited many of the bacteria commonly encountered in food. Certain species, such as *Streptococcus faecalis*, *Proteus vulgaris*, *P. morganii*, and *Bacillus cereus*, were not inhibited significantly. However, these bacteria developed only pinpoint white colonies after 24 hr of incubation and, therefore, did not

![Fig. 1. Colonial morphology of two strains of *C. perfringens*: (A) SEC S40 and (B) NCTC 8449 on SFP agar incubated anaerobically for 24 hr at 35°C.](image-url)
present any problem in the enumeration of large black colonies of C. perfringens with zones of opaque precipitate.

Recovery of C. perfringens inoculated into various foods on SFP agar and SPS agar. It has been reported (10, 11) that various foods and their ingredients modify the selective and differential properties of a medium. The following tests were performed in comparison with SPS agar to evaluate SFP agar in the presence of various foods. A variety of meat and fish foods such as tuna fish salad, roast beef, boiled ham, beef, and roast turkey were inoculated with known strains of C. perfringens, and their recovery ratio was determined on SFP agar and SPS agar obtained from two sources (Difco and BBL).

One milliliter of a 24-hr broth culture of a specific strain was added to a 10-g portion of particular food samples. Five different strains of C. perfringens were used, one for each separate food (Table 3). Inoculated food samples were plated out in appropriate dilution on SPS agar (Difco), SPS agar (BBL), and SFP agar. The number of added C. perfringens per gram of food was determined on blood-agar. The un inoculated foods were also examined on all of the media for the presence of C. perfringens in the original foods. Each food was also examined for aerobic bacterial counts.

The results of the effects of the above foods on recovery of C. perfringens on SFP agar and SPS agars (Difco and BBL) are given in Table 3. As indicated by the C. perfringens recovery ratio, SFP agar performed much better than SPS agars in the presence of five different foods. The average recovery ratio of C. perfringens from all foods was 90.6% as compared to 69.8 and 60.2% for BBL.

| Foods         | C. perfringens inoculated | C. perfringens recovery after inoculationb |
|---------------|---------------------------|-------------------------------------------|
|               | Strain                    | Amt added per g                           | SFP agar | SPS agar (BBL) | SPS agar (Difco) |
|               |                           |                                           | Amt per g | Per cent       | Amt per g | Per cent       | Amt per g | Per cent       |
| Tuna fish salad | SEC-S40                   | 36 x 10⁶                                | 32 x 10⁶ | 90             | 24 x 10⁶ | 67             | 12 x 10⁶ | 33             |
| Roast beef    | Hobbs 7                   | 34 x 10⁶                                | 30 x 10⁶ | 88             | 13 x 10⁶ | 38             | 9 x 10⁶  | 27             |
| Boiled ham    | Hobbs 2                   | 19 x 10⁶                                | 19 x 10⁶ | 100            | 18 x 10⁶ | 95             | 18 x 10⁶ | 95             |
| Corned beef   | Hobbs 12                  | 65 x 10⁶                                | 58 x 10⁶ | 90             | 47 x 10⁶ | 72             | 42 x 10⁶ | 65             |
| Roast turkey  | SEC-S34                   | 26 x 10⁶                                | 22 x 10⁶ | 85             | 20 x 10⁶ | 77             | 21 x 10⁶ | 81             |

a No C. perfringens found on SFP agar, SPS agar (BBL), and SPS agar (Difco) before inoculation.
b No visible growth of aerobic bacteria after 24 hr of anaerobic incubation. Overall average recovery on SFP agar, 90.6%; on SPS agar (BBL), 69.98%; on SPS agar (Difco), 60.2%.

Table 4. Recovery ratio of Clostridium perfringens on SFP agar and SPS agar (Difco) in foods obtained commerciallya

| Food          | C. perfringens per g |
|---------------|----------------------|
|               | SFP agar  | SPS agar |
| Dog food      | 40 x 10⁶  | 0        |
| Roast beef    | 55 x 10⁶  | 0        |
| Roast beef    | 21 x 10⁶  | 0        |
| Gefilte fish  | 52 x 10⁶  | 15 x 10⁶ |
| Roast turkey  | 33 x 10⁶  | 0        |
| Chopped liver | 35 x 10⁶  | 0        |
| Brisket of beef | 30 x 10⁶ | 0        |
| For check-up or survey | 75 x 10⁶ | 0 |
| Pot roast     | 20 x 10¹  | 10 x 10¹ |
| Raw chopped meat | 98 x 10⁶ | 0 |
| Beef mixture  | 28 x 10⁶  | 0        |
| Roast beef    | 17 x 10²  | 0        |
| Raw hamberger | 12 x 10⁶  | 0        |
| Roast beef    | 11 x 10⁶  | 0        |
| Stuffed cabbage | 10 x 10⁶ | 0 |
| Rare hamberger | 80 x 10⁶ | 0 |
| Top sirloin   | 80 x 10⁶  | 0        |
| Raw hamberger | 70 x 10⁶  | 7 x 10⁶  |
| Roast turkey  | 50 x 10⁶  | 11 x 10³ |
| Roast beef    | 50 x 10⁶  | 0        |
| Raw sausage   | 50 x 10⁶  | 24 x 10³ |
| Brisket of beef | 40 x 10⁶ | 0 |
| Raw hamberger | 31 x 10⁶  | 0        |
| Sliced roast beef | 36 x 10⁶ | 0 |
| Boiled ham    | 20 x 10⁶  | 0        |
| Brisket of beef | 20 x 10⁶ | 0 |
| Roast beef    | 10 x 10⁶  | 0        |

a Test included 464 food samples. C. perfringens was found in the 27 samples in this table.
and Difco SPS agars, respectively. This was based on the enumeration and confirmation of large black colonies surrounded by opaque zones on SFP agar and black colonies on SPS agar. Previously, when various batches of SPS agar from two sources were tested for recovery of several pure cultures of \textit{C. perfringens}, we found that SPS agar either was inhibitory to the various strains or produced white colonies instead of the typical black. The low recovery ratio in SPS agars could have been due to two factors mentioned above. In addition, colony sizes of all strains of \textit{C. perfringens} on SFP agar were two to three times larger then colony sizes in SPS agar. The presence of bacterial flora of foods did not interfere with the enumeration of \textit{C. perfringens} on SFP agar and SPS agars because their growth was inhibited or they appeared as pinpoint white colonies.

**Comparative evaluation of SFP agar and SPS agar on commercial food samples.** To compare the performance on SFP agar and SPS agar (Difco), some 464 food samples of meat and poultry products were simultaneously analyzed as previously described. The majority of the samples were part of the New York City Health Department’s routine survey and periodic checkup program of the food establishments. Many of the samples tested were directly associated with cases of food poisoning. Ten black colonies with the characteristics described by Angelotti et al. (1) on SPS agar and 10 black colonies with zones of opaque precipitate on SFP agar were picked and confirmed by the following biochemical tests: fermentation of glucose, maltose, sucrose, lactose, salicin, and mannitol; reduction of nitrate; sporulation; motility; aerobic growth; and morphology. The number of \textit{C. perfringens} per gram of food sample was calculated by the ratio of confirmed colonies to the total number of typical colonies on the plates.

The results are reported in Table 4. Of 464 food samples examined, \textit{C. perfringens} was recovered from 27 samples on SFP agar and from 5 samples on SPS agar. Even the recovery ratios of \textit{C. perfringens} on SPS agar plates were far below those of the corresponding SFP agar plates from the same samples. The bacterial flora of the food was found to be absent most of the time from the plates, and, when bacteria grew, either they were very low in number or the colonies were smaller in size. Almost all of the strains of \textit{C. perfringens} recovered from food samples had larger colony sizes on SFP agar as compared to SPS agar.

The reliability of LM agar as a single confirmatory test for \textit{C. perfringens} isolated from SFP agar (black colonies with zones of opaque precipitate) was compared with the aforementioned biochemical confirmatory tests. The LM agar gave 100% performance when used as a single confirmatory test in conjunction with SFP agar on all known \textit{C. perfringens} strains and on unknown bacteria confirmed as \textit{C. perfringens} by other tests.

**DISCUSSION**

**SFP agar.** Our 3-year laboratory study on SFP agar and the overall results of the study presented in this article indicate that (i) SFP agar promotes luxuriant growth of \textit{C. perfringens}, (ii) it is not inhibitory to various strains of \textit{C. perfringens} as compared to SPS agar, (iii) it is inhibitory to many species of bacteria found in food, and (iv) it has stable ingredients which have the ability to withstand the adverse effect of various food ingredients, autoclaving, and storage conditions.

**LM agar.** Angelotti et al. (1) proposed the use of motility-nitrate medium, sporulation broth, and Noyes veal broth for toxin production to confirm black colonies produced on SPS agar. We, as well as others (3), experienced difficulties in demonstrating nitrate reduction and sporulation by \textit{C. perfringens} consistently. We suggest that all of these tests can be replaced by a single confirmatory test, LM agar, provided SFP agar is used as an enumerating medium. The use of SFP agar eliminates all clostridia except six species, namely, \textit{C. bifermantans}, \textit{C. botulinum}, \textit{C. parabotulinum}, \textit{C. sporogenes}, \textit{C. novyi}, and \textit{C. haemolyticum}, which also demonstrate \textit{H}_2\textit{S} and lecithinase production on SFP agar similar to \textit{C. perfringens} (Berger’s Manual, 7th ed.).
Since all of the species mentioned above are motile and nonlactose fermenters, a single LM agar tube test is sufficient to differentiate \textit{C. perfringens}, which is nonmotile and a lactose fermenter. In this study and in other experiments, the reliability and performance of the LM agar were tested against other confirmatory tests described previously and found to be convenient, time-saving, and satisfactory. Table 5 shows the scheme to differentiate \textit{C. perfringens} from other lecithinase- and H$_2$S-producing clostridia by using SFP agar and LM agar. By employing the combination of SFP agar and LM agar, analysis of food samples for \textit{C. perfringens} is completed within 48 hr as compared to other procedures which take more than 68 hr.

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