COMPARATIVE STUDY OF MINITEK, A MINIATURIZED SYSTEM AND CONVENTIONAL METHOD IN IDENTIFICATION OF ENTEROBACTERIACEAE

by

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INTRODUCTION

Foods, in addition to providing the body with nutrients, serves as vehicles for transmission of microorganisms. Plants and animals, along with their natural microflora, become further contaminated via soil, water, sewage, and air as well as by contact with other plants and animals. Additional contamination of foods occurs during handling and processing. Many of these contaminants are members of the family Enterobacteriaceae, (Guthertz and Okoluk, 1978).

Knowledge of the identity of Enterobacteriaceae in different food is essential for assessing the value of such quality or safety indices as "the coliform count", "fecal coliform", and the "Enterobacteriaceae count" (Hansen et al., 1974). This information also may be of importance in selection of methods for isolation and differentiation of salmonellae and other enteric pathogens from foods. The number and nature of biochemical and other tests required to speciate members of this family have frequently discouraged food microbiologists from obtaining these data (Cox and Mercuri, 1978). There are several commercial "test kits" available for the identification of Enterobacteriaceae. Although they are designed primarily for use in clinical laboratories, many of these techniques can be adapted to food microbiology. The Minitek is an example of these biochemical micromethods. The series of papers published in the March, 1979, issue of Food Technology details rapid methods and automation in food microbiology (Cox and Mercuri, 1979; Goldshmidt and Fung, 1979).

The accuracy of most of the available identification systems was documented previously by Cox et al. (1977), Cox and Mercuri (1979), and Fung and Cox (1981). Some of these systems have since undergone changes as a result of comparative studies. Most manufacturers claimed 90 to 95% accuracy compared to conventional procedures for the identification of Enterobacteriaceae.

Fung and his colleagues have developed a variety of miniaturized tests for use in general microbiology by use of microtiter system and multiple inoculation device (Fung, 1969; Fung and Hartman, 1972 and 1975; Fung, 1976). This system is adaptable to solid and semisolid as well as liquid microbiological testing procedures.

The present study was undertaken to compare the Minitek, the Multiple Inoculation technique developed by Fung, to the corresponding conventional tube test for obtaining biochemical information on Enterobacteriaceae freshly isolated from food. To evaluate the efficiency of Minitek and Fung's miniaturized method for identification of Enterobacteriaceae and to perform a detailed time-and-cost analysis and material required for the diagnosis of Enterobacteriaceae isolated from foods for each specific method.

LITERATURE REVIEW

The progression of diagnostic kit development involved the use of the conventional method, improvement of the conventional method by miniaturization and then development of commercial diagnostic kits. The purpose of this study included a comparison of the efficiency and accuracy of the conventional method with the miniaturized method of D. Y. C. Fung and a commercial kit called Minitek. Other commercial kits are presented after the review of the three systems tested in this study.

Conventional method

For the purpose of this investigation "conventional method" means cultivating bacteria in the test tubes containing approximately 5 ml of sterile media. For each unknown culture a battery of tests in individual tubes will be studied using procedures commonly applied in medical and food laboratories (International Commission on Microbiological Specifications for Foods, 1974; Harrigan and McCance, 1976; Marth, 1978; and Kreig, 1984).

The following section briefly describes the major conventional biochemical reactions of the family Enterobacteriaceae to set the stage for a discussion of miniaturization and development of diagnostic kits. The tests described are those used by Edwards and Ewing (1980) in their classical scheme of biochemical differentiation of Enterobacteriaceae.

Differentiation of the Enterobacteriaceae is based primarily on the determination of the presence or lack of different enzymes coded by the genetic material of the bacterial chromosomes. These enzymes direct the metabolism of bacteria along one of the several pathways that can be detected by special media used in "in vitro" cultures techniques. Substrate upon which these enzymes can react is incorporated into the culture medium, together with an indicator system that can detect either the utilization of the substrate or the presence of specific metabolic

products. By selecting a series of media that measures different metabolic characteristics of the microorganism to be tested, a biochemical "fingerprint" can be determined for making a species identification (Koneman et al., 1983).

With few exception, all members of the Enterobacteriaceae show the following characteristics:

Glucose is metabolized fermentatively

Cytochrome oxidase activity is lacking

Nitrates are reduced to nitrites

A variety of different liquid or agar media can be used to measure the capability of a test organism to utilize carbohydrates fermentatively. The principle of carbohydrate fermentation is based on Pasteur's studies of bacteria and yeast done more than a hundred years ago; the action of many species of microorganism on a carbohydrate substrate results in acidification of the medium. The carbohydrates to be tested (adonitol, arabinose, dextrose, inositol, lactose, raffinose, rhamnose, sorbitol, and sucrose) are filter-sterilized and added aseptically to the basal medium to the final concentration of 0.5 to 1.0%. The phenol red is a pH indicator that turns yellow when the pH of medium drops below 6.8.

Glucose fermentation follows the anaerobic Embden-Meyerhoff-Parnas (EMP) pathway leading to the formation of pyruvic acid from which a variety of organic acids are derived. All Enterobacteriaceae ferment glucose through this pathway, producing a mixed acid fermentation and a yellow color in a medium using phenol red or bromthymol blue as the pH indicator. In addition to producing a pH color shift in fermentation culture media, the production of mixed acids, notably butyric acid, often results on pungent, foul odor in the culture medium. Close studies also reveal that gas formation (H_2 + CO_2) from glucose fermentation occurs only after acid (formic acid) has been formed.

In practice, microorganisms incapable of fermenting glucose are commonly detected by observing the reactions they produce when growing on Triple Sugar Iron Agar (TSI). An alkaline slant/alkaline butt reaction on these media indicates lack of acid production and inability of the test organism to ferment the glucose and other carbohydrate present (lactose + sucrose). This reaction alone is sufficient to exclude an organism from the family Enterobacteriaceae.

Even if an organism is a fermenter and is suspected of being one of the Enterobacteriaceae, a cytochrome oxidase test, and in some instances a nitrate reduction test, should be performed to exclude organism belonging to other genera of fermenting bacteria, such as aeromonads, vibrios and pasteurellas.

Any organism that displays cytochrome oxidase activity is also excluded from the family Enterobacteriaceae. The commercial cytochrome oxidase disc or strips are most commonly used because of their convenience. The developing color reaction must be interpreted within 10 to 20 seconds because many organisms, including selected members of the Enterobacteriaceae, may produce delayed false positive reaction.

All Enterobacteriaceae, with the exception of certain biotypes of Enterobacter agglomerans and Erwinia species, reduce nitrate to nitrite. Because it requires 18 to 24 h to perform the nitrate reduction test, the test is not commonly used in most laboratories to pre-screen unknown bacterial isolates, but it is used either to confirm the correct classification of an unknown microorganism or as an aid in arbitrating the identification of a bacterial species showing atypical reaction in tests measuring other characteristics. Because the enzyme nitroreductase is activated only under anaerobic condition, the use of semisolid agar is recommended. Semisolid media enhance the growth of many bacterial species and provide the anaerobic environment needed for enzyme activation.

Fermentation is an oxidation-reduction metabolic process that takes place in an anaerobic environment, with an organic substrate serving as the final

hydrogen (electron) acceptor in place of oxygen. In bacteriological test system this process is detected by visually observing color changes of pH indicators as acid products are formed.

Many bacteria, including all of the Enterobacteriaceae, utilize carbohydrates by a process called mixed acid fermentation, in which a variety of organic acids are ultimately derived from pyruvic acid. Bacteria differ in the carbohydrates that they can utilize and in the types and quantities of mixed acid produce. These differences in enzymatic activity serve as one of the important characteristics by which the different species are recognized.

The bacterial fermentation of lactose is more complex than that of glucose. Lactose is a disaccharide composed of glucose and galactose connected through an oxygen linkage known as a galactoside bond. Upon hydrolysis this bond is severed, releasing glucose and galactose. In order for bacteria to utilize lactose, two enzymes must be present: (1) ß-galactoside permease, permiting the transmigration of ß-galactoside (lactose) across the bacterial cell wall, and (2) ß-galactosidase, required to hydrolyse the ß-galactoside bond once the disaccharide has entered the cell. The final acid reaction is from the degradation of glucose. Any organism incapable of utilizing glucose cannot form acid from lactose. This explain why glucose is omitted from the formulas of primary isolation media such as MacConkey agar or EMB agar. Otherwise, the ability to detect the lactose fermenting capability of the test would be lost.

A non-lactose fermenting organism is one that lacks ß-galactosidase or cannot attack glucose. So called late lactose fermenters are thought to be organisms that possess the ß-galactosidase activity but show sluggish ß-galactoside permease activity.

Orthonitrophenyl galactoside (ONPG) is a compound structurally similar to lactose, except that the glucose has been substituted by an orthonitrophenyl radical. This rather ingenious manipulation of the molecule forms the basis for the

ONPG test. This test allows for the detection of the enzyme ß-galactosidase far more quickly than the test for lactose fermentation. This is helpful in identifying those late lactose-fermenting organisms that are deficient in ß-galactoside permease. ONPG is more permeable through the bacterial cell wall than is lactose, and under the action of ß-galactosidase ONPG is hydrolyzed into galactose and orthonitrophenol. The ONPG test is not a substitute for the determination of lactose fermentation, since only the enzyme ß-galactosidase is measured.

A very rich medium, with the lack of inhibitors permits the growth of all but the more the fastidious bacterial species (excluding the obligates anaerobes). For this reason, Triple Sugar (glucose, lactose and sucrose) Iron Agar (TSI) can be used only in testing a bacterial species picked from single colony recovered on primary or selective agar plates. Lactose is present in a concentration 10 times that of glucose (the ratio of sucrose to glucose is also 10:1 in TSI). Ferrous sulfate is used as an H_2S detector. The phenol red indicator is yellow below a pH of 6.8. Because the pH of the inoculated medium is buffered at pH 7.4, relatively small quantities of acid production result in a visible color change. Without carbohydrate fermentation, no acids are formed and the amine production in the slant together with the alkaline buffers produce a red color throughout the medium. Bacteria that produce this type of reaction are known as nonfermenters. A negative TSI is one of the important initial indications that an organism does not belong to the family Enterobacteriaceae.

Indole (a benzyl pyrrole) is one of the metabolic degradation products of the amino acid tryptophan. Bacteria that possess the enzyme tryptophanase are capable of hydrolyzing and deaminating tryptophan with the production of indole, pyruvic acid, and ammonia. The indole test is based on the formation of a red color when indole reacts with the aldehyde group of p-dimethylaminobenzaldehyde. This is the active chemical in Kovac's reagent. A medium rich in tryptophan must be used.

Bacteria that follow primarily the mixed acid fermentation route often produce sufficient acid to maintain pH below 4.4 (the acid color-break-point of the methyl red indicator) against the buffer system of the test medium. Bacterial species that produce strong acids are referred to as methyl red positive.

Members of the Klebsiella-Enterobacter-Hafnia-Serratia group produce acetoin as the chief end product of glucose metabolism and form less quantities of mixed acid. In presence of atmospheric oxygen and 40% potassium hydroxide, acetoin is converted to diacetyl, and alpha-naphthol serve as a catalyst to bring out a red color complex.

Sodium citrate is a salt of citric acid, a simple organic compound as one of the metabolites in the tricarboxylic acid cycle (Krebs cycle). Some bacteria can obtain energy in a manner other than the fermentation of carbohydrates by utilizing citrate as a sole source of carbon. The utilization of citrate by a test bacterium is detected in citrate medium by the production of alkaline by-products.

Malonate is another anionic radical commonly used to determine the ability of bacteria to utilize this single compound as a sole of carbon.

Urease is an enzyme possessed by many species of microorganisms that can hydrolyse urea to form ammonia. The ammonia reacts in solution to form ammonium carbonate, resulting in alkalinization and an increase in the pH of the medium. Organisms that hydrolyze urea rapidly may produce positive reactions within 1 or 2 h; less active species may require 3 or more days.

Decarboxylases are a group of substrate-specific enzymes that are capable of attacking the carboxyl (COOH) portion of amino acids, forming alkaline-reacting amines. This reaction, known as decarboxylation, forms carbon dioxide as a second product. Lysine, ornithine, and arginine are three amino acids routinely tested in the identification of Enterobacteriaceae. The specific amine products are as follows:

Lysine----------> Cadaverine

Ornithine----------> Putrescine

Arginine----------> Citrulline

Moeller decarboxylase medium is the base most commonly used for determining the decarboxylase capabilities of the Enterobacteriaceae. The amino acid to be tested is added to the decarboxylase base prior to inoculation with the test organism. A control tube, consisting of only the base without the amino acid, must also be set up in parallel. Both tubes are anaerobically incubated by overlaying with mineral oil.

Phenylalanine is an amino acid that upon deamination forms a keto acid, phenylpyruvic acid. Of the Enterobacteriaceae, only members of the Proteus, Morganella and Providencia genera possess the deaminase enzyme necessary for this conversion. The phenylalanine test depends upon the detection of phenylpyruvic acid in the test medium after growth of the test organism. After incubation at 35° C for 18 to 24 h, 4 to 5 drops of ferric chloride reagent are added directly to the surface of the agar. The immediate appearance of an intense green color indicates the presence of phenylpyruvic acid and a positive test.

The ability of certain bacterial species to liberate sulfur from sulfur-containing amino acids or other compound in the form of H_2S is an important characteristic for their identification. H_2S can be detected in a test system if the following conditions are present:

A. There is a source of sulfur in the medium.

B. There is an H_2S indicator in the medium.

C. The medium supports the growth of the bacterium being tested.

D. The bacterium possesses the H_2S-producing enzyme systems.

The sequence of steps leading to the production and detection of H_2S in a test system is thought to be as follows:

(1) Release of sulfide from cysteine or from thiosulfate by bacterial enzymatic action.

(2) Coupling of sulfide (S^{-2}) with hydrogen ion (H^{+}) to form H_2S.

(3) Detection of the H_2S by heavy metal salts, such as iron, bismuth, or lead, in the form of a heavy metal-sulfide, black precipitate.

Bacterial motility is another important characteristic in making a final species identification. Bacteria move by means of flagella, the number and location of which vary with the different species. Motility media have agar concentrations of 0.4% or less. At higher concentrations the gel is too firm to allow free spread of the organisms.

Fung's Mini System

In an attempt to increase efficiency and reduce material for testing large numbers of bacteria, Fung (1969) described a series of miniaturized tests and inoculation procedures for bacteriology. In general, this system involves preparation of a master plate containing many pure cultures. From the master plate a multiple inoculation procedure allows efficient inoculation of cultures into liquid or solid media contained in multi-well chambers called microtiter plates. After incubation, biochemical changes are observed and used to identify the unknown microorganism with the aid of various diagnostic schemes. In practice the sterile Microtiter Plate (Dynateck Lab., Inc., Alexandria, VA) or similar products with 96 wells (each has a 0.35 ml. capacity) has been proven to be a convenient vessel to cultivate the organism. Resterilization of plates can be done by soaking the plate in 500 ppm sodium hypochlorite solution for 1 h followed by rinsing with sterile distilled water. Other methods of sterilization include UV radiation for 1 h and CO-60 irradiation (2.5 Mrads). Into the sterile Microtiter plate, sterile liquid, semi-solid or solid media can be introduced. Usually one substrate is placed in all 96 wells. On occasion 8 replicates

of 12 different media or 12 replicates of 8 media are placed in the Microtiter plate to make a "kit" for specific identification schemes. Addition of media can be accomplished by a hand pipette or a pipetting machine. A large petri dish (15 x 150 mm) can also be used to accomodate solid media for this system.

A multiple inoculator facilitates mass transfer of test culture into the wells or onto the solid media in the petri plates. This can be purchased or constructed by fixing 96 stainless steel pins (27 mm long) into wood blocks or other material in the same pattern as the microtiter plate. According to Fung and Hartman (1972, 1975) each pin head delivers about 0.0006 ml and each pin-point delivers about 0.0002 ml. Pin heads are suitable for inoculation into liquid media and solid agar surfaces while pin-points are suitable for stabbing into agar or semi-solid agar. Sterilization of the device is achived by dipping the protruding portions of the pins into alcohol for about 20 seconds followed by flaming for about 1 second. The sterile device can then be used to charge inoculum from a "Master Plate" containing test cultures and to transfer the organism to either Microtiter plates containing suitable substrate or petri dishes containing solid agar.

Fung and Miller (1970) described a rapid miniaturized procedure, with the microtiter plate and the Amojell-overlay technique, for the detection of acid and gas production by bacterial cultures. Twelve carbohydrates (dulcitol, fructose, galactose, glucose, glycerol, inulin, lactose, maltose, mannitol, raffinose, sorbitol, and sucrose) were tested for 25 species of bacteria representing a variety of carbohydrate fermentation patterns, in four replicates. They found a 100% correlation between the conventional method and the miniaturized procedure in the detection of acid production by all species, but slight variation in gas detection was observed in 2 species. The authors also stated that the Microtiter-Amojell method requires 50 time less material and ten time less effort compared to the conventional tube method. Fung and Miller (1972) proposed a combined multiple inoculation Microtiter test system for

performing IMViC (indole, methyl red, Voges-Proskauer, citrate) tests. Results of the IMViC tests on 24 bacterial species with the miniaturized test corresponded directly to results obtained with the conventional tests. The authors stated that the incubation period necessary for obtaining definite results were shorter for the miniaturized tests than for the conventional tests. The amount of reagent to be added to the culture after growth proved to be rather critical. Because of the small volume of culture, excess reagent will cause erroneous reading.

Fung (1976) reported some of the advantages and disadvantages of the Microtiter system. Advantages of this system are low cost of operation, flexibility of tests, mass production of data for many unknown, speed of reactions, and saving of space and time. Disadvantages include the need to have large numbers of organisms to test at any one time and a technician who is well trained, experienced and dexterious. With the advent of automated and semi-automated instrumentation in this area, the need for dexterity is eliminated and the design of kits geared for specific laboratories will reduce the number of complete plates to be prepared, i.e. a design for eight or 12 tests in one Microtiter plate could be used for studying eight unknown organisms at one time.

It is apparent that most biochemical tests can be miniaturized and that the multiple inoculation procedure can facilitate mass inoculation of large number of cultures for biochemical studies. Although the miniaturized methods were first done for biochemical tests later Fung and colleagues also used the similar idea for viable cell counts.

Fung and Kraft (1968) developed a microtiter method based on the loop dilution principle, for the evaluation of viable-cell counts of bacterial cultures. They showed that the microtiter method is comparable to the conventional agar plate method in accuracy and precision of counting viable cells of bacterial cultures. Some of the advantages of the microtiter method over the agar plate method were

utilization of one microtiter plate instead of many dilution bottles, utilization of one petri dish instead of several dishes for duplicate plating several dilutions, utilization of one set of loops and dropers instead of many pippettes, occupying only a very small area for operation and storage, easy of sterilization and cleaning and ease and saving of time in enumeration of colonies. Baldock et al. (1968) used the microtiter system to evaluate spore survival after heat treatment. Fung and LaGrange (1969) described the application of the microtiter method to the enumeration of viable cells in milk samples. Statistical analysis showed high correlations between the microtiter method and the conventional Standard Plate Count. The authors also stated that it has been estimated that the cost of supplies for the microtiter method is approximately one-tenth that of the Standard Plate Count Method. Fung and Kraft (1969) developed a rapid procedure for estimating viable cell counts in bacterial cultures by combining the microtiter system and Most Probable Number techniques. They found a correlation coefficient of 0.801, which was significant at the 1% level. In addition to the advantages previously mentioned they found that the microtiter system eliminates the dilution estimates for testing since the method covers the range from 16 to 44 x 10^8 organisms per ml of the original sample. Fung et al. (1976) reported a collaborative study of the Microtiter Count Method and the Standard Plate Count (SPC) method for the viable cell count of raw milk. Statistical analysis showed that at the 95% confidence limit the amended (i.e. agar overlay, 48 h incubation) procedure was reliable compared with the SPC for making a viable cell count of raw milk. The Microtiter Count method was credited with saving time, space, and material. However some negative comments concerning the Microtiter Count Method were: (a) need some time to master the technique, so more skill is involved than in the SPC method; (b) when a laboratory accident occurs more samples will be lost per plate compared with SPC method, and (c) automatic pipetting syringe must be checked frequently for accuracy and proper gravimetric calibration is difficult and tedious.

Goldschmidt and Fung (1978, 1979) reviewed the applications and implications of automated instrumentation as well as miniaturized methods which can be used to detect and characterize microorganisms of importance in the food industry.

Commercial diagnostic kits

In order to provide consumers with easy to use bacteriological kits with reliable data interpretation capabilities, many commercial kits started to appear on the market from late 1960's and continued to the present day. The common features of these kits are 1) multiple media chambers, 2) convenience in inoculation, 3) unique design for incubation, 4) ease of reading reactions, and 5) code books for interpretation of data through construction of data base. The central theme is accurate identification with minimum manipulation and human interpretation. There are two distinct developments. One trend is manual and another trend is with the use of instruments. Table 1 lists the name of the kit, number of tests, time needed for completion of test, whether the kit is manual or instrumental, and the manufacturers with addresses. The biochemical tests used by major commercial diagnostic kits are listed in Table 2 which is an up-date of a similar tabulation by Hartman and Minnich (1981).

The following section contains more detailed description of the kits currently available, their advantages and disadvantages and some of the published evaluations. More emphasis was given to the Minitek system since this is the commercial kit tested in this study. Only data from the past 5-6 years were reviewed since Cox et al. (1979) and Fung and Cox (1981) made detailed reviews of previous years already.

Minitek system

The Minitek system consists of a covered, square, plastic plate containing 20 wells. Into each well a paper disc with a particular substrate is added, after which

Table 1. Commercial Diagnostic Kits Currently Available (1985).

System	No. of Tests	Incubation time (h)	Type of System	Manufacturer
ABBOTT MS-2	17	5	Instrument (nephelometry)	Abbott Diagnostics 4757 Irving Blvd. Dallas, TX 75247
API 20E	20	18-24	Manual	Analytab Products, Inc. Div. of Sherwood Medical 200 Express Street Plainview, NY 11803 800-645-0666
API RAPID 20E/ DMS RAPID E	20	4	Manual	DMS Laboratories RD 3 Box 94 Dart Mill, Flemington, NJ 08822 201-788-5559
AUTOBAC-IDX	18*	3-6	Instrument (nephelometry)	General Diagnostics Morris Plains, NJ 07950
AUTOMICROBIC (AMS)	26	4-8	Instrument (nephelometry & colorimetry)	VITEK Systems, Inc. 595 Anglum Drive Hazelwood, MO 63042 800-325-1977
AUTOSCAN-3	24	18-24	Instrument	MicroScan System of America Sacramento, CA
CATHRA REPLISCAN	16	18-24	Instrument	CATHRA International P.O. Box 4337 Saint Paul, MN 55104

Table 1 (continued).

System	No. of Tests	Incubation time (h)	Type of System	Manufacturer
EIKEN Systek No. 1	21	18-24	Manual	EIKEN System; EIKEN Chemical Co., LTD. Tokyo, Japan
ENTERIC-TEK	14	18-24	Manual	Flow Laboratories 25 Lumber Road Roslyn, NY 11576
ENTERO-SET 20	20	20-24	Manual	Fisher Diagnostics Inolex Corp. for Diagnostic Div. 526 Route 303 Orangeburg, NY 10962
ENTEROTUBE II	15	18-24	Manual	Roche Diagnostic Systems Div. of Hoffmann-La Roche, Inc. 340 Kingland Street Nutley, NJ 07110 201-235-3983
MICRO-ID	15	4	Manual	General Diagnostics Div. of Warner-Lambert Company 201 Tabor Road Morris Plains, NJ 07950 800-638-7010
MINITEK	35	18-24 4	Manual Manual	BBL Microbiology Systems, Becton Dickinson and Co. P.O. Box 243 Cockeysville, MD 21030 301-666-0100

Table 1 (continued).

System	No. of Tests	Incubation time (h)	Type of System	Manufacturer
QUANTUM II	21	4-5	Instrument	Abbott Laboratories North Chicago, IL
r/b	18	24	Manual	Flow Laboratories McLean, VA 22102 703-893-5925
SENSITITRE	24	18-24	Instrument	Seward Laboratory London, England (Manufacturer) GIBCO Laboratories (Distributor) 421 Merrimalk St. Lawrence, MA 01843 617-729-7448
SPECTRUM 10	20	18-24	Manual	Austin Biological Laboratories 6620 Manor Road Austin, TX 78723 800-259-9230 TX 800-531-5106 US

*18 different antimicrobial discs.

Table 2. Biochemical Tests of Currently Available Commerical Systems.*

	Abbott MS-2	API 20E	API Rapid 20E	Automicrobic (AMS)	AutoScan 3	Dynatech	Eiken	Enteric-Tek	Enteric 20	Entero-Set-20	Enterotube II	Micro-ID	MicroMedia Quad	Minitek	Pathotec	Quantum II	r/b System	Replicscan	Sceptor	Sensititre	Spectrum 10
Test of Substrate																					
Acetamide				+									+			+					
Adonitol	+		+	+	+	+	+	+	+	+	+	+	+	+		+			+	+	+
Amygdalin		+											+								
Antibiotics					+								+	+				+			
Arabinose	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+
Arginine		+		+	+	+	+			+			+	+		+		+			+
Celobiose			+											+							
Citrate	+	+	+	+	+	+	+	+	+	+	+		+	+		+	+	+	+	+	+
Control (Growth)	+			+									+					+			
DNase																	+	+			
Dulcitol												+									
Esculin	+		+	+	+	+							+	+	+	+		+	+	+	
Fructose														+							
Galactose														+							
Gelatin		+				+													+		
Glucose	+	+	+	+	+	+	+	+	+	+	+			+		+	+	+	+	+	
Glycerol														+							
H_2S		+		+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+
Indole	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+
Inositol	+	+		+	+	+	+		+	+		+	+	+		+		+	+	+	+
Lactose	+			+				+			+		+	+		+	+				
Lysine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Malonate	+		+	+	+	+	+	+	+	+		+	+	+	+	+			+	+	+
Maltose				+		+	+		+	+				+						+	
Mannitol	+	+		+		+	+		+					+						+	+
Mannose														+							
Melibiose		+	+	+	+	+							+	+					+		
Motility																	+				
Nitrate reduction		+				+						+	+	+	+					+	
ONPG		+	+	+	+	+	+			+	+	+	+	+	+				+	+	+
Ornithine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Oxidase		+	+												+					+	
Phenylalanine			+					+	+	+	+	+		+	+		+				+
Raffinose			+	+	+									+			+				+
Rhamnose	+	+	+	+	+	+	+	+						+		+	+		+	+	+
Salicin										+	+			+							+
Sorbitol	+	+		+	+	+	+	+	+	+	+	+	+	+		+	+		+	+	+
Starch														+							
Sucrose	+	+	+	+	+	+	+			+	+		+	+		+		+	+	+	+
Trehalose			+							+	+			+							
Tryptophane		+		+	+	+			+	+									+	+	
Urea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+
V-P (acetoin)		+	+		+		+				+	+	+	+	+					+	+
Xylose	+		+	+						+											

AMS - contains Glucose (Oxidative) and Glucose (Fermentative); contains growth control and base control.

\+ = gas production

* = Update of Table 2 of Hartman and Minnich (1981)

the test organism is inoculated. After incubation color changes will indicate positive or negative results. The results of these tests are converted to a seven-digit profile number (using the octal system) which is then matched in an index; The Minitek Numerical Taxonomy System. For numbers listed, the corresponding relative likelihood (confidence value) is printed as a percentage together with the absolute likelihood (biotype validity). Supplementary tests for the confirmation of suggested identifications are generally given. Identification may also be made through the use of differential tables or by computer through the offices of the manufacturer.

Kiehn et al. (1974) compared the Minitek and the conventional system for the identification of clinical isolates and stock cultures of Enterobacteriaceae. The Minitek correctly identified 90.1% of the cultures. The author stated that the Minitek provides complete speciation of Enterobacteriaceae but recommended use of a companion DNase plate. False-positive hydrogen sulfide reactions were the major fault with this system. Finklea et al. (1976) used 41 stock organisms and 581 fresh clinical isolates to compared the Minitek system to conventional tubed media to determine if the Minitek is feasible and accurate for a high-volume clinical microbiology laboratory. In addition to comparison with tubed media, they tested the reproducibility of discs, the effect of variation in inoculum size, the effect of the age of culture and the effect of predispensing discs. They demonstrated that reasonable variation in inoculum size and age of culture when inoculated into broth had no effect in the results. Discs may be predispensed to the plastic plates on a weekly basis, refrigerated, and used as needed. Some lot to lot variation in the discs dictates that quality control must be done on all new lot of discs. The carbohydrate fermentation pattern of 24 frequently isolated salmonellae from poultry were reliable as determined by Minitek discs (Cox and Mercuri, 1976).

Cox and Mercuri (1976) developed a rapid procedure for biochemical and serological confirmation of suspect Salmonella colonies with the Minitek system. Both

biochemical and serological testing were completed within 24 h after selecting a suspect colony from a primary isolation agar plate. This techniques provided an alternative to the time-consuming conventional procedures for confirming suspect Salmonella colonies. Cox and Mercuri (1977) compared the efficacy of four commercially available test for ONPG and the conventional ONPG test with Enterobacteriaceae isolates from poultry, human and selected foods. For the 102 cultures from human and poultry sources the Minitek agreed with the conventional test in 98% of the cases. For the 148 food isolates, the Minitek agreed with the conventional in only 88.5% of the cases. The authors stated that the API and the Minitek system may be more sensitive than the conventional one for detecting the presence of ß-galactosidase in some strains of Enterobacteriaceae isolated from food. Guthertz and Okoluk (1978) concluded that the Minitek system could be used by food microbiologist to definitely identify enteric organisms found in foods. The only agreements below 96% were those of 88.8% and 84.3% for citrate and urea respectively. The overall agreement between reactions on the Minitek and those in tube media was 96.9%. McCarthy et al. (1978) compared 11 Minitek biochemical tests with the corresponding conventional tubed media using 1,089 isolates of enteric gram-negative rods. The Minitek biochemical tests demonstrated a 97.4% overall correlation with conventional tests. All the Minitek tests with the exception of citrate yielded correlations of 94.8% or better when compared with conventional biochemical tests. The Minitek biochemical tests for lysine decarboxylase and adonitol fermentation yielded positive reactions earlier than those observed with Moeller lysine decarboxylase broth and 1% adonitol in phenol red broth base. Positive Minitek urease reaction appeared later than those observed on Christensen's urea agar isolates. The authors found that the oil necessary for the overlay, when dropped inadvertently between the wells of the inoculum plate, spread when the cover was replaced. The spreading oil formed a seal between the rims of wells containing discs, resulting in

false-negative reactions for discs not requiring an oil overlay. A year later, Cox et al. (1979), reported an overall agreement between Minitek and conventional biochemical tests of 93.6% which is considered excellent by most laboratories. The Minitek results agreed with the conventional tests for isolates from carrots and lettuce only 88 and 89% respectively, but for other six foods were greater than 93%. The correlation with results of conventional test for each of the 15 Minitek biochemical discs tested was from 93.3-100% for all tests except inositol (78%), ornithine decarboxylase (89.8%) and sorbitol (67%).

Cox et al. (1981) compared the Minitek inoculum broth and the Minitek H_2S/indole disc results for the accuracy of detecting indole and compared these results to those obtained with the conventional indole test. The indole test can be performed in almost any negative disc or in the broth vial following an 18-24 h inoculation. Their results indicated that the indole test with the Minitek inoculum broth is more reliable than the test with H_2S/indole disc. Cox et al. (1983) reported that the most frequent false-positive reaction that could result in an incorrect identification was Voges-Proskauer. And the most frequent false-negative reaction was indole.

Cox et al. (1984) discussed the technical advantages, disadvantages and cost of six presently available commercial systems: API 20E, Enteric-Tek, Enterotube II, Micro-ID, Minitek and Spectrum-10. The most often mentioned advantage of the Minitek system was its versatility. The 20 tests recommended by the manufacturer may be used or a specialized scheme can be divided according to individual needs by using any number and assortment of the 35 available test discs. An abbreviated number of tests may be selected if the user is only interested in an initial screening or in only identifying a specific member of the Enterobacteriaceae family rather than a more detailed species characterization. The Minitek system can also be used as an accessory to other miniaturized systems or to conventional procedures to perform any

additional tests that may be necessary. The ease of reading the reactions and stacking in the incubator were listed as advantages, whereas cost and the requirement of adding reagents were listed as disadvantages. The Minitek was found to be about twice as expensive as the other rapid systems. Fung et al. (1984) reported a 99 and a 97% accuracy for the Minitek on two separate workshops.

API 20E

The API 20E (Analytab Productcs, Inc, Plainview, N.Y.) is a standardized, miniaturized version of the conventional procedures for the identification of Enterobacteriaceae. It is a ready to use, microtube system designed for the performance of 23 standard biochemical tests (Table 2) from isolated colony(ies) of bacteria on plating medium. The API 20E has procedures for same day and 18-24 h identification of Enterobacteriaceae as well as 18-24 or 36-48 h identification of other gram-negative bacteria. The API 20E has been extensively reviewed by Cox and Mercuri (1979), Hartman and Minnich (1981) and others. The API 20E has been one of the more widely used kit system in clinical microbiology laboratory if not the most widely used in the past several years. Researchers had been using the API 20E as the reference or standard methods when evaluating other new systems for the past 3 or 4 years.

Cox et al. (1983) evaluated five miniaturized systems for identification of Enterobacteriaceae from stock cultures and freshly isolated from food sources. The accuracy of identification to genus was 93% and to species 91% using the API 20E. They found that the most frequent false-negative reaction with the API 20E was citrate and Voges-Proskauer. Castillo and Bruckner (1984) evaluated the accuracy and utility of the Eiken Systek No. 1, with 354 Enterobacteriaceae isolates (clinical) with API 20E and conventional methods. The API 20E correctly identified 339 (97.7%) and misidentified 3 (0.9%). There were no identification code for 5 (1.4%) organisms with

the API 20E. Overman et al. (1985) compared the the API Rapid E 4-h system with the API 20E for identification of routine clinical isolates of the family Enterobacteriaceae. The API 20E identified 98.9% (436 of 441) of the isolates without the use of additional biochemicals and was found to be correct in each case of the discrepancy among the 436 isolates.

API Rapid 20E

The Rapid 20E method (a 4 h system) consists of a strip of 20 microtubes containing dehydrated substrates to demonstrate the presence of enzymes or the fermentation of carbohydrates. The substrates are reconstituted when the bacterial suspension is added. The microorganisms react with the contents of the cupules during the 4 h incubation period to yield a metabolic endproduct and produce a color change. The system can not be used after 5 h even if preserved at 4° C. After incubation, indole and acetoin (Voges-Proskauer reaction) reagents are added. The tests are read according to the instruction of the manufacturer, and the identification is determined with the aid of the code book (data base, 3,000 biochemical profiles). Unlisted profiles are interpreted by referring to the manufacturer's computer (data base, 15,000 biochemical profiles).

Mounier and Denis (1983) compared the performance of Micro-ID and the Rapid 20E system in direct identification of Enterobacteriaceae in 120 blood cultures. They concluded that the Rapid 20E system (96.6%) seemed to be better than the Micro-ID system (87%) for the direct identification of Enterobacteriaceae in blood culture, particularly when red blood cells were removed before inoculation of the strip. Izard et al. (1984) compared the Rapid 20E and API 20E systems with conventional methods for identifying clinical Enterobacteriaceae isolates. The Rapid 20E yielded correct identification with 95.9% of the isolates tested. Keville and Doern (1984) found that the Rapid 20E

correctly identified 97.2% to species level within 4 h, and 94.6% were correctly identified with the API 20E after overnight incubation. Murray et al. (1984) found the Rapid E system to be accurate (94.1%) for the identification of Enterobacteriaceae. The lowest list price of the Rapid E is $1.93. They stated that the identification accuracy of the Rapid E system can be further improved by expanding the data base to include more biochemically variant strains of Enterobacteriaceae and to exclude oxidase-negative strains of organisms not specifically identified by the system (e.g., Acinetobacter spp.).

Cox and Bailey (1985) evaluated the Rapid 20E with food isolates and stock cultures. A total of 232 cultures, representing 13 genera of Enterobacteriaceae were used in this study. The Rapid 20E correctly identified 94.4% of the cultures to species. Ten of the thirteen errors in identification occurred with Enterobacter species and were due to a false negative reaction with the Voges-Proskauer test. Overman et al. (1985) compared the Rapid 20E to the API 20E, the Rapid 20E gave the same identification as the API 20E for 94.2% of the isolates, misidentified only 3%, and gave a correct but low selectivity identification for the remaining 2.8%. Appelbaum et al. (1985) evaluated the API 20E, Micro-ID, and the Rapid 20E for their ability to provide useful same day identification of 161 clinically isolated Enterobacteriaceae. The Rapid 20E correctly identified 96.9% of strains. The Micro-ID was found to be the easiest to set up, and the Rapid 20E had the advantage of requiring a lower inoculum than the API 20E or Micro-ID. The author concluded that both Micro-ID and Rapid 20E were accurate, and reliable methods for routine same-day identification of Enterobacteriaceae in the clinical laboratory.

Eiken Systek No. 1

The Eiken Systek No. 1 (Eiken Systems; Eiken Chemical Co., Ltd., Tokyo, Japan) is designed for identification of members of the families Enterobacteriaceae

and Vibrionaceae. The system consists of a white, opaque plastic tray containing 20 reaction wells covered with a plastic cover. This system can be stored at room temperature for up to 1 year. Inoculation does not require the use of pipettes, and the one-step inoculation method frees the technologist from tedious manipulation. The 21 biochemical tests included in the system are listed in Table 2.

Castillo and Bruckner (1984) conducted a clinical comparison, with 345 Enterobacteriaceae isolates, of the Eiken system with API 20E. The Eiken system correctly identified 79.5% and misidentified 3.7% of the isolates. There were no identification codes for 16.8% of the microorganisms with the Eiken system. Although the Eiken system correctly identified only 79.5% of the isolates tested, the low accuracy was the result of an inadeaquate computer code book data base.

Castillo and Bruckner (1984) concluded that the Eiken system, in its present form, does not have the accuracy of the API 20E; however, the ease of inoculation and the refrigerator space-saving capabilities make the Eiken system an attractive new development. Improvements in translation of the instructions in the code book are needed and with minor adjustments in decarboxylase and oxidase test interpretation and supplementary code numbers for the Proteus spp., Morganella sp., and Providencia rettgeri, the accuracy and utility of the Eiken system would be comparable to these qualities of the API 20E.

Enteric-Tek

The Enteric-Tek system (Flow Laboratories, Inc., Roslyn, NJ) is comprised of a circular plastic plate with 11 independently sealed peripheral wells and a central well, each of which contains agar based media. Fourteen biochemical characteristics can be determined altogether (Table 2). The wells are inoculated with a suspension of the test organism in distilled water using a Pasteur pipette. The plate is incubated for 18 to 24 h at 35 to 37° C and then examined for a color change

of the individual media; only the indole test requires the addition of a reagent. Tests are read in conjuction with a description of the appropriate color changes. Results may be interpreted with a differential chart or they may be converted to a profile number which is matched in an index, the Enteric Computer Code Book, provided by the manufacturer. Computer-assisted identification is also available from the offices of the manufacturer.

Appelbaum et al. (1982) compared the ability of the API 20E, Minitek and Enteric-Tek, to accurately and completely identify 368 clinically isolated Enterobacteriaceae without supplemental tests. The Enteric-Tek correctly identified 97% of the strains to species level with 3.0% spectrum identification. Bruckner et al. (1982) found that the Enteric-Tek system correctly identified 96.1% of strains tested to species level. They found the Enteric-Tek system to be a convenient method for rapid identification of the Enterobcteriaceae. The most serious disadvantage of the Enteric-Tek is that the phenylalanine deaminase and H_2S reaction take place in the same well. The phenylalanine reaction may be obscured by organisms producing H_2S. Goldstein et al. (1982) found that identification with the Enteric-Tek system agreed with those made with conventional biochemical 97% of the time. At a 95% confidence level the Enteric-Tek was able to identify 75% of the isolates within 18 h without the aid of additional tests. When additional biochemical tests were required for organisms identified at a confidence level below 95%, the Enteric-Tek needed an average of 2.05 additional tests to achieve a final identification. Esaias et al. (1982) found that the Enteric-Tek system correctly identified 264 (97.8%) of the 270 common or typical strains and 26 (83.9%) of the 31 unusual or atypical strains tested, demonstrating an overall identification accuracy rate of 96.3%. There were 26.6% correctly identified strains requiring additional tests. They also compared the biochemical reactions for the various tests in the Enteric-Tek with those obtained by the conventional method. Of

the 14 tests, 10 showed more than 97% agreement with their conventional counterparts, whereas urease, citrate, adonitol, and lactose agreed 83.4, 91.4, 94.0, and 94.4%, respectively. These four tests comprised 75% of all of the test discrepancies.

Cox et al. (1983) compared the Enteric-Tek system to conventional procedures for identification of Enterobacteriaceae from stock cultures and freshly isolated from food sources. The Enteric-Tek correctly identified 94 and 93% of the organisms to genus and species levels respectively. Fung et a. (1984) evaluated ten commercial bacterial diagnostic systems in two separate workshops (July, 1981 and July, 1982), the Enteric-Tek system correctly identified only 67% of the isolates. They stated that the deficiency in Enteric-Tek was probably related to the difficulty participants had in reading and interpreting color reaction. The system performed better, however, in the 1982 workshop with an accuracy of 89%. Cox et al. (1984) provided current information on technical advantages, disadvantages, and costs of six presently available commercial systems, including the Enteric-Tek system.

Entero-Set 20

The Entero-Set 20 (Fisher Diagnostics) is a 20 test kit for overnight identification of members of the Enterobacteriaceae. The system is a combination and reformation of the Entero-Set 1 and Entero-Set 2 (Inolex Corp.) kits, which are used for screening and identification to species, resoectively, of enteric isolates. D'Amato et al. (1981) described this system in detail.

The configuration of the Entero-Set 20 test strip is similar to that of the API 20E in that there are 20 biochemical tests (Table 2) impregnated into a plastic strip. Inoculation of the strip is easy. Three to five drops of inoculum are added to the top of each tube, which is filled by capillary action due to a small "venting" hole at the opposite end of the tube. Aldridge et al. (1981) compared the Entero-Set 20

kit with the API 20E using 303 stock and 202 clinical strains of Enterobacteriaceae, and concluded that the Entero-Set 20 performed with high degree of accuracy and reproducibility when compared with conventional tube media. When compared with the API 20E, the Entero-Set 20 performed equally well and offered the advantages of being easier to inoculate and requiring less reagents.

Enterotube II

The Enterotube II system (Roche Diagnostics, Nutley, NJ) contains 12 compartments and permits the determination of 15 biochemical parameters (Table 2). An inoculating needle, with an inoculating tip on one end and a handle on the other, extend through the entire length of the tube. Each end of the tube is picked onto the tip of the inoculating needle, and the media in the tube are inoculated by withdrawing the needle through the compartments. The inoculating needle is reinserted through all 12 compartments and is again withdrawn until the tip is in the H_2S/indole compartment. The end of the needle is broken off at a prescored position and the caps are loosely replaced. A strip of tape covering certain compartments is removed to provide aerobic conditions and the tube is incubated at 35 to 37° C for 18 to 24 h. After incubation, appropriate reagents are added to appropriate chambers for the indole and Voges-Proskauer tests. Test results are converted into a 5 digit profile number and identification is made from an identification manual, the Computer Coding and Identification System, through the use of differential charts, or by computer.

Hayek and Willis (1984) compared the API 20E and the Enterotube II, using the results with 235 cultures of fresh clinical isolates. On first testing, the Enterotube II only correctly identified 90.6 or 91.9% of the organisms examined, depending upon which profile index was used. The Enterotube II correctly identified all enteric pathogens, although identifying the lactose-fermenting Salmonella as

Arizona. They found that the dependence on extra tests to be carried out on nearly half the cultures examined has now been greatly reduced to about 25%. This is obviously due to the inclusion of four extra tests in the Enterotube battery. They criticized that the manufacturer have now complicated matters by making available two profile indexes which do not quite match. One index includes all tests while the alternative index lists those profiles reached without the use of the Voges-Proskauer test avoiding the necessity of waiting for the 10 minutes required for any color change to develop.

Micro-ID

The Micro-ID system (General Diagnostics Division, Warner-Lambert Company, Morris Plains, New Jersey) consists of a molded styrene tray containing 15 reaction chambers each containing an individual test substrate (Table 2) and a hinged cover. The first five chambers contain a substrate disc and a detection disc. The remaining ten reaction chambers each contain a single, combination substrate/detection disc. Discs contain all substrate and detection reagents required to perform the indicated biochemical test except for the Voges-Proskauer test. The Micro-ID system tests for preformed enzymes and is inoculated with a dense bacterial suspension, equal to at least a 0.5 McFarland turbidity standard prepared from morphologically identical, well isolated colonies. After inoculation the kit unit is placed upright in a support rack and is incubated for 4 h at 35 to 37° C. After incubation, 2 drops of 20% KOH is added to the Voges-Proskauer well. After reading the tests, the results are converted into a five-digit profile number using the octal coding system. The profile number may be interpreted by using an index, the Micro-ID Identification Manual, which lists for each profile number the four most likely taxa and indicates separately the absolute likelihoods and relative likelihoods.

Cox et al. (1979) studied the overall agreement of the 15 biochemical test in the Micro-ID system, and into the corresponding test in the Minitek system with the same 15 conventional test on 400 isolates (50 from each of 8 food), the overall agreement was 96.8% with Micro-ID and 93.6% with Minitek. Three laboratory technicians independently evaluated and recorded the results of each of the 18,000 test reactions; they were in complete agreement on 99.7% of the conventional tests, 99.3% of the Micro-ID tests and 98.9% of the Minitek tests. Also reported that Micro-ID plates could be inoculated in the afternoon and incubated overnight (16 h at 22° C) without decreasing the accuracy of identification. In another study Cox et al. (1980) evaluated the accuracy of the Micro-ID with 10 genera of Enterobacteriaceae grown in 11 selective plating media. Although the manufacturer cautioned against using certain of these media (Brilliant Green Sulfa Agar and Bismuth Sulfite Agar), correct identification were obtained from all media tested. They concluded that Brilliant Green Sulfa and Bismuth Sulfite agars, two media widely used by food microbiologist, may be used with the Micro-ID system. The inoculating procedure for Micro-ID also was modified by selecting only one colony (instead of several as recommended) from a primary isolation agar plate, incubating this colony in 0.2 ml of nutrient broth for 4 h, then adding 3.3 ml of physiological saline solution to the broth culture before inoculating the Micro-ID system. That modification minimized the chances of using a mixed culture from the primary isolation agar plate. Cox et al. (1981) compared the Micro-ID to the RC (a rapid confirmation 24-h biochemical and serological procedure involving the Minitek system) procedure for the identification of Salmonella with stock cultures, artificially inoculated poultry carcasses, and samples naturally contaminated with Salmonella, the Micro-ID correctly classified 141 of 144 known Salmonella stock cultures. When 113 suspect Salmonella isolates from naturally contaminated samples were examined the Micro-ID correctly classified all except one.

Manford Gooch and Hill (1982) reported a comparison between Micro-ID and API 20E as systems for same-day identification of member of Enterobacteriaceae. Overall agreement with conventional identification at genus and species levels was 93.5% with the Micro-ID system; 94.3% of isolates were identified on the day of inoculation with the Micro-ID. Telephone consultations with the manufacturers to resolve unprinted octal codes required a maximum of 15 min with Micro-ID and from 2 to greater than 48 h with API 20E. Bailey et al. (1983) evaluated the Micro-ID and concurrent serological tests to confirm suspect-Salmonella colonies in 8 h, and compared the Micro-ID serology procedure to the time-consuming conventional procedure. Of the 244 isolates confirmed to be Salmonella by conventional testing, 236 (97%) were also confirmed by the 8 h procedure. During the same year the Micro-ID was compared to conventional procedures for identification of Enterobacteriaceae from stock cultures and freshly isolated from food sources. The accuracy of identification to genus was 98%, and 97% to species level (Cox et al., 1983).

Cox et al. (1984) reported information on the technical advantages, disadvantages and cost of commercial systems from 23 professional microbiologists who had previous experience with the systems. The 4 h incubation period, ease of inoculation and minimal time required for addition of reagents were listed as advantages. The only disadvantage mentioned was that some of the reactions, primary the carbohydrates, are difficult to interpret.

Harrison and Williams (1985) evaluated the Micro-ID system for its ability to recognize Yersinia pestis of various biotypes. They tested a total of 100 cultures of Yersinia pestis that were originally identified by conventional procedures. The Micro-ID system indicated Y. pestis as a possibility for the identification of 89 (89%) of the cultures examined, although not always as the first choice. Micro-ID test of nine cultures resulted in a five digit code, 20071, that is not listed in the

Manual. When they accepted code 20071 as indicative of Y. pestis, the reliability of the Micro-ID system for identification of their cultures increased to 98%. They suggested that the Micro-ID system can be a useful and reliable procedure for presumtive identification of Y. pestis in clinical laboratories, providing that its limitations are recognized.

r/b

The r/b media (name derived from names of co-inventors William Rollender and Orville Beckford) is manufactured by Flow Laboratories, Inc., Roslyn, N.Y.. The r/b system is basically a set of composite agar media in screw-capped tubes specially constricted near their base to permit the determination of two or more reactions in each tube. The r/b system consists of the 2 basic tubes, the r/b 1 and r/b 2, as well as the Expanders, Cit/Rham and Soranase. These constricted tubes contain sterile media and are used to determine 14 biochemical parameters (Table 2). The tubes are inoculated with a needle, using well isolated colonies. All four tubes are inoculated to the base, and when applicable, the slant is streaked on withdrawal of the needle. The tubes are incubated in an upright position for 18 to 24 h at 35 to 37° C and the reactions are read. Results may be interpreted by means of a percentage chart or dichotomus keys provided by the manufacturer. An index, the r/b Computer code book, is also available, (D'Amato et al., 1981).

The work done with the r/b system has been reviewed by Cox et al. (1977), Cox and Mercuri (1979), and Fung and Cox (1981).

Spectrum 10

The Spectrum-10 system consist of 20 bichemical or carbohydrate tests contained in two separate clear plastic trays with 10 wells (tests) per tray. The biochemical and carbohydrate tests are shown in Table 2. A bacterial suspension is

prepared by suspending a single isolated colony into 3 ml of sterile water (pH 6.8 to 7.2). One ml of this suspension is dispensed into the back section of each tray with a sterile calibrated pipette. After inoculation, the trays are tilted back to a 45° angle and gently rocked from left to right to evenly distribute the inoculum along the back section of the tray. The trays are then gently tilted forward to allow the inoculum to flow forward evenly into each of the test wells. Two strips of cellophane tape cover the front and back sections of the trays. The front section is never removed, thus preventing contamination or possible hazard to the user. Four drops of sterile mineral oil are added to each of the following wells: ADH (arginine dihydrolase), LDC (lysine decarboxylase), ODC (ornithine decarboxylase), H_2S and URE (urease). The trays are incubated for 18 to 24 h at 35 to 37° C. Following incubation, the reaction of each test is recorded on a report form to obtain a specific code. The organism is identified by comparing the codes to the Spectrum-10 code manual.

Cox et al. (1985) found that in comparison to the Micro-ID and API-20E systems, the Spectrum-10 identified 95 to 96% of the stock cultures to genus and species, whereas 93% of the fresh isolates were identified to genus and 82% to species. Primarily the incorrect identifications were due to false-positive reactions with LDC, ODC or ADH. Cox et al. (1985) suggested that the LDC, ODC and ADH test should be improved to eliminate or at least minimize false-positive reactions and also additional instruction or a color comparison chart could prove helpful in further assisting the user in reduction of errors.

The system does offer several advantages: least expensive of the various commercially available miniaturized systems (Cox et al., 1984). As the data base expands and the few necessary improvements are incorporated, Spectrum-10 should prove to be a very useful tool for the food microbiologist.

Abbott MS-2

The MS-2 Microbiology System, developed by Abbott Laboratories, is one system now available that is designed to function in several applications. The MS-2 can be used to perform susceptibility studies, minimum inhibitory concentration determinations, urine screening and in bacterial identifications. It is designed to perform these functions simultaneously. The system measures and analyzes turbidimetric or colorimetric changes resulting from bacterial growth over a defined time period. The system maintains a controlled temperature environment for incubation of bacterial organisms. The major components of the MS-2 are the cuvette cartridge, the disc/loader sealer, the analysis module and the control module. Depending on the applications desired, several accessories and disposable components are essential. In bacterial identification, special single-use disposable cartridge are available, each containing seventeen biochemical substrates in a lyophilized form. Abbott indicated that the MS-2 system is limited to analysis of rapidly growing aerobic facultative bacteria from isolated pure cultures.

McCracken et al. (1980) evaluated the MS-2 Bacterial Identification (BID) system by using 150 coded unknown organisms and 1,154 recent clinical isolates. The MS-2 identified 86% of the isolates correctly compared with conventional manual tube methods. An additonal 8 to 9% of the organisms were correctly identified, but with a lower percent likelihood (less than 80%). These strains required additional bichemical testing to confirm the first-choice identification. They also stated that the MS-2 system was rapid and simple to operate and produced printed result of bacterial identification in 5 h. In addition the cost of disposable components ($2.10), compared favorably with commercialy, visually read systems for identifying Enterobacteriaceae.

In a collaborative study by DiPersio et al. (1983) who evaluated the impact of an expanded data base and related software improvement in the identification accuracy of the MS-2 BID system. The updated MS-2 software correctly identified 94.4% of the isolates tested. API 20E and the original MS-2 software correctly identified 91 and 85.3% of the strains respectively. MS-2 responses were considered to be equivocal (needing additional tests for verification) if the percent likelihood values were less than 80%. The percentage of equivocal responses was reduced from 6.5% with the original software to 2.2% with the updated software, and the percentage of incorrect identifications was reduced from 8.2 to 3.4% with the original and updated software, respectively. DiPersio et al. (1983) concluded that a high level of accuracy, coupled with a 4 to 5 h identification, makes the newer MS-2 BID system an attractive alternative for routine use in microbiology laboratories.

Autobac IDX

The Autobac identification system consist of five main components: light-scattering photometer, incubator-shaker, data terminal, and 19-chamber cuvette. The preparation of the cuvette and the 18 inhibitory agents used in the identification system have been described by Sielaff et al. (1982).

To perform an identification with the Autobac system, the gram-negative bacilli must first be isolated on both a sheep blood agar plate and a MacConkey agar plate: whether growth occurred; and if growth occurred, whether lactose was fermented; and whether the bile salts in the medium were precipitated. One observation, presence or absence of swarming growth, is made from the blood agar plate, and two rapid biochemical tests, a spot indole and a spot oxidase, are performed with a colony from that plate.

This system utilizes growth inhibition profiles to a panel of differentially inhibitory chemical agents. Cuvettes are read after 3 to 6 h of incubation. A probability of 0.80, calculated by the data terminal with two-stage quadratic discriminant analysis, is considered satisfactory for identification (Sielaff et al., 1982).

Barry et al. (1982b) evaluated the Autobac system for rapid identification of gram-negative bacilli and found and overall accuracy of 95.3% for identification of reference strains and clinical isolates during experimental conditions. Kelly et al. (1984) compared the Autobac IDX system with the identification methods in routine use in four laboratories. The study included 1,515 organisms representing 30 species of enteric and nonenteric bacteria. Overall, 98% of the organism were correctly identified by the routine method, and 93% were correctly identified by the IDX system. After adjustment for frequency of clinical occurrence of the organism tested, the IDX system was performed with 95% accuracy. Results with the IDX system were available in 3 to 6 h. Results with the comparative methods were available in 4 to 48 h. Costigan and Hollick (1984) evaluated the accuracy of the Autobac IDX system to identify 290 gram-negative bacilli from 18 different genera. They found an overall sensitivity of 95.8%. Late lactose-fermenting Escherichia coli, Citrobacter freundii, and Proteus mirabilis accounted for over 90% of the misidentifications.

Automicrobic (AMS) System

The AutoMicrobic system (AMS, Vitek System, Inc., Hazelwood, MO.) is a fully automated computerized bacteriological system that was introduced in 1976 for automated detection and identification of organism in urine. The AMS instrument has six components: diluent dispenser, filling module, reader-incubator, computer, cathode ray tube-keyboard module, and card sealer. The system has been diversified to also enumarate, identify and determine the antibiotic susceptibility of microorganisms.

In January 1979 the Enterobacteriaceae Biochemical Card (EBC) for the identification of organisms of the family Enterobacteriaceae from cultures was released for clinical use in the AMS. The AMS-EBC consist of a plastic card with 30 wells for biochemical test media. This EBC has 26 different biochemical tests (Table 2) that are monitored automatically once the card is inoculated and placed in the instrument. The biochemical reactions are analyzed by a computer module which issues an identification within 8 h. The AMS-EBC was first evaluated for identification of Enterobacteriaceae by Isenberg et al. (1980). Kelly and Latimer (1980) also evaluated the identification accuracy of the AMS instrument compared with conventional biochemical identification methods; the AMS correctly identified 97% of the organisms tested.

Benfari Ferraro et al. (1981) investigated presumptive identification of certain organisms within 4 h; they concluded that the AMS-EBC has the economical advantage of providing preliminary identification within 4 h as well as complete definitive identification after an additional 4 h of incubation of the same subculture test module without further cost to the user. Goldstein et al. (1982) evaluted the AMS system with the Enteric-Tek system for the identification of Enterobacteriaceae freshly isolated from clinical specimens, the AMS identified 92% of the isolates in 8 h without the aid of additional biochemical tests. Technologist time was reduced approximately 57% compared with the Enteric-Tek.

Schifman and Ryan (1982) evaluated the automated identification of gram-negative bacilli directly from blood culture bottles by using the AMS Enterobacteriaceae-plus nonfermenter identification card, an addition designed to identify clinically important oxidase positive or glucose-nonfermenting, or both, organism in addition to Enterobacteriaceae. The AMS-EBC+ correctly identified 92.6% of the organisms within 8 to 13 h of the first reading. Of 69 identifications analyzed after 6 h of incubation, 91% were correct. The EBC+ card has 30

microwells containing 29 biochemical broths and a positive control broth. Barry et al. (1982a) presented the results of two independent evaluations of the AMS, using the EBC+ and identical standard reference methods, the reference tests were considered to be 96% accurate and the AMS was 97% accurate, so they concluded that the AMS, with the EBC+, was perfectly reliable for identifying the most common gram-negative bacilli, provided that inconclusive (equivocal) identifications are recognized and that supplementary test systems are available to confirm the identities of such strains. Malloy et al. (1983) demonstrated that the AMS-EBC+ identification card can be used to identify Enterobacteriaceae directly from the broth of positive blood cultures within 5 h. They stated that some of the advantages of the AMS include minimal set-up time and automatic reading and computation of results, which require little to no technician time and reduce transcription error. Disadvantages include initial cost and the space requirements.

In two separate workshops (July, 1981 and July 1982) consisting of 40 participants the AMS was found to be 97 and 100% accurate respectively in the identification of 12 coded enteric bacteria (Fung et al., 1984). Bailey et al. (1985) evaluated the accuracy of the AMS for the identification of Enterobacteriaceae from foods and feeds. The AMS correctly identified to species 99.3% of the stock cultures and 98.2% of the fresh isolates. All Salmonella cultures tested were correctly identified by AMS. They stated that the selection of biochemicals and the data base of the AMS appeared to be adequate to insure a highly acceptable percentage of correct identifications with isolates from foods.

Harris and Graves (1985) developed the Enteric Pathogen Card (EPS), a cost effective rapid screen for colonies suspected of being Salmonella, Shigella, or Yersinia enterocolitica for the Vitek Automicrobic System. A subset of 10 substrates from the Gram Negative Identification (GNI) Card has been utilized in triplicate in the EPS card which allows for simultaneous testing of three separate

isolates. Preliminary screen results are available in 1-7 h with a final report automatically printed at 4-8 h.

Quantum II

The Quantum II system (Abbott Laboratories, Diagnostic Division, Irving, Texas) consists of a 20-chamber disposable plastic cartridge, a multipunch cartridge perforator, and a dual wavelength photometer that measures colorimetric changes in the individual cartridge chambers. The lyophilized biochemical reagents in the cuvette are listed on Table 2. The readings for the individual biochemical reaction are automatically interpreted by the photometer and compared with a probability matrix by an internal microcomputer, and the most likely identification, additional test information (e.g., percent likelihood of identification, supplemental tests), and a biotype are automatically printed (DeCresce and Blatt; 1984).

Murray et al. (1984) tested a total of 492 clinical isolates from the family Enterobacteriaceae, 97% of these isolates were correctly identified with the Quantum II system. An additional 48 non-Enterobacteriaceae isolates were tested with the Quantum II system and 83.3% were correctly identified. The majority of incorrect identification were caused by a single aberrant biochemical reaction. They recommended incubating the Quantum II for 5 h before reading results. The lowest list price for the Quantum II is $1.64, and it includes lease of the instrument from Abbott Laboratories. The major advantage of the Quantum II photometer was the standardized interpretation of the test reactions rather than the speed or reduction of technical processing time.

Sherlund et al. (1985) evaluated the Quantum II for accuracy of identifying enteric pathogens, excluding Salmonella typhi; 99% of 84 Salmonella isolates were identified correctly to the genus level (probability greater than .80). Of 43 Salmonella typh, 98% were identified with a probability of greater than 0.75. With

indole-negative Shigella, 92% of 73 isolates were identified correctly to genus level. The authors concluded that the Quantum II System provides a rapid, reliable method for identifying routine isolates of Salmonella and Shigella. Lineback et al. (1985) compared identification of 342 clinical and 116 selected isolates of Enterobacteriaceae by Quantum II and API 20E used according to manufacturer's instruction. The authors stated that for Quantum II setup and reading time was about one minute longer than API 20E but supplies were slightly less expensive. The Quantum II compared favorably to the overnight API 20E. Weiser et al. (1985) compared the Quantum II System with either Micro-ID, API 20E, Vitek and conventional replicator method. The overall correlation between identification with the Quantum II System and the other system was 96%.

Hardy et al. (1985) evaluated and compared three optical scanning automated systems (the API Data Management System, MicroScan AS4, and the Quantum II) for their ability to correctly identify gram-negative microorganisms (93 member of Enterobacteriaceae in 18 h and require 36-48 h for identification of non-fermenters). The Quantum II requires only 4 and 5 h incubation, respectively, for identification of the Enterobacteriaceae and non-fermenters. Of the 114 isolates surveyed, agreement with conventional API 20E was 97% with the API-DMS, 94% with MicroScan, and 93% with the Quantum II. The level of disagreement was more apparent with non-fermenter and was most evident with the Quantum II due to the absence of some species from the data base.

Sensititre

The Sensititre identification system (manufactured by Seward Laboratory, London, England, and distributed in the United States and Canada by GIBCO Laboratories) consists of a microplate containing a pattern of 24 biochemicals repeated four times together with an automatic inoculation device and a

microcomputer-assisted data interpretation component. The Sensititre identification plate consist of a 96-well plastic microtiter plate packaged in a moisture-proof aluminum foil pouch, containing 24 dried biochemical test substrate (Table 2) arranged in three vertical columns repeated four times across the plate. Staneck et al. (1983) described in details the system components, the method of use, and the computer-assisted derivation of identification.

A total of 1,415 isolates of Enterobacteriaceae plus 6 isolates of other glucose-fermenting gram-negative bacilli were tested in three hospital laboratories in parallel with API 20E. Sensititre yielded correct identification at the species level with 94.6% of the isolates and at the genus level with an additional 1.9%. Among the distinct advantages of the Sensititre equipment is its flexibility of use. It can be utilized either as a manual system providing distinct economic advantages or as a semiautomated microcomputer-assisted system offering economical running costs and the flexibility of use with microtiter antimicrobial susceptibility testing plus commercially available or user-written data management programs (Staneck et al.,1983).

Summary of major studies concerning accuracies of various commercial systems compared with the conventional system is presented in Table 3. Table 4 is a summary of advantages and disadvantages of major commercial diagnostics kits from various sources.

Cost Analysis

A number of miniaturized kits are commercially available since late 1960's for the diagnosis of Enterobacteriaceae in the clinical laboratory. Many of these kits had been adapted to food microbiology. Evaluation of these kits has generally demonstrated that they are accurate when compared to the conventional biochemical tests performed in individual tubes. The consideration of cost is extremely important when considering the adoption of a new miniaturized or rapid system.

Robertson et al. (1976) performed a detailed time and cost analysis comparing a conventional 17 tube (20 tests) system and the API 20E for the diagnosis of Enterobacteriaceae isolates. They found that performing 20 tests with the API 20E cost $3.02, whereas the comparable 17-tube method cost $7.98 per isolate. A conventional 7-tube (10 tests) setup cost $3.60, whereas the comparable API 10S cost $2.33. They also compared the API 10S and the API 20E, and found that the API 20E increased cost by 30% while increasing the number of isolates identified correctly by 3%. The authors also stated that performing 20 tests with the API 20E kit cost less than performing only 10 tests with the tubed media. Bartlett et al. (1979) presented a system of cost analysis in which materials and costs are separately computed, and to which are added the effects of fringe benefits, decreased productivity resulting from administration, quality control, education and development and the additional expense of indirect costs that are allocated to laboratory procedures by accepted and standardized hospital accounting methods. Bale and Matsen (1981) studied the assess time, motion, and cost required for identification of Enterobacteriaceae using a conventional (average 7 tubes) system, the API 20E and Micro-ID kit methodologies. They found that the Micro-ID method required less technologist time (4.5 min) for set-up and interpretation than did either the API 20E method (6 min) or conventional method (7 min). Total direct costs (June 1981) per organism identified were: Micro-ID, $4.30; API 20E, $4.96; conventional biochemicals with commercially prepared media, $5.66. An estimate of 80% technologist time efficiency was made in all procedures.

Table 3. Performance of Miniaturized Systems With Clinical and Food Isolates

System and Source of Isolates	% Correlation	Reference
COMMERCIAL DIAGNOSTIC SYSTEMS		
API 20E		
Food	82.0	Cox and Mercuri (1979)
Clinical	92.0	Kelly and Latimer (1980)
Stock + Clinical	95.0	Aldridge et al. (1981)
Clinical	93.7	Appelbaum et al. (1982)
Clinical	92.4	Bruckner (1982)
Stock + Clinical	90.2	Manford and Hill (1982)
Food	93.0 (genus)	Cox et al. (1983)
Food	91.0 (species)	Cox et al. (1983)
Clinical	91.0	DiPersio et al. (1983)
Blood culture	60.0	Malloy et al. (1983)
Clinical	97.8 (genus)	Staneck et al. (1983)
Clinical	91.1 (species)	Staneck et al. (1983)
Clinical	97.7	Castillo et al. (1984)
Clinical	98.0	Izard et al. (1984)
Food	89.0 (1981)	Fung et al. (1984)
Food	97.0 (1982)	Fung et al. (1984)
Clinical	98.7	Hayek and Willis (1984)
Clinical	94.6	Keville and Doern (1984)
Clinical	98.0	Murray et al. (1984)
API 20E RAPID 20E/DMS RAPID E		
Blood Culture	91.7	Mounier and Denis (1983)
Clinical	95.9	Izard et al. (1984)
Clinical	97.2	Keville and Doern (1984)
Clinical	94.1	Murray et al. (1984)
Stock & Feed	94.4	Cox and Bailey (1985)
Clinical	97.0	Overman et al. (1985)
Clinical	96.9	Appelbaum et al. (1985)
EIKEN		
Clinical	79.5	Castillo and Bruckner (1984)
ENTERIC-TEK		
Clinical	97.0	Appelbaum et al. (1982)
Clinical	96.1 (species)	Bruckner et al. (1982)
Stock	96.3	Esaias et al. (1982)
Clinical	75.0	Goldstein et al. (1982)
Food	93.0	Cox et al. (1983)
Food	87.0 (1981)	Fung et al. (1984)
Food	89.0 (1982)	Fung et al. (1984)

Table 3 (continued).

System and Source of Isolates	% Correlation	Reference
ENTERO-SET 20		
Stock + Clinical	97.0 vs tube	Aldridge et al. (1981)
	92.0 vs API	Aldridge et al. (1981)
	91.0 vs API vs tube	Aldridge et al. (1981)
ENTEROTUBE II		
Food	86.0 (genus)	Cox et al. (1983)
Food	79.0 (species)	Cox et al. (1983)
Food	91.0 (1981)	Fung et al. (1984)
Food	97.0 (1982)	Fung et al. (1984)
Clinical	91.9	Hayek and Willis (1984)
MICRO-ID		
Food	96.8	Cox and Mercuri (1979)
Clinical	92.4	Edberg et al. (1979a)
Blood culture	96.1	Edberg et al. (1979b)
Salmonella	99.0	Cox et al. (1981)
Stock + Clinical	93.5-95.8	Manford and Hill (1982)
Food (Salmonella)	97.0	Bailey et al. (1983)
Food	98.0 (genus)	Cox et al. (1983)
Food	97.0 (species)	Cox et al. (1983)
Blood culture	90.0	Malloy et al. (1983)
Food	97.0	Fung et al. (1984)
Yersinia pestis	83.0-98.0	Harrison and Williams (1985)
MINITEK 24h		
Food	96.8	Cox and Mercuri (1979)
Food	93.6	Cox and Mercuri (1979)
Clinical	96.0	Appelbaum et al. (1982)
Food	95.0 (genus)	Cox et al. (1983)
Food	94.0 (species)	Cox et al. (1983)
Food	99.0 (1981)	Fung et al. (1984)
Food	97.0 (1982)	Fung et al. (1984)
MINITEK 4h		
Food	92.0 (1982)	Fung et al. (1984)
R/B		
Food	72.0	Cox and Mercuri (1978)
SPECTRUM 10		
Food	91.0 (1982)	Fung et al. (1984)
Stock + Food	95.0 (stock)	Cox et al. (1985)
	93.0 (genus-fresh)	Cox et al. (1985)
	82.0 (species-fresh)	Cox et al. (1985)

Table 3 (continued).

System and Source of Isolates	% Correlation	Reference
INSTRUMENTAL SYSTEMS		
ABBOTT MS2		
Clinical	94.0	McCracken et al. (1980)
Clinical	94.4	DiPersio et al. (1983)
Blood culture	44.0	Malloy et al. (1983)
ABBOTT QUANTUM II		
Clinical	97.1	Murray et al. (1984)
Clinical	94.6	Boshard et al. (1985)
Clinical	93.0	Hardy et al. (1985)
Clinical	95.0	Kelley et al. (1985)
Clinical	95.0	Pfaller et al. (1985)
Clinical	96.0	Weiser et al. (1985)
Clinical + Stock	85.3	Brandt et al. (1985)
AUTOBAC IDX		
Stock + Clinical	95.3	Barry et al. (1982)
Clinical	93.1	Sielaff et al. (1982)
Clinical	95.8	Costigan and Hollick (1984)
Clinical	93.0	Kelly et al. (1984)
Clinical	96.3	Boshard et al. (1985)
AUTOMICROBIC (AMS)		
Clinical	96.4	Isenberg et al. (1980)
Clinical	97.0	Kelly and Latimer (1980)
Stock + Clinical	96.0	Benfarri Ferraro et al. (1981)
Stock + Clinical	99.2	Burdash et al. (1981)
Clinical	97.0	Barry et al. (1982)
Clinical	92.0	Goldstein et al. (1982)
Stock + Clinical	92.6	Schifman and Ryan (1982)
Blood culture	92.0	Malloy et al. (1983)
Food	97.0 (1981)	Fung et al. (1984)
Food	100.0 (1982)	Fung et al. (1984)
Stock + Food	99.3 (stock)	Bailey et al. (1985)
	98.2 (fresh)	Bailey et al. (1985)
Clinical	97.9	Boshard et al. (1985)
Clinical	96.0	Pfaller et al. (1985)
AUTOSCAN-3		
Stock + Clinical	95.1 (genus)	Woolfrey et al. (1983)
	94.9 (species)	Woolfrey et al. (1983)
	97.9 (genus)-visual	Woolfrey et al. (1983)

Table 3 (continued).

System and Source of Isolates	% Correlation	Reference
AUTOSCAN IV		
Stock + Clinical	96.4	Brandt et al. (1985)
SCEPTOR		
Stock + Clinical	96.8 (genus)	Woolfrey et al. (1983)
	93.4 (species)	Woolfrey et al. (1983)
SENSITITRE		
Clinical	96.5 (genus)	Staneck et al. (1983)
	94.6 (species)	Staneck et al. (1983)

Table 4. Advantages and Disadvantages of Some Commercial Diagnostic Systems.

SYSTEM	ADVANTAGES	DISADVANTAGES
API RAPID 20E	1. The provision of final identification after 4 hours. 2. Incubation time of 4 hours. 3. Miniaturization and sensitivity enable identification from a primary plate with one colony, versus 3 or 4 with the Micro-ID system.	1. The main disadvantage is the inability of user to obtain results after 5 hours.
Future - The utility of the API Rapid 20E system for identification of less common Enterobacteriaceae awaits further investigation.		
EIKEN	1. System can be stored at room temp. for up to 1 year. 2. Inoculation does not require the use of pipette and the one step inoculation method free the technologist from tedious manipulation.	1. Kit directions are difficult to interpretate. 2. Inadequate computer code book data base. 3. Requires strip manipulation.
ENTERIC-TEK	1. Only one reagent is needed to complete the biochemical tests and generate a five-digit number for computer code identification 2. Requires only a small amount of inoculum. 3. Ease of inoculation 4. Minimal manipulation 5. Ease of reading the color changes in the media.	1. Phenylalanine deaminase and H_2S reaction take place in the same well. The phenylalnine reaction may be obscured by organisms producing H_2S. 2. Need for slightly more storage space. 3. Easy to contaminate oneself while inoculating and handling.
Problems - The inaccurate testing of bacterial strains that produce only small quantities of indole. H_2S may be falsely negative.		
ENTERO-SET	1. Can truly be called a rapid test kit in that reaction can be interpreted within 3 to 4 hours after inoculation.	1. Has not gained popularity in clinical laboratories because of persistent difficulties in accurately interpreting some of the reactions.

Table 4 (continued).

SYSTEM	ADVANTAGES	DISADVANTAGES
ENTEROTUBE II	1. Easiest to inoculate. 2. Takes up little space in storage. 3. Risk of contamination is minimal.	1. A minor problem exist in differentiating the elevation of the wax overlay in the glucose chamber (an indicator of gas production) from shrinkage of the media during storage. 2. Indole and V-P reagents must be added with a needle and syringe through the thin plastic backing. If this is not done carefully, the added reagent can leak into other chambers, resulting in altered reactions.
MICRO-ID	1. Only one reagent (20% KOH) needs to be added to one chamber prior to interpreting the results. 2. Short incubation time. 3. Easy to inoculate. 4. Minimal time required for addition of reagents.	
MINITEK	1. For microbiologists who desire freedom of choice of the physiological characteristics to use for identification. 2. Versatility. 3. Rigid construction. 4. Easily stacked in incubators.	1. The system requires several manual manipulations in the set up, incubation and interpretation steps. 2. The need to overlay the discs and culture medium within the reaction well with mineral oil adds an extra step to the procedure, and is considered messy by manual users. 3. Difficult and time consuming to inoculate. 4. Expensive.

Table 4 (continued).

SYSTEM	ADVANTAGES	DISADVANTAGES
r/b		1. Since the tubes contain hydrated agar medium, the shelf life is shorter than for systems that utilize dehydrated substrates. There is a tendency for the media to dehydrate in storage if the caps are not tightly secured.
FUNG'S MINIATURIZED METHOD	1. Low cost of production. 2. Flexibility of test. 3. Mass production of data for many unknowns. 3. Savings of space and time.	1. Need to have large number of organisms to test at any one time. 2. Technician needs to be well trained, experienced and dexterous. 3. Chances of cross-contamination. 4. Requires several manual manipulations in the set up, incubation and interpretation steps.
CONVENTIONAL	1. Widely accepted or classic methodology. 2. Versatile or flexibility in biochemical testing. 3. Format can be altered to include short sets concommitent with morphology criteria to actually be cheaper than any kit method for most gram-negative isolates.	1. Difficult and time-consuming to inoculate. 2. Results are time-consuming to read. 3. Requires an inordinate amount of incubator space. 4. Media preparation, clean up, quality control of media and reagent also very time-consuming. 5. Short storage time. 6. Longer incubation time required.

MATERIAL AND METHODS

Stock cultures analysis

The stock cultures of named Enterobacteriaceae analyzed in this study are presented in Table 5. Each of the stock cultures was inoculated into tubes of Brain Heart Infusion (BHI) broth (Difco) and incubated for 24 h at 37° C. Each was then streaked for isolation onto plates of MacConkey agar (Difco) and incubated for 24 h at 37° C. One isolated colony was transferred from the plate to a 1.5 ml vial of Minitek inoculum broth, and another isolated colony was transferred to a BHI broth (Difco) and incubated for 24 h at 37° C for use in the microtiter system the next day. The remaining isolated colonies on the MacConkey plate were sampled with a loop to inoculate the conventional tube system with a loop. All cultures were correctly re-identified by the conventional method.

Fresh isolates from raw foods

Samples of ground beef, shrimp, carrots, tomatoes, green onion and broiler carcasses were obtained from a local supermarket, and also beef trim was obtained from the meat processing laboratory at Kansas State University. Each of the ground beef, beef trim, shrimps, carrots and tomatoes (50 g) was blended in sterile blendor jars (Osterizer blender at blend speed) with 450 ml of sterile buffered peptone (0.1%) for 1 min. Green onions and broiler carcasses meat were shaken vigorously for 1 min with 100 ml of sterile buffered peptone in stomacher bags. Following preparation of samples, serial dilutions were made and Enterobacteriaceae count were obtained using Violet Red bile agar (Difco) containing 1% glucose (VRBG) (Mossel et al. 1962). After 24 h of incubation at 37° C, isolated colonies were ramdomly selected from VRBG. These colonies were then streaked onto MacConkey agar (Difco) plates and incubated for 24 h at 37° C to produce pure, isolated colonies. Cell suspensions were prepared as described above.

Table 5. Stock Culture Organisms Used in This Study.

Organism	No. of Strains	Fung's Mini System Correct Identification To Genus	Fung's Mini System Correct Identification To Species	Minitek Correct Identification To Genus	Minitek Correct Identification To Species
Citrobacter diversus	2	2	2	2	2
Citrobacter freundii	3	3	3	3	3
Enterobacter aerogenes	2	2	2	1	1
Enterobacter cloacae	3	3	3	3	3
Enterobacter gergoviae	3	3	3	3	3
Enterobacter sakazakii	3	3	3	3	3
Escherichia coli	5	5	5	5	5
Hafnia alvei	3	3	3	3	3
Klebsiella oxytoca	3	3	3	3	3
Klebsiella pneumoniae	5	4	4	5	5
Morganella morganii	2	0	0	2	2
Proteus mirabilis	2	2	2	2	2
Proteus vulgaris	3	1	1	3	3
Providencia stuartii	2	2	2	1	1
Providencia rettgeri	3	3	3	3	3
Salmonella III = Arizona	2	2	2	2	2
Salmonella enteriditis	4	4	4	4	4
Serratia liquefaciens	2	2	2	2	2
Shigella sonnei	2	2	2	2	2
Shigella dysenteriae	2	2	2	2	0
Shigella flexneri	2	2	2	2	0
Total	58	53 (91.38%)	53 (91.38%)	56 (96.55%)	52 (89.66%)

Conventional system

The conventional biochemical tests for bacterial identification and corresponding Minitek discs evaluations are shown on Table 6. The following media were prepared according to the manufacturer's (Difco) instructions: Triple-sugar iron agar, Simmons citrate agar, malonate broth, phenylalanine agar, motility test medium, urea broth, nitrate agar, MR/VP broth (BBL). Moeller decarboxylase broth base (Difco) was used as a basal medium for the testing of lysine and ornithine decarboxylases and arginine dihydrolase. A 1% tryptone broth (Difco) was used as the medium for indole production. Phenol red broth base (Difco) was used as the medium for the testing of adonitol, arabinose, dextrose, inositol, lactose, raffinose, rhamnose, sorbitol and sucrose fermentations. All organisms were tested for cytochrome oxidase activity (Difco).

All tubes were inoculated by loop with isolated colonies from a MacConkey agar plate and were incubated at 37° C. After 18 to 24 h of incubation, reagents were added for indole, Voges-Proskauer (acetoin), phenylalanine and nitrate tests. Tubes which were not positive were incubated at 37° C and re-examined daily for 3 days before being discarded. Bacteria were identified according to the schema of Edwards and Ewing (1980).

Fung's mini system

Miniaturized microbiological techniques using the Microtiter system developed by Fung and Hartman (1975) were used to study carbohydrate fermentation, indole production, Voges-Proskauer test, citrate utilization, H_2S production, motility test, nitrate reduction, urea hydrolysis, phenylalanine deamination, amino acid decarboxylation and malonate utilization tests.

For all the tests, sterile microtiter plates (Dynatech Laboratories, Inc., Alexandria, VA) as growth chambers were used and the volume of liquid was 0.2 ml per well unless otherwise stated. Media for the amino acid decarboxylation test was

Table 6. Conventional Biochemical Tests Used For Bacterial Identification and Corresponding Minitek Discs Evaluated.

Test	Conventional Medium	Minitek Disk
Adonitol	Phenol Red Broth base + 1% Adonitol (DIFCO)	AD
Arabinose	Phenol Red Broth base + 1% L-arabinose (DIFCO)	AR
Arginine	Decarboxylase medium, Moeller base + .5% L-arginine (DIFCO)	ARG
Citrate Utilization	Simmon Citrate Agar (DIFCO)	CIT
Dextrose	Phenol Red Broth base + 1% Dextrose (DIFCO)	DEX/without nitrate
Hydrogen Sulfide Production	Triple Sugar Iron Agar (DIFCO)	H_2S/IND
Indole Production	Tryptone (DIFCO)	H_2S/IND
Inositol	Phenol Red Broth base + 1% Inositol (DIFCO)	I
Lactose	Phenol Red Broth base +1% Lactose (DIFCO)	L
Lysine decarboxylase	Decarboxylase medium, Moeller base + .5% L-lysine (DIFCO)	LY
Malonate	Malonate Broth (DIFCO)	MAL
Nitrate Reductase	Nitrate Agar (DIFCO)	NR
ONPG	ONPG	ONPG
Ornithine decarboxylase	Decarboxylase medium, Moeller base + .5% L-ornithine (DIFCO)	OR
Phenylalanine deaminase	Phenylalanine Agar (DIFCO)	PA
Raffinose	Phenol Red Broth base + 1% Raffinose (DIFCO)	RA
Rhamnose	Phenol Red Broth base + 1% Rhamnose (DIFCO)	R
Sorbitol	Phenol Red Broth base + 1% Sorbitol (DIFCO)	SO
Sucrose	Phenol Red Broth base + 1% Sucrose (DIFCO)	SU
Urease activity	Urea Broth (DIFCO)	UR
Voges-Proskauer	MR-VP Broth (BBL)	VP
Motility	Motility Test Medium (DIFCO)	Not Tested

added to each well of the microtiter plate, inoculated with organisms, and overlayed with sterile mineral oil (ca. 2 drops). For phenylalanine deamination, citrate utilization, nitrate reduction, TSI (H_2S production) and motility test, 0.2 ml of the appropriate media was added to each well.

A "master" plate was prepared by placing four drops (ca. 0.2 ml) of a bacterial culture (BHI) into each well of a microtiter plate. Twenty-four cultures were tested in quadruplicate on each plate. Mass inoculation of bacteria from the "master" plate to solid and liquid media was achieved by use of a sterile multipoint inoculator. After incubation at 37° C for 18 h, growth/no growth, color reactions, and other typical biochemical reactions similar to the conventional methods were observed. In specialized tests, reagents were added before reactions were read. To read nitrate reduction test, one drop of sulfanilic acid solution was added using a pasteur pipette, then one drop of naphthylamine solution was added. Red or pink color indicated a positive reaction. For those wells producing no red or pink color a small pinch of zinc dust was added. The absence of red color after addition of zinc indicated a confirmed positive nitrate reduction. For the phenylalanine deamination test, a drop of ferric solution was added to the agar; a pale green color indicated a positive reaction. For the Voges-Proskauer test a drop of 40% KOH was added followed by a drop of 5% alpha-naphthol. If a pink or red color developed within 10 to 15 minutes that indicated a positive reaction. To detect indole production, 2 drops of Kovac's reagent were added using a pasteur pipette. Red layer formation on top of the broth indicated a positive reaction.

Minitek system

The Minitek system (BBL Microbiology Systems, Becton Dickinson and Co., Cockeysville, MD) consists of a covered, square, plastic plate containing 20 wells. The manufacturer also supplies a multiple disc dispenser, a pipetter with disposable tips, and vials containing 1.5 ml of inoculum broth. For use, the plate is placed in the disc

dispenser and the preselected set of discs is released into the wells. Discs selected for this study included: nitrate reductase, phenylalanine, H_2S/indole, Voges-Proskauer, citrate, ONPG, urease, lysine, arginine, ornithine, dextrose without nitrate, malonate, adonitol, arabinose, inositol, raffinose, sorbitol, lactose, rhamnose, and sucrose.

A single colony of the isolate was picked with a loop and emulsified in a vial of the inoculum broth. Approximately one ml of the inoculum was withdrawn from the vial by means of the pipetter and distributed into each well in portions of 0.05 ml, except for arginine which requires 0.1 ml (2 shots). At least 0.1 ml but not more than 0.15 ml of sterile mineral oil was placed into wells containing urea, lysine, ornithine, and hydrogen sulfide-indole discs. Lids were replaced on plate(s). The inoculated Minitek plate was placed into the humidor (with saturated sponge in place) and covered with humidor lid. Incubation was for 18-24 h at 35 to 37° C. Reactions were read either by observing color changes in the discs or, in certain cases (indole, Voges-Proskauer, phenylalanine, and nitrate reductase), the color produced in the well after the addition of the appropriate reagents.

The results of these tests were converted to the seven-digit profile number (using octal system) which was then matched in an index (Minitek Numerical Taxonomy System).

Cost analysis

The evaluation of cost (Table 14) was an important part of this study. All the conventional media used in this study were prepared in our laboratory, with careful quality control carried out on each batch of media. However for cost comparison purposes, the prices of tubed media charged by GIBCO (GIBCO Laboratories, 2801 Industrial Drive, Madison, Wisconsin) were used as the basis of analysis. Such an approach was used by Bale and Matsen (1981). An average of 21 tubes were used for cost comparison purposes. The cost of media and kit shown in Table 14 reflect market price for a moderate user in 1985.

No allowance were made for overhead cost such as electricity, water, cost of ancillary personnel, fringe benefits for personnel, equipment cost, depreciation of equipment, or the cost of obtaining an isolated colony ready for identification and post identification clean-up. Direct material and labor costs during the identification process were reported. It is very important to keep in mind that technologist salaries, cost of materials and other factors are different based on laboratory volume, geographical location, institution (university, hospital, food industry or private laboratory etc.), instrument cost, and others and this will affect the final cost per identification for a given system.

The cost of the conventional method was calculated using the price of already prepared media (GIBCO) and already prepared reagents (BBL). Labor cost was calculated by determining the total time spent on setting-up, processing and identifying one isolate using the conventional tube system. The total time for performing one identification was then divided by 60 minutes and then multiplied by the hourly rate ($6.25).

The cost of Fung's mini system includes the cost for the microtiter plates, plate sealers, and cotton plug pipettes. For media cost we calculated that Fung's mini system used 25 times less media than the conventional system. So we divided the total media cost of conventional media by 25 and we determined the media cost for Fung's mini system. Reagent cost and labor cost were calculated as previously described.

The cost for the Minitek system was calculated using the list price for the Enterobacteriaceae Set II that is good for 50 isolates. Reagent and labor costs were determined as described above.

The $6.25 hourly rate for technologist time corresponded to an average of the Kansas State Civil Service Basic Salary plan of a Lab Technician I, a Lab Technician II, and a Microbiologist I.

Time analysis

Each method was divided into its individual steps. Each step of the procedure was timed on 20 different occasions and an average time was calculated, and the total time needed for identification of one isolate was calculated by adding the various component parts. No predetermined time standards were used because of unique laboratory layouts; so the analyst used the basic stopwatch time studies.

RESULTS AND DISCUSSION

A total of 295 isolates were tested, of these Enterobacteriaceae, 230 were fresh food isolates and 58 were from the Kansas State University food microbiology laboratory stock culture collection (Table 5). In addition there were 7 nonfermenter fresh isolates (2 strains of Pseudomonas aeruginosa from carrot and 5 strains of Pseudomonas maltophilia from beef trim). The organisms isolated in this study are listed in Table 7. According to Cox and Mercuri (1978) the Klebsiella, Enterobacter, Serratia group of the Enterobacteriaceae family are usually the most difficult to classify accurately. In this study, 164 of the 237 isolates belonged to that group. Table 8 lists the most frequently encountered species of the Enterobacteriaceae family from each of the foods as identified by the conventional method. The Fung's mini system identified all but 5 cultures (4 strains of Serratia liquefaciens from ground beef and 1 strain of Enterobacter cloacae from beef trim). Similarly the Minitek identified all but 5 cultures (4 strains of Enterobacter agglomerans from tomatoes and 1 strain of Enterobacter cloacae from carrots). The predominant Enterobacteriaceae from each food type samples were, respectively: processed broiler carcasses (Escherichia coli), carrots (Enterobacter agglomerans and Enterobacter sakazakii), green onion (Enterobacter agglomerans), ground beef (Serratia liquefaciens), been trim (Enterobacter agglomerans), shrimp (Enterobacter cloacae) and tomatoes (Enterobacter agglomerans).

Stock cultures

Although the conventional method may not be perfect, it is used as the "standard" in comparing accuracy of Fung's mini system and Minitek system. With the Minitek system 56 of the 58 (96.55%) of the stock cultures were correctly classified to genus level and 52 of 58 (89.66%) to species level (Table 5). One of the incorrectly classified organism exhibited a false-positive urea test in conjuction with a false-negative ornithine decarboxylase and was keyed as Klebsiella pneumoniae instead

Table 7. Gram Negative Food Isolates.

Genus and Species	No. of Isolates	Fung's Mini System Correct Identification To Genus	Fung's Mini System Correct Identification To Species	Minitek Correct Identification To Genus	Minitek Correct Identification To Species
Enterobacteriaceae					
Citrobacter diversus	2	2	2	2	2
Citrobacter freundii	14	14	14	14	14
Enterobacter agglomerans	59	59	59	55	55
Enterobacter cloacae	38	37	37	37	37
Enterobacter sakazakii	4	4	4	4	4
Escherichia coli	45	45	45	45	45
Hafnia alvei	1	1	1	1	1
Klebsiella oxytoca	8	8	8	8	8
Klebsiella pneumoniae	17	17	17	17	17
Providencia rettgeri	4	4	4	4	4
Serratia liquefaciens	30	26	26	30	30
Serratia odorifera	6	6	6	6	6
Serratia rubidae	2	2	2	2	2
Others					
Pseudomonas aeruginosa	2	2	2	2	2
Pseudomonas maltophilia	5	5	5	5	5
TOTAL	237	232 97.89%	232 97.89%	232 97.89%	232 97.89%

Table 8. Enterobacteriaceae Isolated From Each Food.

Source	Predominant Organisms	Other Organisms Isolated
Broiler carcasses	Escherichia coli (21)[a]	Citrobacter freundii (4) Enterobacter cloacae (5)
Carrots	Enterobacter agglomerans (7) Enterobacter sakazakii (4)	Citrobacter freundii (1) Enterobacter cloacae (2) Serratia rubidaea (2)
Green onion	Enterobacter agglomerans (10)	Enterobacter cloacae (6)
Ground beef	Serratia liquefaciens (24)	Citrobacter freundii (2) Enterobacter cloacae (6) Escherichia coli (5) Hafnia alvei (1) Klebsiella oxytoca (8) Klebsiella pneumoniae (13) Serratia odorifera (6)
Beef Trim (KSU)	Enterobacter agglomerans (21)	Enterobacter cloacae (7) Escherichia coli (16)
Shrimp	Enterobacter cloacae (9)	Citrobacter diversus (2) Citrobacter freundii (7) Enterobacter agglomerans (3) Klebsiella pneumoniae (4) Providencia rettgeri (4) Serratia liquefaciens (6)
Tomato	Enterobacter agglomerans (18)	Enterobacter cloacae (3) Escherichia coli (3)

[a]Numbers in parentheses indicate the number of each type of organism found in each food source.

of the correct Enterobacter aerogenes. The other incorrectly classified organism was a Providencia stuartti, because of false-positive arabinose and false-negative sucrose tests, it keyed out as Salmonella enteriditis. In addition two strains of Shigella dysenteriae and two strains of Shigella flexneri keyed out as Shigella species and suggested serology as a confirmatory test.

The Fung's mini system correctly classified 53 of the 58 (91.38%) of the stock cultures analyzed to the genus and species level (Table 5). Two strains of Proteus vulgaris were not identified due to false-negative hydrogen sulfide production in conjuction with false-negative phenylalanine deaminase and false-positive inositol and raffinose test. Two strains of Morganella morganii were not identified due to false-negative phenylalanine deaminase in conjuction with a false-positive raffinose, and a strain of Klebsiella pneumoniae was also misidentified due to false-negative lysine and ornithine decarboxylase tests.

Fresh isolates from food

Reactions were recorded as correct if they agreed with conventional media after a 18 to 24 h incubation period. The overall agreement of Minitek and Fung's mini system with conventional tube test with Enterobacteriaceae freshly isolated from seven different food sources is shown in Tables 9 and 10. The overall individual biochemical test agreement between results of Minitek and conventional biochemical tests was 96.9% with a range of 94.8% (broiler carcasses) to 98.6% (tomatoes). The overall agreement between Fung's mini system and conventional biochemical tests was 98% with a range of 97.0% (ground beef) to 99.5% (beef trim). The agrrement between results of the conventional tests and each of the individual 21 Minitek biochemical discs was very good (94.1 to 100%) (Table 11) for all tests except sorbitol (90.3%) and malonate (89%). The correlation between results of conventional test and each of the individual 21 tests on the Fung's mini system was very good (93.25 to 100%) (ONPG was not tested in Fung's mini system but motility test was added) (Table 11). There

Table 9. Number of Discrepant Biochemical Reactions with Minitek and Fung's Mini System Compared with Conventional Methods.

Test* (N=237)	Minitek		Fung's Mini System	
	False (+)	False (-)	False (+)	False (-)
CIT	11	1		16
UR	4		3	1
ONPG		5	Not tested	Not tested
LY	3	3	4	
ARG	1	7	4	4
OR	4	5	5	3
MAL	2	24	2	2
AD	3		2	
AR	2	7	3	
I	14		15	1
RA	3	10	2	1
SO		23	3	1
L		2	1	1
R		1	6	
SU	2	3	4	
VP	2	7	5	5
IND	2	1	1	
MOT	Not Tested	Not Tested	7	
TOTAL[1]	53 (35%)	99 (65%)	67 (65.7%)	35 (34.3%)

*See Table 13 for abbreviations.

[1]152/4977 total discrepancies for Minitek systems = 97%; 102/4977 total discrepancies for Fung's Mini system = 98%.

Table 10. Overall Agreement of Minitek and Fung's Mini System with Conventional Tube Test with Enterobacteriaceae from Different Food Sources.

Food Source	No. of Isolates	No. of Tests	Minitek		Fung's Mini System	
			Number of Discrepancies	Overall Agreement (%)	Number of Discrepancies	Overall Agreement (%)
Broiler carcasses	30	630	33	94.8	16	97.5
Carrots	18	378	18	95.2	11	97.1
Green onion	16	336	9	97.3	5	98.5
Ground beef	65	1365	53	96.1	41	97.0
Raw red meat	49	1029	15	98.5	5	99.5
Shrimp	35	735	17	97.7	17	97.7
Tomato	24	504	7	98.6	7	98.6
TOTAL	237	4977	152/4977	96.9	102/4977	98.0

Table 11. Overall Percentage Agreement of Individual Biochemical Tests on Minitek and Fung's Mini Systems Compared to Conventional Methods.

	Stock Culture (N=58)		Fresh Isolates (N=237)	
	Minitek	Fung's	Minitek	Fung's
ARG	96.55	93.10	96.62	96.62
H_2S	96.55	94.83	100.00	100.00
IND	100.00	100.00	98.73	99.58
L4	93.10	98.28	97.47	98.31
OR	94.83	96.55	96.20	96.62
UR	89.66	96.55	98.31	98.31
AD	98.28	96.55	98.73	99.16
AR	91.38	100.00	96.20	98.73
CIT	94.83	96.55	94.94	93.25
DEX	94.83	100.00	100.00	100.00
I	86.21	74.14	94.09	93.25
L	100.00	100.00	99.16	99.16
MAL	94.83	100.00	89.03	98.31
ONPG	96.55	Not Tested	97.89	Not Tested
RA	98.28	91.38	94.51	98.73
R	100.00	100.00	99.58	97.47
SO	79.31	91.38	90.30	98.31
SU	93.10	91.38	97.90	98.31
NR	98.28	100.00	100.00	100.00
PA	100.00	93.10	100.00	100.00
VP	81.03	97.93	96.20	95.78
MOT	Not Tested	100.00	Not Tested	97.04

were only 102 discrepancies from a total of 4977 tests between Fung's mini system and conventional. Of these 102, false negative reactions accounted for approximately 34.3% (35/102) and false positive reactions accounted for the other 65.7% (67/102). There were only 152 discrepant reactions between the Minitek system and the conventional method; 65% (99/152) of them were false negative reactions and 35% (53/152) were false-positive reactions. The false-positive reactions on Fung's mini system are probably due to the differences in amount of substrate between Fung's mini system and conventional (conventional method use about 25 times more media than Fung's mini system). The most frequent false-positive reactions (Table 12) encountered in this study were inositol (Minitek, Fung's mini system), citrate (Minitek), Voges-Proskauer (Minitek); the most frequent false-negative reaction were citrate (Fung's mini system), sorbitol (Minitek), malonate (Minitek), and raffinose (Minitek).

Escherichia coli was responsible for nine of the eleven false-positive citrate reaction; two of the four urea false-positive reaction and twenty-one of the twenty-three false negative sorbitol reaction encountered with the Minitek. Enterobacter cloacae was responsible for six of the seven false-negative ornithine reaction and also responsible for the nine false-negative arabinose reaction. Fifteen of the twenty-three false-negative malonate and the seven false-negative Voges-Proskauer were caused by isolates from ground beef. Enterobacter agglomerans caused six of the fourteen false-positive inositol in Minitek.

On Fung's mini system Serratia liquefaciens (isolated from ground beef) was responsible for ten of the sixteen false-negative citrate and for five of the six false-positive raffinose reaction. Of the sixteen discrepancies witn inositol, Enterobacter cloacae was responsible for eight false-positive reactions, and Enterobacter agglomerans and Citrobacter freundii were also responsible for two false-positive citrate reactions each.

Table 12. Most Frequently Encountered Discrepant Biochemical Reaction with Each System

System	Inaccurate Tests[a]	
	False-positive	False-negative
Minitek	CIT, I, VP	MAL, SO, RA
Fung's Mini System	I	CIT

[a]CIT, Citrate Utilization; I, Inositol Fermentation; MAL, Malonate Utilization; RA, Raffinose Fermentation; SO, Sorbitol Fermentation, VP, Voges-Proskauer.

Table 13. Biochemical Tests Used in This Study

Test Sequence	Biochemical Test	Abbreviation
1	Arginine (decarboxylase activity)	ARG
2a	Hydrogen sulfide production	H_2S
2b	Indole production	IND
3	Lysine (decarboxylase activity)	LY
4	Ornithine (decarboxylase activity)	OR
5	Urease activity	UR
6	Adonitol (acid production)	AD
7	Arabinose (acid production)	AR
8	Citrate utilization	CIT
9	Dextrose	DEX
10	Inositol (acid production)	I
11	Lactose (acid production and gas)	L
12	Malonate utilization	MAL
13	O-Nitrophenyl-B-D-galactosidase activity	ONPG
14	Raffinose (acid production)	RA
15	Rhamnose (acid production)	R
16	Sorbitol (acid production)	SO
17	Sucrose (acid production)	SU
18	Nitrate reductase activity	NR
19	Phenylalanine deaminase activity	PA
20	Voges-Proskauer (acetoin production)	VP
21	Motility	MOT

The Minitek system correctly identified all strains tested except five (97.9%), whereas the Fung's mini system also correctly identified all but five (97.9%). The species misidentified by the Minitek were four strains of Enterobacter agglomerans (Tomato), all having the same profile number (4341543), and one strain of Enterobacter cloacae (carrot) which did not key out. Similarly the Fung's mini system misidentified four strains of Serratia liquefaciens due to a false-negative citrate in conjuction to a false positive raffinose test. Also one Enterobacter cloacae was not correctly identified due to a false-positive lysine decarboxylase test. The Pseudomonas species mentioned in Table 7 represent nonfermenters often encountered in food samples. All seven strains were correctly identified by the two system.

Most of the color changes on the Minitek system were clear and easy to read, but the color comparator card was necessary until one became familiar with the color changes. All the tests except malonate, citrate, and ONPG gave strong positive reactions in all cases when positive. Instruction for use of the Minitek were found very easy to understand and all the processes flowed smooth and easy.

With Fung's mini system most of the reaction were simple to read. The following test needed special consideration. For amino acid decarboxylation tests, a purple color indicated a positive reaction, and a light blue or very light purple color was considered a negative reaction. For the citrate utilization test we checked for growth in addition to color change from green to blue on medium. Blue color or growth indicated a positive reaction. For the carbohydrate fermentation test, only yellow (either bright or clear) was considered as a positive reaction. Any orange, gold or light pink shadow was considered negative.

Time and cost analysis

Calculation of cost and time did not include "Enterobacteriacea count", isolation of pure culture and clean-up. As previously indicated, all media used in this study (Table 6) were prepared in our laboratory. But for cost and time comparison we used a price for already prepared media which we would have been charged for had we used a commercial manufacturer's. Fung's mini system and conventional system require media preparation and sterilization. Media and reagent preparation includes weighting and mixing, heating, distribution into appropriate container and sterilization. Also include were the preparation of heat sensitive media and reagents. All this may include the possibility of many common errors in media preparation and control such as improper weighting, use of out dated media, overheating, improper pH and sterility control. This is the main reason why we decided to use the cost of already prepared media from a commercial manufacturer.

Direct cost represents the most valid means of comparing alternative methods (Bartlett et al. 1979). Table 14 shows the cost figures used for material and technological time. Table 15 details the time analysis in seconds expended for each step of the conventinal tube media method, Table 16, Minitek; and Table 17, Fung's mini system.

The cost and time analysis required for the identification of Enterobacteriaceae isolates indicated that performing twenty-one tests with the Minitek kit costs $4.08,; Fung's mini system (4 replicates/identification) $4.47, (Fung's mini system using 96 different cultures costs approximately $1.39/identification); conventional system with commercially prepared media costs $12.61. The Minitek and Fung's mini system (for 96 different cultures) required approximately the same time for set-up and identification (Minitek, 4.89 min; Fung's 5.6 min), Fung's mini system (4 replicates/identification) took approximately 12.21 min and the conventional method

Table 14. Total Cost Analysis (21 Tests).

System	Material Cost	Media Cost	Reagent Cost	Labor Cost	Total Cost ($)
Minitek	$3.43*	-	.12	.53	4.08
Fung's (4 replicate/ID)	$1.21	1.51	.48	1.27	4.47
Fung's (96 dif. cultures)	$0.32	0.38	.12	0.58	1.39
Conventional	-	9.42	.76	2.43	12.61

*Does not include initial cost of equipment (multiple disc dispenser; pipetter; humidor).

Table 15. Time Analysis for Conventional 21-Tube Method.

STEP	TIME (Sec.)
Set up or assemble tubes 2.49 sec/tube X 21	52.29
Label one tube 4.35 sec/tube X 21	91.35
Inoculate tube 35.92 sec/tube X 21	754.95
Mineral oil addition 27.40/tube X 3	82.20
Reagent addition	153.80
Read and record results	102.00
Identify organism	158.30
Record Identification	6.00
TOTAL	1400.89 (23.35 min)

Table 16. Time Analysis for Minitek Procedures.

STEP	TIME (Sec.)
Place cartridge in dispenser and lock in place (311/50)	6.22
Dispense discs into Minitek 20-well plate	15.84
Label Minitek plate	5.75
Label one MIB bottle	4.32
Pick one colony and emulsify in MIB*	32.91
Inoculate one plate	57.44
Overlay arginine, urea, ornithine, lysine and H_2S/Indole with mineral oil	17.76
Humidify the sponge (13.53/humidor)	1.35
Place Minitek plate in humidor and incubate	5.12
Addition of reagent	46.94
Read results and record	69.04
Identify organism	23.53
Record identification	6.95
TOTAL	293.17 (4.89 min)

Table 17. Time Analysis for Fung's Mini System (4 Replicates/Identification)

STEP	TIME (Sec.)
Preparation of "master" plate (1212.9/plate) = 12.63/2311 X 4	50.54
Preparation of substrate microtiter plate (340.49/plate) = 3.547/well X 4 wells X 21 tests	297.95
Aseptic multiple inoculation into liquid and solid media (13.15/plate) = 0.137/well X 4 wells X 21 tests	11.51
Sealing microtiter plate(20.56/plate) = 0.214/well X 4 wells X 21 tests	17.98
Overlay of decarboxylases with 2 drops sterile mineral oil (241.4/plate) = 2.51/well X 4 wells X 3 tests	30.12
Reagent addition	30.03
Collection of data (143/plate) = 1.49/well X 4 wells X 21 tests	125.16
Label one plate (5.75/plate) = .06 well X 4 wells X 21 tests	5.04
Interpretation	158.30
Record identification	6.00
TOTAL	732.63 (12.21 min)

took approximately 23.35 min. Minitek saved 79% of the time compared to conventional system and 60% compared to Fung's mini system (4 replicates). Fung's mini system (4 replicates) saved 47.7% time compared to conventional system. The Minitek system could identified approximately 12.27 isolates in 1-h period, compared to 4.91 for Fung's mini system (4 replicates or 10.71 isolates per h without replicates) and only 2.57 with the conventional system.

A range of 0.49 to 2.5 minutes has been observed or recomended as applicable to processing individual tubes used for test in tubes by Bartlett et al., 1979. In our study however, we calculated 1.13 minutes per tube. No allowance for operator factors was included when calculating the cost of technologist time. Normally the times would be increased by an arbitrary 15 to 20%. If we include this allowance, the difference between Minitek and Fung's mini system would be even greater when compared to conventional system. Timing including removal of tubes from refrigerators and removal from incubator were not included in this study.

Compared with conventional tube system, the Minitek kit and Fung's mini system offers savings in both time and material cost. In addition the miniaturized system requires less storage and incubator space.

Conclusions

In conclusion, a flexible miniaturized system (Fung's mini system) and flexible commercial system (Minitek system) provided highly reliable identification of laboratory cultures and fresh food isolates as compared with the conventional procedure. Further more the cost per isolate identified was substantially less and the time in operation as well as in obtaining final data were considerably reduced. Thus, miniaturized microbiological techniques are definitively more advantageous than the corresponding conventional procedures.

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COMPARATIVE STUDY OF MINITEK, A MINIATURIZED SYSTEM AND CONVENTIONAL METHOD IN IDENTIFICATION OF ENTEROBACTERIACEAE

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ABSTRACT

A total of 295 Enterobacteriaceae isolates [58 stock cultures and 237 fresh isolates from broiler carcasses (30), carrots (18), green onions (16), ground beef (65), beef trim (49), shrimp (35), and tomatoes (24)] were inoculated into the 21 biochemical tests of the Minitek, and into 20 corresponding tests in Fung's mini system and into the 21 corresponding tests in the conventional tube method. The overall agreement between Minitek and conventional test was 96.4% (94% stock and 96.9% fresh isolates), whereas the agreement between Fung's miniaturized system and conventional tests was 97.4% (95.3% stock and 98% fresh isolates). The most frequently encountered Enterobacteriaceae from these foods were Escherichia coli (broiler carcasses), Enterobacter agglomerans (carrots, green onions, beef trim, and tomatoes), Enterobacter cloacae (shrimp), Enterobacter sakazakii (green onions) and Serratia liquefaciens (ground beef).

The cost and time analysis required for the identification of Enterobacteriaceae isolates indicated that using the Minitek (21 tests) costs $4.08, Fung's mini system (4 replicates/identification) costs $4.47, (Fung's mini system using 96 different cultures cost approximately $1.39/identification) and a similar identification using the conventional 20-tube (21 tests) set-up with commercially prepared media costs $12.61/identification. The Minitek and Fung's mini system (for 96 different cultures) require approximately the same time for set-up and identification (Minitek, 4.89 min; Fung's 5.6 min), but Fung's mini system (4 replicates) takes approximately 12.21 min per identification and conventional method takes approximately 23.35 min. No estimate on technologist time efficiency was added in this study.

Thus, the Minitek system and the Fung's mini-system are highly accurate in identifying Enterobacteriaceae, and at the same time save considerable amount of time and expenses compared with the conventional method.