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Coagglutination and Enzyme Capture Tests for Detection of *Escherichia coli* β-Galactosidase, β-Glucuronidase, and Glutamate Decarboxylase†

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Polyclonal antibodies to *Escherichia coli* β-galactosidase, β-glucuronidase, and glutamate decarboxylase were used in coagglutination tests for identification of these three enzymes in cell lysates. Enzyme capture assays were also developed for the detection of *E. coli* β-galactosidase and β-glucuronidase. The enzymes were released by using a gentle lysis procedure that did not interfere with antibody-enzyme interactions. All three enzymes were detected in 93% (51 of 55) of the *E. coli* strains tested by coagglutination; two of the three enzymes were identified in the remaining 7%. Of 42 non-*E. coli* tested by coagglutination, only four nonspecifically agglutinated either two or three of the anti-enzyme conjugates. Thirty-two (76%) non-*E. coli* isolates were negative by coagglutination for all three enzymes. The enzyme capture assay detected the presence of β-galactosidase in seven of eight and β-glucuronidase in all eight strains of *E. coli* tested. Some strains of β-galactosidase-positive *Citrobacter freundii* and *Enterobacter cloacae* were also positive by the enzyme capture assay, indicating that the antibodies were not entirely specific for *E. coli* β-galactosidase; however, five other gas-positive non-*E. coli* isolates were negative by the enzyme capture assay. The coagglutination tests and enzyme capture assays were rapid and sensitive methods for the detection of *E. coli* β-galactosidase, β-glucuronidase, and glutamate decarboxylase.

Escherichia coli is often used as an indicator of fecal contamination because it is specific to the intestinal tracts of warm-blooded animals (1, 2, 6, 11) and is usually present in numbers greater than other coliforms and pathogens (1). However, the isolation of *E. coli* from a mixture of coliforms and verification of the isolates require considerable time and expense. Although improvements in *E. coli* detection have been made (3, 9, 19), additional methods that are more rapid or can supplement existing methods are needed.

Enzyme immunoassay and coagglutination tests have provided a rapid means of identifying bacteria (13, 21, 27, 29). Most enzyme immunoassay and coagglutination tests utilize antibodies directed to cell surface somatic (4), capsular, flagellar (21), or other surface antigens (18, 26) common to the species being detected. Immunological identification of *E. coli* is difficult because numerous serogroups exist (25) and cross-reacting antigens are shared by other members of the family *Enterobacteriaceae* (8, 28).

Intracellular constituents of *E. coli*, unlike the surface components, include several antigens common to *E. coli*. Three antigens, β-galactosidase (GAL), β-glucuronidase (GUD), and glutamate decarboxylase (GAD), are produced by a majority of *E. coli* (8, 10, 16). Previous studies with α-amylase (14) and GAL (22) have shown that similar enzymes from different sources are immunologically distinct. Therefore, this study was undertaken to determine if polyclonal antibodies could be used to detect GAL, GUD, and GAD from *E. coli* and if detection of one or all of these enzymes could be used to confirm the presence or identification of *E. coli*. These antibodies were also tested for their ability to distinguish *E. coli* GAL, GUD, and GAD from similar enzymes produced by other procaryotes and eucaryotes.

MATERIALS AND METHODS

Stock cultures and media. Environmental strains of *E. coli*, numbered 1 through 72 (23), were obtained from S. A. Minnich (IGEN, Inc., Rockville, Md.). The remaining cultures were obtained from P. Feng (IGEN, Inc.) and were from food and water samples and the culture collection at Iowa State University.

Media were commercial products (Difco Laboratories, Detroit, Mich.), except for enzyme induction broth, which consisted of the following: lauryl tryptose broth, 3.56 g; 4-methylumbelliferyl-β-D-glucuronide (MUG; Hach Co., Ames, Iowa), 0.015 g; L-glutamic acid, 0.05 g; distilled water, 100 ml; pH 6.8 to 7.0.

Antibody production. *E. coli* enzymes used for immunization were obtained commercially (Sigma Chemical Co., St. Louis, Mo.). Rabbits were injected intramuscularly with 500 μg of GAL, GUD, or GAD in complete Freund adjuvant. Four weeks after the initial injection, the rabbits received an identical dosage of enzyme in incomplete Freund adjuvant. Finally, 500 μg of enzyme in 0.01 M phosphate-buffered saline (PBS; pH 7.2) was administered intramuscularly 5 to 7 days prior to bleeding. The sera were tested for anti-enzyme antibody by indirect immunoassay (30).

Protein A purification of immunoglobulin G antibodies. Blood was clotted at 4°C, and the erythrocytes were removed by centrifugation. Clarified sera were divided into aliquots and stored at −20°C. Before addition to the column, a serum sample was melted and clarified by centrifugation. Four milliliters of clarified serum was added to 1 ml of 0.05 M PBS (pH 8.2), and the mixture was applied to an protein A-Sepharose (Sigma) column (8 by 1.25 cm) equilibrated with 0.01 M PBS (pH 8.2). Eluate was monitored at 280 nm.

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After unwanted proteins had passed through the column, as exhibited by a return to base-line absorbance values, immunoglobulin G was eluted with an acid solution (glacial acetic acid, 2.5 ml; NaCl, 4.5 g; NaN_3, 0.2 g; distilled water, 500 ml). When the pH of the eluate became acidic (shortly after an increase in A_{280}), 1-ml fractions were collected in tubes containing 0.2 ml of 0.5 M PBS (pH 8.2). Additional PBS was added if fractions were not completely neutralized. The fractions were collected until absorbance values returned to base-line values. The fractions were pooled, adjusted to pH 7.0, and stored in 2-ml volumes at −20°C. Once thawed, sodium azide was added to 0.2% and the preparations were stored at 4°C.

Quantities of immunoglobulin G recovered from the protein A column were estimated by using A_{280} values and an extinction coefficient of 1.4 (15).

Preparation of staphylococcal anti-enzyme conjugates. To 300 μl of a 10% *Staphylococcus aureus* Cowan strain suspension (Sigma) in 0.05 M PBS (pH 7.5), 300 μg of one of the protein A-purified anti-enzyme antibodies was added. The volume of the mixture was adjusted to 1 ml with 0.01 M PBS (pH 7.2). The mixture was incubated at room temperature for 2 to 4 h with continuous agitation and then washed three times with 0.01 M PBS (pH 7.2). After the final wash, the staphylococcal antibody conjugates were suspended to 1 ml in 0.01 M PBS (pH 7.2) and sodium azide was added to 0.2%. The conjugates were stored at 4°C until used.

Cell lysis. Cultures to be tested were grown for 24 h in enzyme induction broth at 35°C. The cells from 0.5 ml were pelleted by centrifugation in a Microfuge (Beckman Instruments, Inc., Fullerton, Calif.) for 3 min. The cell pellet was suspended to its original volume in lysis buffer (0.2 M Na_2HPO_4, 25 ml; 0.2 M $NaHPO_4$, 25 ml; sucrose, 8 g; EDTA, 0.93 g; Triton X-100, 50 μl; distilled water, 50 ml; pH 7.0). Then an equivalent volume of RNase solution (20 mg of Sigma bovine pancreas RNase A per ml of 0.01 M PBS containing 0.05% Tween 20 and 3% bovine serum albumin, pH 7.0) was added. A combination of RNase and bovine pancreas DNase 1, both at 10 mg/ml, was also satisfactory. Finally, 15 μl of lysozyme solution (10 mg of lysozyme per ml) was added to the cell pellet; the pellet was suspended by agitation and incubated at 35°C for 20 min.

To ensure that lysis was not denaturing the enzyme, particulate material was removed by centrifugation following lysis, and 500 μl of 4-methylumbelliferyl-β-D-galactopyranoside (MUGAL; 100 μg/ml; Sigma) was added. The mixture was incubated for 30 min at 37°C. Fluorescence under long-wave UV light was recorded as positive for the presence of GAL.

Coagglutination. To identify the enzymes by agglutination, 25 μl of cell lysate was added to 25 μl of 0.01 M PBS (pH 7.0) on a glass slide; 12 to 15 μl of the respective staphylococcal anti-enzyme conjugate was added, and lysates and conjugate were mixed. The mixture was allowed to react for 30 to 60 s and then rocked (1 to 2 min) until the *E. coli* control agglutinated. Agglutination reactions were observed with the aid of a bacterial colony counter.

Crude preparations of GAL, GUD, and GAD of various origins were obtained commercially (Sigma). The enzyme preparations were diluted to an equivalent of 0.5 enzyme unit per ml of lysis buffer. The diluted enzyme preparations were mixed directly with 12 to 15 μl of staphylococcal anti-enzyme conjugate, and the agglutination procedure was performed as described above.

Enzyme capture. Protein A-purified antibodies to *E. coli* enzymes were diluted in carbonate buffer (pH 9.6); 100 μl was added to a well of a microtitration plate (Immunoplate 1; Nunc, Roskilde, Denmark), and the plates were incubated at room temperature for 2 h or overnight at 4°C. The optimal dilution of antibody was determined by using 1:2 dilutions of the antibody and commercial enzyme diluted in lysis buffer. The highest dilution of antibody that produced a detectable reaction with a minimum quantity of enzyme (usually 1:500 to 1:1,000 dilution) was used. Following attachment, the remainder of the antibody suspension was removed, and remaining free binding sites were blocked with 250 μl of carbonate buffer containing 2% bovine serum albumin for 1 h at room temperature. Plates coated with antibody could be stored at 4°C for up to 1 week.

After the nonspecific binding sites were blocked, the plates were washed three times with 0.01 M PBS containing 0.05% Tween 20 (pH 7.4). Cultures to be tested were grown for 24 h in enzyme induction broth and lysed. A 100-μl portion of the cell lysate was added per antibody-coated well, and the plates were incubated at room temperature with shaking for 1 h. The plates were washed three times and then 100 μl of PBS, containing substrate for the enzyme captured, was added to each well. To detect the presence of captured GAL, either MUGAL (200 μg/ml) or *O*-nitrophenyl-β-D-galactopyranoside (300 μg/ml; Sigma) was added. GUD was detected by adding either MUG (200 μg/ml; Hach) or *p*-nitrophenyl-β-D-glucuronide (300 μg/ml; Sigma). Plates were incubated with substrate for 30 min at 35°C. To stop reactions and enhance fluorescence, 100 μl of 0.2 N NaOH was added to each well. The A_{409} of wells incubated with the colorimetric substrates, *O*-nitrophenyl-β-D-galactopyranoside and *p*-nitrophenyl-β-D-glucuronide, were determined with a Minireader (Dynatech Laboratories, Inc., Alexandria, Va.). Wells incubated with MUG and MUGAL were read visually under long-wave UV light. Because reactions with MUG and MUGAL were more easily detected, subsequent tests were conducted only with the fluorogenic substrates.

RESULTS AND DISCUSSION

Release of intracellular enzymes. Four lysis procedures were evaluated for their ability to release GAL in an enzymatically active form (data not shown). Cells were lysed by using EDTA-sodium dodecyl sulfate-lysozyme, toluene-sodium deoxycholate, 3% potassium hydroxide, and EDTA–Triton X-100–lysozyme (5, 20). MUGAL was added to lysates to detect enzyme activity. The EDTA–Triton X-100–lysozyme procedure released active GAL from all four *E. coli* tested and was most suitable for the purposes of this research (data not shown). These results are in agreement with a previous report on the denaturing effects of detergents (12) on proteins. Nonionic (Triton X-100), weakly ionic (sodium deoxycholate), and strongly ionic (sodium dodecyl sulfate) detergents, in this order, correlate with increasing solubilizing power as well as disruption of protein-protein interactions and denaturation (12).

Detection of GAL and GUD by enzyme capture. Because colorimetric and fluorogenic substrates were available for GAL and GUD and both enzymes were active following lysis with EDTA–Triton X-100–lysozyme, an enzyme capture assay was developed. The assay was easy to perform and could be completed within 90 min. Instead of adding a second anti-enzyme antibody, as in the direct and indirect enzyme immunoassay procedures, substrate to the captured enzyme was added. MUGAL and MUG were used to detect the presence of captured GAL and GUD, respectively. The

TABLE 1. Production of gas from lactose, serological detection of GAL, cleavage of MUG, and serological detection of GUD

Organism	Gas[a]	Anti-GAL[b]	MUG[c]	Anti-GUD[b]
Citrobacter freundii	−	−	−	−
C. freundii 1404573	+	+	−	−
C. freundii TF146	+	−	−	−
Enterobacter aerogenes MC12	−	−	−	−
Enterobacter agglomerans	−	−	−	−
E. agglomerans Carol 4	+	−	−	−
Enterobacter cloacae F31	+	±	−	−
E. cloacae F32	+	+	−	−
Escherichia coli B	+	+	+	+
E. coli CPM	+	+	+	+
E. coli ET3C	+	+	+	+
E. coli KK2B	+	+	+	+
E. coli PM1B	+	+	+	+
E. coli 31	+	+	+	+
E. coli 50	±	±	+	+
E. coli 60	+	+	+	+
Klebsiella oxytoca Ohio 6	−	−	−	−
K. ozanae L901	−	−	−	−
K. pneumoniae WSSC 26	−	−	−	−
K. pneumoniae F29	+	−	−	−
K. pneumoniae Ohio 3	+	±	−	−
Shigella sp. F21	−	−	−	−

[a] Gas production was recorded after incubation at 35°C for 24 h in enzyme induction broth.

[b] Capture antibody to *E. coli* GAL or GUD.

[c] The MUG reaction was determined after incubation at 35°C for 24 h in enzyme induction broth. Fluorescence under long-wave UV light was recorded as positive.

sensitivity of the enzyme capture method was excellent; 175 ng of GAL per ml produced a strong reaction when MUGAL was used as substrate.

Both GAL and GUD were detected in cell lysates by the enzyme capture procedure (Table 1). Seven of the eight *E. coli* tested were strongly positive for GAL; the eighth strain was weakly positive. Among the GAL-positive non-*E. coli*, only *Citrobacter freundii* 1404573 and *Enterobacter cloacae* F32 yielded positive tests for GAL. *Enterobacter cloacae* F31 and *Klebsiella pneumoniae* Ohio 3 exhibited some GAL activity, but the reactions were weak. *C. freundii* TF146, *Enterobacter agglomerans* Carol 4, and *K. pneumoniae* F29 produced gas from lactose, but were negative by the GAL capture assay. The polyclonal antibodies exhibited some specificity in binding, suggesting that antigenic differences exist among GALs from different bacterial genera. However, quantities of GAL produced by different cultures might also account for these differences. The hydrolysis of MUG and the presence of GUD were restricted to *E. coli*. Further tests on purified GAL and GUD from various bacterial genera as well as other origins are needed. In addition, we have produced monoclonal antibodies to *E. coli* GAL and GUD and have begun studies on the specificities of these antibodies.

Identification of GAL, GUD, and GAD by coagglutination. Staphylococcal anti-enzyme conjugates were used to detect GAL, GUD, and GAD in *E. coli* lysates. The sensitivity of the coagglutination procedure was good when purified enzyme preparations were used, although sensitivity varied between conjugates (data not shown). The GAL conjugate was the most sensitive, agglutinating as little as 50 ng of GAL per ml of lysis buffer. The GAD conjugate agglutinated approximately 2 to 3 μg of GAD per ml of lysis buffer. Agglutination reactions for GAL and GAD were optimal at enzyme concentrations above 7.5 μg/ml. The GUD conjugate was the least sensitive of the conjugates, requiring approximately 31 μg/ml for agglutination; concentrations of 500 μg/ml or higher were optimal. Because of the reduced sensitivity, agglutination with the GUD conjugate was the most difficult of the conjugates to visualize.

Concentrations of inducers required for induction of GUD and GAD were determined qualitatively by assaying cell lysates for the presence of each of the respective enzymes (data not shown). The highest levels of MUG (150 μg/ml) tested yielded the highest levels of enzyme after the shortest period of growth. GUD activity was detected in cell lysates within 4 h of inoculation; however, levels of GUD were much higher after 24 h of growth. Concentrations of 0.05, 0.1, and 0.5% glutamate all resulted in detectable GAD activity after 24 h of growth. Differences in the strength of the GAD reactions could not be determined. Because lactose was already present in enzyme induction broth, an inducer for GAL was not added. GAL activity attained maximal levels 4 h after inoculation. Based on the results from enzyme induction tests, lauryl tryptose broth was supplemented with 0.5% glutamate and 150 μg of MUG per ml. Also, cultures were incubated for 24 h prior to testing.

Coagglutination tests were used to examine 42 non-*E. coli* belonging to 25 different species (Table 2). Of 11 gas-positive bacteria, 7 were agglutinated by the GAL conjugate. The GAL conjugate also agglutinated a strain each of *C. freundii* and *Enterobacter aerogenes* that were negative for gas production after 24 h; however, the *Enterobacter aerogenes* strain was positive for gas production after 48 h. The GUD conjugate agglutinated cell lysates from *Acinetobacter calcoaceticus*, *C. freundii*, *Enterobacter cloacae*, and *Serratia fonticola*, all of which were MUG negative. One unusual, MUG-positive strain of *Hafnia alvei* was not agglutinated. The GAD conjugate agglutinated lysates of one strain each of *Enterobacter cloacae*, *H. alvei*, and *S. fonticola*. GAD activity in these species is probably uncommon because Freier et al. (10) did not detect GAD in the *Hafnia* spp., *Enterobacter cloacae*, and *Serratia* spp. they tested. *Enterobacter cloacae* and *S. fonticola* were the only non-*E. coli* to agglutinate all three conjugates. Thus, a small number of bacteria tested produced a cross-reacting antigen or some other material that agglutinated the staphylococcal anti-enzyme conjugates. The apparent cross-reactivity may have been caused by a minor protein contaminant(s) in the enzyme preparations used for immunization.

Originally, lysates of pseudomonads and klebsiellae were viscous. This prevented proper mixing of the lysates with the anti-enzyme conjugate and resulted in a filamentous, agglutination-like reaction (data not shown). The addition of RNase to the lysates reduced their viscosities, and false-positive reactions were eliminated (Table 2).

Numbers of *E. coli* and non-*E. coli* lysates reacting with three, two, one, or none of the staphylococcal anti-enzyme conjugates are shown in Table 3. Of the 55 *E. coli* lysates tested by coagglutination, 51 (93%) were agglutinated by all three conjugates and the remaining 7% were agglutinated by two of the three conjugates. Only two *E. coli* were negative for GAD, one was negative for GAL, and one was negative for GUD. Individually, their anti-enzyme conjugates agglutinated 96 to 98% of the *E. coli* tested, which agrees with reports on the distribution of GUD and GAD in *E. coli* (10, 16). Ewing (8) reported that 90% of *E. coli* were positive for GAL. Percentages of *E. coli* positive for GAL exceeded 90% in this study because two gas-negative *E. coli* were positive for GAL by coagglutination. Apparently, some gas-negative *E. coli* contain a nonfunctional GAL which is still recognized

TABLE 2. Numbers of non-*E. coli* examined and members producing positive reactions in five different tests

Organism	No. of strains	No. producing positive reactions				
		Gas[a]	Anti-GAL conjugate[b]	MUG[c]	Anti-GUD conjugate[b]	Anti-GAD conjugate[b]
Acinetobacter calcoaceticus	2	0	0	0	1	0
Arizona sp.	1	1	0	0	0	0
Bacillus cereus[d]	1	0	0	0	0	0
Bacillus thuringiensis	1	0	0	0	0	0
Citrobacter freundii	2	1	2	0	1	0
Enterobacter aerogenes	1	0	1	0	0	0
Enterobacter agglomerans	2	0	0	0	0	0
Enterobacter cloacae	5	4	3	0	1	1
Hafnia alvei	2	1	1	1[e]	0	1
Klebsiella oxytoca	1	0	0	0	0	0
Klebsiella pneumoniae	6	3	1	0	0	0
Proteus mirabilis	1	0	0	0	0	0
Pseudomonas aeruginosa	1	0	0	0	0	0
Pseudomonas putida	1	0	0	0	0	0
Salmonella enteritidis	1	0	0	0	0	0
Serratia fonticola	2	1	1	0	1	1
Serratia liquefaciens	1	0	0	0	0	0
Serratia plymuthica	1	0	0	0	0	0
Shigella flexneri	1	0	0	0	0	0
Shigella spp.	2	0	0	0	0	0
Staphylococcus aureus[d]	1	0	0	0	0	0
Streptococcus faecalis[d]	1	0	0	0	0	0
Vibrio fluvalis	1	0	0	0	0	0
Yersinia enterocolitica	2	0	0	0	0	0

[a] Gas production was recorded after incubation at 35°C for 24 h in enzyme induction broth.
[b] Coagglutination with staphylococcal anti-enzyme conjugate.
[c] The MUG reaction was determined after incubation at 35°C for 24 h in enzyme induction broth. Fluorescence under long-wave UV light was recorded as positive.
[d] Sparse growth in enzyme induction broth after 24 h at 35°C.
[e] Very weak activity.

by antibody. Similarily, lysates from five of six MUG-negative *E. coli* were positive when tested with the anti-GUD conjugate. Thus, coagglutination appears to detect the presence of enzyme(s) in some strains that are negative when examined by using conventional tests.

Among the 42 non-*E. coli* (Table 3), only *S. fonticola* and *Enterobacter cloacae* (5%) agglutinated all three conjugates; *C. freundii* and *H. alvei* (5%) agglutinated two of the three conjugates. Six (14%) of the non-*E. coli* were agglutinated by a single conjugate, five were agglutinated by the anti-GAL conjugate, and one was agglutinated by the anti-GUD conjugate (Table 3). This was not unexpected because 11 of the non-*E. coli* tested were GAL positive (produced gas from lactose). The results show that coagglutination tests can specifically detect GAL, GUD, and GUD in *E. coli* lysates.

The specificities of the coagglutination tests were examined further by using a variety of commercial enzymes (Table 4). The enzyme preparations were impure and contained other cellular constituents; therefore, preparations were diluted to an equivalent of 5 enzyme units per ml. The anti-GAL conjugate did not agglutinate GAL from *Saccharomyces fragilis* or bovine liver but did agglutinate GAL from *Aspergillus niger* and *E. coli*. The anti-GUD conjugate agglutinated only *E. coli* GUD. The anti-GAD conjugate agglutinated GAD from *Clostridium welchii* and *E. coli* equally well. These results and those described above indicate that polyclonal antibodies differ in their ability to distinguish between enzymes from different origins.

The significance of distinguishing between *E. coli* GUD and GUD from the other sources is that some foods, such as

TABLE 3. *E. coli* and non-*E. coli* examined and numbers producing positive reaction with the staphylococcal anti-enzyme conjugates

Isolates	No. of strains tested	No. (%) of lysates agglutinated by given no. of conjugates			
		3	2	1	0
E. coli	55	51 (93)	4 (7)	0	0
Non-*E. coli*	42	2 (5)[a]	2 (5)[b]	6 (14)[c]	32 (76)

[a] Cultures identified as *Serratia fonticola* and *Enterobacter cloacae* agglutinated all three anti-enzyme conjugates.
[b] *C. freundii* and *H. alvei* agglutinated the anti-GAL and -GUD conjugates and the anti-GAL and -GAD conjugates, respectively.
[c] *C. freundii*, *K. pneumoniae*, *Enterobacter aerogenes*, and two strains of *Enterobacter cloacae* agglutinated the anti-GAL conjugate; one strain of *A. calcoaceticus* agglutinated the anti-GUD conjugate.

TABLE 4. Reactions of staphylococcal anti-enzyme conjugates with enzymes from various sources

Source and enzyme	Agglutination with staphylococcal anti-enzyme conjugate		
	Anti-GAL	Anti-GUD	Anti-GAD
E. coli GAL	+	NA[a]	NA
Saccharomyces fragilis GAL	−	NA	NA
Bovine liver GAL	−	NA	NA
Aspergillus niger GAL	+	NA	NA
E. coli GUD	NA	+	NA
Bovine liver GUD	NA	−	NA
Chlamys opercularis GUD	NA	−	NA
Abalone entrails GUD	NA	−	NA
E. coli GAD	NA	NA	+
Clostridium welchii GAD	NA	NA	+

[a] NA, Not applicable.

shellfish, contain endogenous GUD. When large samples of these foods are added to media containing MUG, endogenous enzyme causes fluorescence in all tubes. Therefore, the examination of shellfish for *E. coli* is restricted to the use of MUG in confirmatory EC media (17). By using an antibody specific for *E. coli* GUD, tests might be made directly from presumptive tubes to obtain a 1-day assay.

The coagglutination test also has promise as a rapid confirmatory test for colonies from agar plates or membrane filters. Preliminary results showed that colonies could be suspended in a small volume of lysis buffer containing RNase solution on a glass slide and then tested with the anti-enzyme conjugates. Thus, colonies could be verified within minutes by testing for GAL, GUD, or GAD or for all three. Verification may require the use of only one conjugate, possibly GUD or GAD, because GAL-positive colonies are usually identified directly on lactose-containing differential media. The coagglutination and enzyme capture procedures could also be used to verify the presence of *E. coli* in most-probable-number tubes. Because of the simplicity and rapidity of the assays, all growth-positive most-probable-number tubes could be examined to increase the accuracy of the procedure (7, 24).

Both the coagglutination test and the enzyme capture assay provide rapid and sensitive means for detection of GAL, GUD, and GAD. Tests of mixed cultures and food and water samples are needed, however, to determine the practical effectiveness of these procedures for the confirmation and identification of *E. coli*.

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