Journal of Food Protection, Vol. 53, No. 12, Pages 1037-1046 (December 1990)
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Detection of Salmonellae and Other *Enterobacteriaceae* by Commercial Deoxyribonucleic Acid Hybridization and Enzyme Immunoassay Kits

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(Received for publication May 14, 1990)

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ABSTRACT

Cultures of 294 *Salmonella* and 100 other Enterobacteriaceae isolates were comparatively analyzed by the GENE-TRAK[R] *Salmonella* Assay, the Report[R] *Salmonella* Visual Immunoassay, and the Organon Teknika Salmonella-Tek[R] assay. Of the *Salmonella* isolates, 99.0% were reactive in the GENE-TRAK assay, 98.0% were reactive in the Report assay and 100% were reactive in the Organon Teknika assay. The three strains missed by the GENE-TRAK method belonged to *Salmonella* subgenus V. Four of seven strains that were nonreactive by the Report assay were nonmotile isolates; some nonmotile isolates were reactive. Of the non-*Salmonella* isolates assayed, 6.0% were consistently positive by the Report Method and 16.0% were consistently positive by the Salmonella-Tek method, while none were consistently positive by the GENE-TRAK method. Variable assay results were obtained with 19.0% of the strains using the Report assay, 20.0% of the strains in the Salmonella-Tek assay, and 7.0% of the strains by the GENE-TRAK assay.

Several rapid method test kits utilizing either deoxyribonucleic acid hybridization or enzyme immunoassay techniques for the detection of *Salmonella* in foods are available from commercial sources (*4,5,7,12,14,15,16,18*). Two of the methods, the GENE-TRAK *Salmonella* (deoxyribonucleic acid hybridization, DNAH) Assay and the Organon Teknika Salmonella-Tek enzyme-linked immunosorbent assay (ELISA) or enzyme immunoassay (EIA), represent second generation test kits. The new GENE-TRAK assay differs significantly from the first generation test kit. The new assay includes probe sequences which hybridize to *Salmonella* ribosomal ribonucleic acid (rRNA) in place of the chromosomal-target probes used previously. These probes do not hybridize to rRNA from subgenus V salmonellae (*5*) which are believed to be rarely pathogenic to man (*22*). GENE-TRAK Systems has developed probes for the detection of the group V salmonellae (P. Groody and M. Mozola, GENE-TRAK Systems, personal communication). Hybrids are captured on a plastic dipstick and are detected colorimetrically. The first generation test utilized filter hybridization and the detection of a radioisotope. The revised Organon Teknika assay utilizes the same antibodies as in the previous assay. In the new assay antibody coated microtiter plate wells are used in place of magnetic beads. This microtiter dish format is similar to the format used by the other EIA methods. Antibodies and colorimetric detection substrates represent the significant differences among the various immunoassays.

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The rapid *Salmonella* methods have been compared to standard culture methods in studies conducted in accordance with guidelines recommended by the Association of Official Analytical Chemists (*1,19,20*). These studies illustrated the efficacy of the methods in several laboratories. By design, a limited number of food types and *Salmonella* serotypes are analyzed in these studies. Moreover, these studies only describe comparisons to the AOAC culture method and do not include comparisons between rapid methods. The application of the rapid methods to a broader range of products and strains has been described in other studies (*3,6,8,9,10,13,14,17,21,24*). These studies, like the AOAC studies, generally compare the rapid methods to one or more culture methods.

A direct comparison of the rapid *Salmonella* methods would be beneficial to laboratories doing *Salmonella* analysis of foods. Two of the AOAC studies included comparisons of rapid methods (*4,7*). The comparisons in both cases were between first and second generation immunoassays. In both studies, decimal dilutions of broth cultures of 25 different *Salmonella* isolates were prepared with enrichment cultures of 150 food samples. *Salmonella* was detected at significantly lower levels by the second generation Salmonella-Tek assay than by the Bio-Enzabead assay (*4*). As a consequence, the Salmonella-Tek method has replaced the Bio-Enzabead method which is no longer available. For the first and second generation Dynatech immunoassays (*7*), no significant differences were observed. Similar studies of other rapid methods, especially between assays from the various commercial sources, might be useful in illustrating the strengths and weaknesses of the methods.

This study describes results obtained from the analyses of 294 *Salmonella* and 100 non-*Salmonella* Enterobacteriaceae isolates by a DNAH assay and two EIA assays. Fresh overnight cultures of the test organisms were prepared and assayed. Because the growth of the salmonellae was not hindered by low numbers or injury as when in

foods and because the organisms did not have to overcome selective agents and competing organisms during growth, the organisms should be at an optimal level for detection. Failure to detect a *Salmonella* isolate under these conditions would clearly indicate the assay was unreliable for detection of the nonreactive isolate. Levels of other Enterobacteriaceae are also optimal and represent the worst case one might encounter in a food culture. Reactivity of the assays with any of these isolates would demonstrate potential cross-reactivity problems.

MATERIALS AND METHODS

Salmonella *rapid assay test kits*

GENE-TRAK *Salmonella* (Colorimetric) Assay kits were supplied by GENE-TRAK Systems, Framingham, MA. Salmonella-Tek immunoassay kits were purchased from Organon Teknika Corporation, Durham, NC, and Report Salmonella Visual Immunoassay kits were purchased from 3M, St. Paul, MN. The Report assay is identical to the TECRA *Salmonella* Visual Immunoassay of Bioenterprises, Pty Ltd, Sydney, Australia. Assays were performed according to instructions include with kits. Cultures were prepared with GN broth for the DNAH assay and with M broth for the EIAs.

Test strains

Two hundred and ninety-four *Salmonella* and 100 other Enterobacteriaceae strains were tested (Tables 1 and 2). The *Salmonella* selection included examples of all 51 somatic (O) antigen groups described in Bergey's Manual (*22*) plus several naturally-occurring biochemical and morphological variants. The Enterobacteriaceae isolates included 6 genera and 19 species. All of the test strains are maintained in the Silliker Laboratories Research culture collection in Chicago Heights, IL.

Preparation of cultures for assays

For each strain a single colony selected from a Hektoen enteric agar plate was used to inoculate 10 ml of tryptic soy broth (Difco, Detroit, MI). The broth culture was incubated 24 h at 35°C and then diluted 1:100 for inoculation of culture tubes containing 10 ml of GN broth (Difco) and 10 ml of M broth (Difco). After 24 h at 35°C, the number of cells in the GN and M broths was determined by direct microscopic count (DMC) using a hemacytometer. Twenty-five strains, usually 20 *Salmonella* and 5 other Enterobacteriaceae, were prepared per day for assay by all three methods.

For each organism, its GN broth culture was analyzed in duplicate using the DNAH assay and its M broth culture was analyzed in duplicate by both EIAs. If the test organism was *Salmonella* and any of the tests were nonreactive or if the organism

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TABLE 1. *Reactivity of* Salmonella *isolates in rapid assays.*

Serovar	Number of isolates	Assay results: GENE-TRAK DNAH	Report EIA	Sal-Tek EIA
S. adelaide	3	++[a]	++	++
S. agona	6	++	++	++
S. ajiobo	1	++	++	++
S. alachua	4	++	++	++
S. albany	1	++	++	++
S. amager	1	++	++	++
S. amsterdam	2	++	++	++
S. anatum (328)[b]	1	+++	--+	++-
S. anatum	6	++	++	++
S. arizonae	2	++	++	++
S. arizonae	1	+++	---	+++
S. arizonae 18:z_4:z_{32}:-	1	++	++	++
S. arizonae 38:(k):z_{35}	1	++	++	++
S. arizonae 48:i:z	1	++	++	++
S. arizonae 48:z_4,z_{23}:-	1	++	++	++
S. arizonae 50:k:z	1	++	++	++
S. arizonae 50:z_4,z_{23}:-	1	++	++	++
S. arizonae 61:k:1,5,(7)	1	++	++	++
S. arizonae 62:z_4,z_{23}:-	1	++	++	++
S. arizonae 62:z_{36}:-	1	++	++	++
S. arizonae 63:z_{41}:z_{29}	1	++	++	++
S. arizonae 65:(k):z	1	++	++	++
S. artis	1	++	++	++
S. bareilly	4	++	++	++
S. barry	1	++	++	++
S. belem	1	++	++	++
S. bere	1	++	++	++
S. berta	1	++	++	++
S. betioky	1	++	++	++
S. binza	2	++	++	++

S. blockley	1	++	++	++
S. bockenheim	1	++	++	++
S. bovismorbificans	1	++	++	++
S. bradenburg	2	++	++	++
S. braenderup	1	++	++	++
S. bredeney	3	++	++	++
S. brookfield	1	---	+++	+++
S. cerro	3	++	++	++
S. chester	1	++	++	++
S. choleraesuis	4	++	++	++
S. choleraesuis v. *kunz*	2	++	++	++
S. crossness	1	++	++	++
S. cubana	1	++	++	++
S. decatur	1	++	++	++
S. derby	3	++	++	++
S. djugu	1	++	++	++
S. drypool	5	++	++	++
S. dublin	3	++	++	++
S. durban	1	++	++	++
S. eastbourne	1	++	++	++
S. eko	1	++	++	++
S. enteritidis	6	++	++	++
S. eschweiler	1	++	++	++
S. ferlac	2	++	++	++
S. gallinarum	1	+++	---	+++
S. give	1	++	++	++
S. haardt	1	++	++	++
S. hadar	4	++	++	++
S. hartford	1	++	++	++
S. havana	3	++	++	++
S. heidelberg	5	++	++	++
S. houten	1	++	++	++
S. indiana	2	++	++	++
S. infantis	4	++	++	++
S. inverness	1	++	++	++
S. java	1	++	++	++
S. javiana	2	++	++	++
S. jerusalem	1	++	++	++
S. johannesburg	2	++	++	++
S. kentucky	2	++	++	++
S. kottbus	1	++	++	++
S. krefeld	1	++	++	++
S. kristianstad	1	++	++	++
S. lexington	1	++	++	++
S. livingstone	2	++	++	++
S. locarno	1	++	++	++
S. london	1	++	++	++
S. madelia	1	++	++	++
S. malawi	1	---	+++	+++
S. manhattan	1	++	++	++
S. manila (386)	1	++	++	++
S. maregrosso	1	---	+++	++-
S. mbandaka	3	++	++	++
S. meleagridis	1	+++	+++	+-+
S. meleagridis	2	++	++	++

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S. miami	1	++	++	++
S. michigan	1	++	++	++
S. minnesota	1	++	++	++
S. mississippi	1	++	++	++
S. montevideo	5	++	++	++
S. muenchen	3	++	++	++
S. meunster	1	++	++	++
S. nesszional	1	++	++	++
S. newbrunswick	2	++	++	++
S. newhaw	1	++	++	++
S. newington	1	++	++	++
S. newport (347, 551)	6	++	++	++
S. nienstedten	1	++-	---	+++
S. nienstedten	2	++	++	++
S. norwich	1	++	++	++
S. ohio	1	++	++	++
S. oranienburg	2	++	++	++
S. othmarschen	2	++	++	++
S. ouakam	1	--+	+++	+++
S. panama	1	++	++	++
S. paratyphi A	1	+++	---	+++
S. paratyphi A	1	++	++	++
S. paratyphi B	1	++	++	++
S. paratyphi C	2	++	++	++
S. phoenix	1	++	++	++
S. pomona	2	++	++	++
S. poona	1	++	++	++
S. pretoria	1	++	++	++
S. pullorum (328)	1	+++	---	+++
S. raus	2	++	++	++
S. reading	1	++	++	++
S. rubislaw	3	++	++	++
S. saint-paul	3	++	++	++
S. sandiego	2	++	++	++
S. saphra	1	++	++	++
S. schwarzengrund (368)	2	++	++	++
S. senftenberg	1	+++	--+	++-
S. senftenberg	9	++	++	++
S. setubal	1	++	++	++
S. shangani	1	++	++	++
S. stanley	1	++	++	++
S. sundsvall	1	++	++	++
S. tennessee	8	++	++	++
S. tranoroa	1	++	++	++
S. treforest	1	++	++	++
S. typhi	2	++	++	++
S. typhimurium (548)	20	++	++	++
S. typhimurium v. lopen	1	++	++	++
S. uccle	1	++	++	++
S. uppsala	1	++	++	++
S. urbana	1	++	++	++
S. utrecht	2	++	++	++
S. vietnam	1	++	++	++
S. virchow	2	++	++	++
S. vrindaban	1	+++	+++	+++

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S. vrindaban	1	++	++	++
S. wandsworth	1	++	++	++
S. wassenaar	1	++	++	++
S. weltevreden	1	++	++	++
S. weslaco	1	++	++	++
S. worthington	3	++	++	++
S. B:g comp	1	++	++	++
S. Cl:g comp	3	++	++	++
S. Cl:z_{29} lac$^+$	1	++	++	++
S. El:eh	1	++	++	++
S. O:z_4	1	++	++	++
S. 1,9,12,(46),27:z_4,z_{24}:1,5	1	++	++	++
S. 4,5,12:-:- (331)	1	+++	--+	+++
S. 4,12:-:-	1	++	++	++
S. 6,7:-:-	1	++	++	++
S. 30:1,z_{28}:z_6	1	++	++	++
S. 40a,40b:a:-	1	++	++	++
S. 44:z_{36}:-	1	++	++	++
S. 43:z_{29}:-	1	++	++	++
S. 58:d:z_6	1	++	++	++
S. 64:k:e,n,x,z_{15}	1	++	++	++
S. -:e,h:1,2	1	++	++	++
S. -:-:-	1	++	++	++
S. -:-:-	1	+++	---	+++
S. spp.	1	++	++	++

[a]+:reactive assay result, -: nonreactive assay result.
[b]Number in parenthesis identifies isolate used to determine test sensitivity in Table 3.

was a non-*Salmonella* isolate and it tested positive, the organism was reanalyzed. Fresh GN and M broth cultures of the isolate were prepared for the reanalysis. Culture broths were analyzed without replication by the appropriate procedures.

For analysis of diluted cultures, overnight GN and M broth cultures of the organisms were prepared, diluted with the appropriate growth broth, and then analyzed using the appropriate test kit.

RESULTS

Comparison of M-broth and GN-broth cultures of Salmonella *by the GENE-TRAK DNAH assay*

For the analysis of food products, the GENE-TRAK DNAH and the Report and Salmonella-Tek EIA methods specify identical pre-enrichment and selective enrichment protocols but different postenrichment procedures. GN broth enrichment is used in the DNAH method and M broth is specified for the EIA methods. M broth is used to stimulate the production of certain motility proteins which are reactive with the antibodies in the immunoassays. This activity is not essential for the DNAH assay which targets rRNA. Thus, the DNAH method used GN broth which selects for bile resistant Enterobacteriaceae. Since the selective properties of the GN broth were not needed for this comparative study which used pure cultures, a series of DNAH assays were performed on M broth cultures to enable a direct comparison of the DNAH and EIA methods. The M broth and GN broth cultures of 49 *Salmonella* strains were prepared and analyzed by DNAH. Forty of the M broth cultures were positive by DNAH and nine were nonreactive, while all 49 strains grown in parallel GN broth cultures were reactive by DNAH (data not shown). All 49 M broths were reactive in both immunoassays. By direct microscopic count, the number of cells in the M broth cultures was comparable to those in the GN cultures. Because the DNAH results were not the same for the two growth media, the comparative study was conducted using GN broth for the DNAH assay and M broth for the EIAs.

Densities of overnight test cultures

The concentration of *Salmonella* in each of the 294 GN broth and 294 M broth cultures was determined by DMC. In GN broth the mean $\log_{10}$ count was 9.050 ± 0.194 cells/ml, densities ranged from 8.079 to 9.964 $\log_{10}$ cells/ml. The M broth cultures contained 9.029 ± 0.167 cells/ml and densities ranged from 8.114 to 9.991 $\log_{10}$ cells/ml. The difference between the mean log counts for the two broth types was 0.021 ± 219 cells/ml and 97.3% of the differences were less than 0.5 log. The cell densities of the 100 non-*Salmonella Enterobacteriaceae* isolates were 8.983 ± 0.181 in GN broth and 8.994 ± 0178 in M broth. The culture densities ranged from 8.519 to 9.505 $\log_{10}$ cells/ml in GN broth and 8.531 to 9.944 $\log_{10}$ cells/ml in M broth.

GENE-TRAK DNAH assay

Assays with absorbance readings at 450 nm of 0.1 or higher than the absorbance of the negative control are

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considered reactive for *Salmonella*. Assays with net absorbance below 0.1 are interpreted as nonreactive for *Salmonella*. Of the 294 *Salmonella* strains that were analyzed, 291 (99.0%) were reactive (Table 1). The three nonreactive isolates were *S. brookfield*, *S. malawi*, and *S. maregrosso*. Each was nonreactive upon analysis of a fresh GN-broth culture. These three strains have the somatic antigen 066 and belong to *Salmonella* "subgenus V" (*22*). This subgenus has previously been reported as nonreactive by DNAH assay (*5*). The *S. ouakam* isolate was nonreactive when initially tested but reactive when retested using a fresh culture. It is likely that the negative result represented a technical error in which culture was not added to the assay tubes. There were 12 other strains that were retested because

TABLE 2. *Reactivity of selected Enterobactertiaceae isolates by three rapid* Salmonella *assay methods.*

Species	Number of isolates	Assay results: GENE-TRAK DNAH	Report EIA	Sal-Tek EIA
Citrobacter amalonaticus	2	++-[a]	--+	++-
Citrobacter diversus	1	--	--	--
Citrobacter freundii (605)[b]	1	++-	--+	++-
Citrobacter freundii	1	---	++-	++-
Citrobacter freundii	1	---	---	++-
Citrobacter freundii (587)	2	-+-	+++	++-
Citrobacter freundii	2	--	--	--
Citrobacter freundii	2	---	---	+++
Citrobacter freundii	3	---	--+	+++
Citrobacter freundii (581, 595)	4	---	--+	++-
Citrobacter freundii	5	--	--	++
Enteric Group D	2	--	--	--
Enterobacter aerogenes	3	--	--	--
Enterobacter aerogenes	1	---	---	+--
Enterobacter agglomerans	1	---	+++	---
Enterobacter agglomerans	2	--	--	++
Enterobacter agglomerans	4	--	--	--
Enterobacter cloacae	1	---	+++	---
Enterobacter cloacae	1	---	++-	---
Enterobacter cloacae	2	--	--	--
Enterobacter hafnia	1	---	---	+++
Enterobacter intermedium	1	--	--	--
Enterobacter spp.	1	--	--	++
Escherichia coli	1	+--	+--	--+
Escherichia coli	1	--	--	-+
Escherichia coli	1	---	++-	+++
Escherichia coli	1	---	++-	---
Escherichia coli (55)	1	---	-+-	--+
Escherichia coli	1	---	--+	+++
Escherichia coli (472)	1	---	--+	++-
Escherichia coli	1	---	---	++-
Escherichia coli	1	---	---	-+-
Escherichia coli	3	---	+++	---
Escherichia coli	33	--	--	--
Klebsiella oxytoca	1	--	--	-+
Klebsiella oxytoca	4	--	--	--
Klebsiella planticola	1	--	--	--
Klebsiella pneumoniae	1	--	--	--
Klebsiella spp.	1	--	--	+-
Klebsiella terrigena	1	--	--	--
Proteus mirabilis	1	--	--	--
Proteus vulgaris	1	--	--	--

[a]+: reactive assay result, -: nonreactive assay result.
[b]Number in parentheses identifies isolate used to determine test sensitivity in Table 3.

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TABLE 3. *Reactivity of diluted culture dilutions in rapid* Salmonella *assays.*

Isolate	cells/ml GN broth	Assay results GENE-TRAK	cells/ml M broth	Assay results Report	Sal-Tek
C. freundii (581)[a]	2.2 x 10^9	-+	2.8 x 10^9	--	++
	2.2 x 10^8	--	2.8 x 10^8	--	++
	2.2 x 10^7	--	2.8 x 10^7	--	++
C. freundii (587)	8.4 x 10^8	--	1.2 x 10^9	--	++
	8.4 x 10^7	--	1.2 x 10^8	--	++
	8.4 x 10^6	--	1.2 x 10^7	--	++
C. freundii (595)	2.0 x 10^9	--	7.3 x 10^8	--	--
	2.0 x 10^8	--	7.3 x 10^7	--	--
	2.0 x 10^7	--	7.3 x 10^6	--	--
C. freundii (605)	3.0 x 10^9	++	1.5 x 10^9	--	++
	3.0 x 10^8	--	1.5 x 10^8	--	++
	3.0 x 10^7	--	1.5 x 10^7	--	++
E. coli (55)	1.6 x 10^9	--	1.3 x 10^9	--	--
	1.6 x 10^8	--	1.3 x 10^8	--	-+
	1.6 x 10^7	--	1.3 x 10^7	--	--
E. coli (472)	2.3 x 10^9	--	1.6 x 10^9	--	++
	2.3 x 10^8	--	1.6 x 10^8	--	++
	2.3 x 10^7	--	1.6 x 10^7	--	-+

[a]Number in parenthesis identifies isolate used.
[b]+: reactive assay result, -: nonreactive assay result.

SLR Strain	cells/ml GN broth	Assay results GENE-TRAK	cells/ml M broth	Assay results Report	Sal-Tek
S. anatum (334)[a]	1.1 x 10^9	-+[b]	1.0 x 10^9	--	++
	1.1 x 10^8	--	1.0 x 10^8	--	++
	1.1 x 10^7	--	1.0 x 10^7	--	++
S. pullorum (328)	4.3 x 10^8	++	5.5 x 10^8	--	++
	4.3 x 10^7	--	5.5 x 10^7	--	++
	4.3 x 10^6	--	5.5 x 10^6	--	++
S. 4,5,12:-:- (331)	9.8 x 10^8	++	7.5 x 10^8	--	++
	9.8 x 10^7	+-	7.5 x 10^7	--	++
	9.8 x 10^6	--	7.5 x 10^6	--	++
S. manila (386)	1.8 x 10^9	++	1.4 x 10^9	++	++
	1.8 x 10^8	++	1.4 x 10^8	++	++
	1.8 x 10^7	++	1.4 x 10^7	++	++
S. newport (347)	1.8 x 10^9	++	1.4 x 10^9	++	++
	1.8 x 10^8	++	1.4 x 10^8	++	++
	1.8 x 10^7	-+	1.4 x 10^7	--	++
S. newport (551)	8.9 x 10^8	++	1.0 x 10^9	++	++
	8.9 x 10^7	++	1.0 x 10^8	++	++
	8.9 x 10^6	++	1.0 x 10^7	++	++
S. schwarzengrund (368)	1.4 x 10^9	++	1.2 x 10^9	++	++
	1.4 x 10^8	++	1.2 x 10^8	++	++
	1.4 x 10^7	--	1.2 x 10^7	++	++
S. typhimurium (548)	1.1 x 10^9	++	1.2 x 10^9	++	++
	1.1 x 10^8	++	1.2 x 10^8	++	++
	1.1 x 10^7	--	1.2 x 10^7	--	++

[a]Number in parenthesis identifies isolate used.
[b]+: reactive assay result, -: nonreactive assay result.

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of aberrant results by one of the EIA procedures; all were reactive by DNAH upon retest.

Of the 100 *Enterobacteriaceae* strains assayed, 93 were nonreactive and 7 were sometimes reactive (Tables 2 and 3). None of the 100 strains were consistently reactive. Variable results were obtained with the two *Citrobacter amalonaticus* isolates, four *Citrobactor freundii* isolates, and one *Escherichia coli* isolate. One of the *C. freundii* isolates and both *C. amalonaticus* isolates were positive in duplicate assays initially but not when retested. There were 27 other strains that were DNAH negative initially but retested because a positive result was obtained by one of the EIA procedures. All of the strains were again negative by DNAH.

Report EIA assay

Assay wells that have a green color equivalent to or darker than color 3 on the color card included with the kit are reactive for *Salmonella*, and wells that are faintly green or colorless are interpreted as nonreactive. The assay kit does not include instructions for use of a photometer, thus all results were read visually. Two hundred eighty-eight of the *Salmonella* strains (98.0%) were reactive in the assay (Table 1). The nonreactive strains included single isolates of *S. gallinarum, S. arizonae, S. paratyphi* A, *S. niensteden* and two nonspeciated, nonmotile strains. Single *S. anatum* and *S. senftenberg* isolates and another nonspeciated, nonmotile strain were nonreactive upon initial assay and strongly reactive upon retest. A third set of cultures of the *S. anatum* isolate and one of the nonmotile isolates were tested and found to be nonreactive (Table 3). Assays that were negative in duplicate and then positive may be explained in terms of culture density or the precipitation of antigen during sample preparation. In many instances, precipitation has been observed following sample boiling. If it is not properly resuspended before withdrawing the test sample, a negative result may occur. It is unlikely that the variable results occurred as a consequence of not adding sample to the test wells, since it is easy to identify wells that do not contain sample.

Of the 100 *Enterobacteriaceae*, 6% of the strains, including isolates of *C. freundii, E. agglomorans, E. cloacae*, and *E. coli* were repeatedly positive (Tables 2 and 3). Another 19% of the strains were positive in one or more assays. Interestingly, for 12 of the 19 strains, the cross-reaction events occurred during the second round of analysis but not in the first round. That 12 strains were initially nonreactive and then reactive upon retest warrants notice. These strains were not randomly selected for retest. Instead each was selected based upon results obtained for one of the other assays. Eight of the retest scores were scored as color 3; color 2 is negative. Since the determination of color is made visually, it is possible that borderline samples may be read either way. Another possibility is that reagents in the kit used for the second round were more reactive than those in the initial kits. This explanation is consistent with the results obtained for certain *Salmonella* strains upon retest.

Organon-Teknika assay

Assays that have an absorbance at 450 nm that are 0.25 units greater than the negative control are considered reactive for *Salmonella*. Lower absorbances indicate a nonreactive result. All 294 *Salmonella* isolates tested in the study were reactive by the assay. However, variable results were obtained for four strains. Isolates of *S. anatum, S. maregrosso*, and *S. senftenberg* were reactive initially and then negative upon retest. A *S. meleagridis* isolate was reactive in one of two initial duplicate assays and upon reassay. Strains positive initially and negative upon retest suggest that difficulties occurred when the assay was conducted. The most likely explanation is that the boiled antigen preparations were not mixed thoroughly before the sample for assay was withdrawn. Other possibilities include lower cell densities in the second set of cultures than in the initial set or degradation of assay materials. Lower densities in the retest broths might easily explain reactivity result.

The EIA assay results exhibited a high degree of repeatability. The mean difference between the absorbances of the duplicate assays of the 294 cultures was 0.021 at A_{450} with a standard deviation of 0.114. Differences per culture ranged from 0.00 to 1.812 A_{450}. Only two pairs of assays had differences that were >0.5 A_{450}. For 224 isolates, readings of 2.000, the maximum absorbance possible, were obtained for both samples.

Of the 100 *Enterobacteriaceae* strains assayed, 36.0% (36 strains) were reactive in the assay (Tables 2 and 3). The majority of the *C. freundii* strains, 19 of 21, were reactive. Nine of these strains reacted variably, sometimes positive and sometimes negative, and 10 strains were consistently reactive. The reactions of some of the cultures were very strong, having absorbance readings that were greater than twice the cutoff value. Although high readings were repeatable for a single culture, none of the organisms produced cultures with consistently high readings. Typically, the false positive reactions were 0.1 to 0.2 absorbance units above the cutoff. Other reactive strains included 2 strains of *C. amalonaticus*, 8 strains of *E. coli*, 5 enterobacters, and 2 klebsiellae. One *Enterobacter* isolate, two *E. aerogenes* isolates, one *E. hafnia* isolate, and two *E. coli* isolates were consistently reactive, and five other strains were variably reactive. As seen for the *C. freundii* cultures, assay absorbances were typically just above the cutoff, some cultures produced higher readings, but no one organism was consistently highly reactive. It is clear from these results that the assay has the potential of cross-reacting with a variety of nonsalmonellae. Moreover, whether a strain will be reactive in a given assay appears to be unpredictable, since many strains were reactive in only one of two repeats.

Analysis of diluted cultures for determination of assay sensitivity

The reactivities of several strains described in Tables 1 and 2 were determined by the DNAH assay and the immunoassays following serial dilution. Five *Salmonella* strains that were consistently reactive in all three assays, two strains which were variable in at least one of the assays, and one strain which was nonreactive by the Report assay were chosen for analysis (Table 3). In addition, four

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C. freundii isolates and 2 *E. coli* isolates were selected. Each of these strains was reactive in at least one of the assays. Overnight cultures were prepared and tested undiluted and at dilutions of 1:10 and 1:100.

The DNAH assay was reactive with the *Salmonella* cultures containing at least 1.4 x 10^8 cells per ml. Some strains diluted below this level were reactive while others were not. The highest sensitivity was achieved for one of the *S. newport* isolates and the *S. manila* isolate. These strains were reactive at densities of 8.9 x 10^6 and 1.8 x 10^7 cells per ml (Table 3). Higher dilutions were not analyzed. Of the nonsalmonellae, only undiluted cultures of two *C. freundii* isolates were reactive.

In the Report assay, three of eight of the *Salmonella* strains were nonreactive (Table 3). All three had been nonreactive or variably reactive previously. Of the reactive strains all were detected at levels of at least 1.4 x 10^8 cells per ml. At densities in the range of 1.0 x 10^7 to 1.4 x 10^7 cells per ml, the highest dilutions tested, 3 strains were reactive and 2 were nonreactive. The 4 *C. freundii* and 2 *E. coli* strains were all nonreactive. Previously, each of the strains was reactive one or more times using the Report assay.

Using the Salmonella-Tek assay, all *Salmonella* dilutions were positive (Table 3). The highest dilution resulted in a density of 5.5 x 10^6 cells per ml and all strains were detectable at densities of 1.4 x 10^7 or more cells per ml. Of the nonsalmonellae strains tested, only one *C. freundii* isolate was nonreactive. The other three *C. freundii* strains were reactive, even at the highest dilution. Each was reactive with as few as 2.8 x 10^7 cells per ml. Of the *E. coli* isolates, one was reactive in one of the duplicate assays at 1.6 x 10^8 cells per ml and nonreactive at 10-fold higher and lower densities. Another *E. coli* isolate was reactive at 1.6 x 10^7 cells per ml in one of two assays and in duplicate assays at 10- and 100-fold higher densities. Interestingly, no strong dilution effect was observed for the reactive nonsalmonellae. In each case the signal was just above cutoff, and upon dilution a slight nonproportional decrease in assay signal was observed. This "tailing" phenomenon is characteristic of some immunoassay systems (*2,11*).

DISCUSSION

A greater percentage of *Salmonella* strains was reactive by the Salmonella-Tek Immunoassay (100%), than by either the Report Immunoassay (98.0%) or the GENE-TRAK DNAH assay (99.0%). The subgenus V isolates were nonreactive by the DNAH assay. Seven nonmotile salmonellae were employed in the study, including *S. gallinarum, S. pullorum,* and five nonmotile variants. The Report assay was nonreactive with *S. gallinarum, S. pullorum,* and one of the nonmotile variants. Two of three cultures of the nonmotile strain were nonreactive.

Promotional information distributed with the DNAH and EIA test kits suggests that these assays will readily detect *Salmonella* in cultures containing fewer than 10^7 cells/ml. This level was readily attained by all strains included in this study. The data clearly show that GN broth which is recommended for the DNAH assay cannot be replaced by M broth which is recommended for the enzyme immunoassays. Since *Salmonella* grows equally well in both media, culture density does not explain the difference. Thus, it is likely the differences relate to the amount of rRNA available for hybridization. Culture may differ in copies of rRNA per cell, RNAse activity, or susceptibility to lysis.

Among the nonsalmonellae strains assayed, 93.0% were consistently nonreactive by the GENE-TRAK assay, 75.0% were nonreactive by the Report assay, and 64.0% were nonreactive by the Salmonella-Tek assay. *C. amalonaticus* strains were reactive by all three assay procedures. Several of the *C. freundii* strains were reactive with the GENE-TRAK probes. Several *C. freundii, Enterobacter,* and *E. coli* strains were reactive with the Report polyclonal antibodies. Several *Enterobacter, E. coli,* and *Klebsiella* isolates and the majority (19 of 21) of the *C. freundii* isolates were reactive with the Salmonella-Tek monoclonal antibodies. Per assay method 244 assays were performed on undiluted cultures of nonsalmonellae (Table 2 and 3). The false positive rates for the methods were 4.9% for GENE-TRAK, 17.6% for Report, and 33.2% for Salmonella-Tek.

Inconsistent results were obtained with a number of the test isolates. Both reactive and nonreactive results were obtained on occasions for 2 *Salmonella* and 6 non-*Salmonella* isolates by the GENE-TRAK assay, for 3 *Salmonella* and 19 non-*Salmonella* isolates by the Report assay, and 4 *Salmonella* and 20 non-*Salmonella* by the Salmonella-Tek assay. The extent of the variability was not anticipated. This variability is of concern, since on occasion some *Salmonella* strains may be nonreactive resulting in a false negative result and some non-*Salmonella* strains may be reactive causing a false positive result.

Several *Salmonella* strains were nonreactive in the Report immunoassay. Several of the isolates were nonmotile; however, not all nonmotiles were nonreactive. Among the other nonreactive isolates, no relationships could be established. That some isolates were nonreactive in this assay was unexpected. The package insert states that the test was reactive using 45 serotypes obtained from the *Salmonella* Reference Laboratory, Adelaide, S.A., Australia, and over 300 strains from the Center for Disease Control, Atlanta, Georgia. It is possible that strains used in the present study differed from those analyzed previously. A relatively high number of false positive results was obtained when non-*Salmonella* strains were analyzed. No relationships among the reactive strains were evident.

This study essentially confirms the utility of the MOPC467 and 6H4 monoclonal antibodies in the Salmonella-Tek for the detection of *Salmonella (23)*. This study also demonstrates that one or the other of these antibodies has a high potential for cross-reacting with *C. freundii* strains and many other Enterobacteriaceae. Food samples that harbor these strains will likely be associated with high false positive rates. The frequency of these isolates in foods is not known. In a survey of 125 *Salmonella*-free samples of soy flour, pasta noodles, nonfat dry milk, ground black pepper, and milk chocolate, five (4.0%) of the samples were reactive (*4*). Since the samples were collected from

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a variety of suppliers, the results indicate that the cross-reacting strains may not occur at high frequency in these foods, had not grown to detectable levels, or are less reactive when subjected to a full *Salmonella* enrichment protocol. It is entirely possible that had samples been selected from only a few suppliers, a higher incidence of false positive results would have been observed if problematic strains were present.

The results demonstrate that each of the assays may generate false positive assay results by cross-reaction with non-*Salmonella* bacteria. It is possible that food samples that test positive and cannot be confirmed by isolation of *Salmonella* represent failures of the confirmation procedure, i.e., *Salmonella* was present in the sample causing a positive assay but could not be isolated by the culture method employed. It is also possible that one of many cross-reacting organisms generated the reactive result. Because all strains do not show the same degree of reactivity for each assay method and the distribution of these organisms in food and the environment is unknown, it is not surprising that different studies have generated different false positive and false negative rates for these assays.

The sensitivities of the GENE-TRAK and Report assays were lower for some organisms than was expected. Each of these assays, according to the suppliers, should detect fewer than 10^7 cells per ml for all strains. These claims are based on viable counts. The results of this study are based on microscopic cell counts. Differences between these two methods of determining organism levels may account for these observations.

ACKNOWLEDGMENTS

The authors wish to thank Dwayne Ford and Luanne Fanning for technical assistance, GENE-TRAK Systems for financial assistance, Russ Flowers for helpful comments and suggestions, and Mark Mozola and George Parsons for their considerable patience during the completion of this study.

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