JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 2008, p. 685–688 Vol. 46, No. 2
0095-1137/08/$08.00+0 doi:10.1128/JCM.01808-07


# Evaluation of Commercial Antisera for *Salmonella* Serotyping▽

Kimmi N. Schrader, Ali Fernandez-Castro, Wendy K. W. Cheung, Claudia M. Crandall, and Sharon L. Abbott*

*Microbial Diseases Laboratory, Division of Communicable Disease Control, California Department of Public Health, Richmond, California 94804*

Received 11 September 2007/Returned for modification 5 October 2007/Accepted 7 December 2007

**We compared a set of commercial *Salmonella* somatic and flagellar serotyping antisera to in-house-prepared antisera from the Microbial Diseases Laboratory, California Department of Public Health, using 327 *Salmonella enterica* strains belonging to subgroups I, II, IIIa, IIIb, and IV. The sensitivities of Denka Seiken (Tokyo, Japan) somatic and flagellar antisera (using a tube agglutination assay) were 94.0% and 99.2%, respectively, and the specificity was 100% for both sets of sera. Polyvalent O and O1 antiserum sensitivity and specificity were >90%, with the exception of polyvalent O1 antiserum, for which sensitivity was 88.9%. When Denka Seiken flagellar antisera were used in a slide agglutination assay, the sensitivity and accuracy dropped to 88.9% and the specificity fell to 91%. Overall, Denka Seiken commercial antisera performed very well and, together with the comprehensive range of factors available, offer laboratories quality reagents suitable for serotyping strains of salmonellae.**

Infections caused by nontyphoidal *Salmonella* strains generally vary from asymptomatic conditions to a self-limiting gastroenteritis characterized by mildly watery stools, mucus, and occasionally occult or visible blood. Bacteremia occurs in ~1% to 4% of immunocompetent patients, and 5% to 10% of these persons will develop other extraintestinal complications, including reactive arthritis, central nervous system infections, endocarditis, osteomyelitis, and urinary tract infections (2, 5). These infections have a significant societal impact in terms of both personal suffering and economic consequences. Between 1996 and 1999, the Food-Borne Diseases Active Surveillance Network estimated that nontyphoidal *Salmonella* strains were responsible for ~1.4 million human cases of gastroenteritis per year in the United States, resulting in 168,000 physician office visits, 15,000 hospitalizations, and 400 deaths annually (6). The annual cost of these infections is estimated to range between $0.5 billion and $2.3 billion (1). These figures are based on medical expense and loss of productivity due to salmonellosis and do not include the substantial additional costs incurred by the food industry (due to product recall, litigation, etc). Although most cases of salmonellosis are sporadic, in 2004 over 120 food-borne outbreaks of salmonellosis in the United States were reported to the Centers for Disease Control and Prevention (CDC) (4). Many salmonella infections and subsequent deaths can be prevented if outbreaks are identified rapidly and epidemiologically linked food products are removed from the market. Since the strain of salmonella involved in an outbreak is typically tracked by its serotype and the molecular subtype of that serotype, it is essential that serotyping be performed with precision to ensure that all strains involved in an outbreak are recognized. Most clinical and public health laboratories rely on commercially prepared antisera to serotype salmonellae. However, in our laboratory, where we serotype between 3,600 and 5,400 salmonella isolates per year, we use high-quality monoclonal and polyclonal antisera prepared in-house by our Biologics Unit. In this study we used our in-house-prepared antisera to evaluate commercial salmonella antisera produced by Denka Seiken Co., Ltd. (DS), Tokyo, Japan.

## MATERIALS AND METHODS

**Bacterial strains.** A total of 327 *Salmonella* strains were tested in this study; some strains were used for both somatic and flagellar testing. Both rare serotypes and organisms from among the 10 most commonly encountered serotypes (Table 1 and 2) were included to ensure a wide a selection of homologous strains. Heterologous strains, selected according to WHO guidelines, were tested to check for cross-reactions (3). With the exception of two quality control (QC) strains of serotype Rostock, the organisms tested represent clinical isolates submitted to the California Department of Public Health Microbial Diseases Laboratory (MDL) for *Salmonella* serotyping. Strains were previously identified with MDL in-house-prepared antisera, using an alcohol antigen for somatic slide agglutination testing and a tube agglutination assay for flagellar testing. Most strains were received in 2005 and 2006 and maintained in motility deeps; some serotypes with infrequently seen antigenic factors were kept at −70°C and retrieved for testing.

**Antisera.** *Salmonella* antisera from DS was evaluated against MDL in-house monoclonal or polyclonal antisera. DS *Salmonella* antisera were provided by the manufacturer at working concentrations and maintained according to the manufacturer's instructions; the antisera tested and their lot numbers are given in Tables 1 and 2. MDL antisera were prepared using immunizing strains originally obtained from the CDC and were absorbed according to the guidelines of the WHO Centre for Reference and Research on Salmonella at the Institut Pasteur, Paris, France. The MDL Biologics Unit has made in-house diagnostic reagents for more than 50 years and currently supplies the CDC with salmonella serotyping reagents. Each reagent undergoes rigorous QC testing before it is put into use. All polyclonal and monoclonal reagents prepared in-house are tested by the Biologics Unit with a battery of QC strains obtained from the CDC over the years. When properly absorbed so that no cross-reactions are noted with these QC strains, the salmonella serotyping reagents are sent to the Enteric Bacteriology Unit. There they undergo further QC testing with an assortment of as many as 30 to 50 wild-type salmonella strains. Strains used for QC testing in the Enteric Diagnostic Laboratory are recommended by the WHO and are selected to check for an appropriate homologous reaction for each reagent as well as to determine that no heterologous reactions are present. The final titer used is determined by testing with wild-type strains. Undiluted MDL antisera are preserved and diluted to the predetermined titer as needed.

**Testing procedure.** For testing, each strain was grown on in-house-prepared brain heart infusion (BHI) slants or broths (for somatic or flagellar factors, respectively) which were incubated overnight at 35°C. Somatic antigen suspen-

* Corresponding author. Mailing address: Microbial Diseases Laboratory, 850 Marina Bay Parkway, Room E164, Richmond, CA 94804. Phone: (510) 412-3737. Fax: (510) 412-3902. E-mail: Sharon.Abbott@cdph.ca.gov.

▽ Published ahead of print on 19 December 2007.

TABLE 1. Organisms used for homologous testing of somatic antisera

| Group (no. of strains) | Lot no. | No. of strains of the indicated *Salmonella* serotype(s) and/or subgroup(s) |
|---|---|---|
| A (10) | 03-6026 2008.3 | 9 Paratyphi A, 1 Nitra |
| B (20) | 04-6026 2008.2 | 8 Typhimurium (4 variant Copenhagen), 4 Schwartzengrund, 3 Sandiego, 2 Saintpaul, 1 Heidelberg, 1 Lagos, 1 Paratyphi B |
| C1 (10) | 04-6026 2008.2 | 2 Montevideo, 1 Braenderup, 1 Choleraesuis, 1 Infantis, 1 Mbandaka, 1 Ohio, 1 Oranienberg, 1 Tennessee, 1 Thompson |
| C2 (10) | 04-6026 2008.2 | 3 Newport, 1 Albany, 1 Blockley, 1 Corvallis, 1 Hadar, 1 Istanbul, 1 Kentucky, 1 Muenchen |
| D (15) | 04-6026 2008.2 | 9 Enteritidis, 1 Berta, 1 Dublin, 1 Javiana, 1 Lomalinda, |
| | 06-6091 2008.9 | 1 Napoli, 1 Panama |
| E1 (10) | 03-6026 2008.2 | 2 Anatum, 2 Give, 1 Amager, 1 Falkensee, 1 Meleagridis, 1 Muenster, 1 Weltevreden, 1 Yaba |
| E4 (7) | 03-6026 2008.2 | 7 Senftenberg |
| F (7) | 04-6026 2008.2 | 2 Aberdeen, 1 Abaetetuba, 1 Chandans, 1 Luciana, 1 IIIb 11:l,v:$z_{53}$, IV 11:g,$z_{51}$:− |
| G (10) | 04-6026 2008.2 | 2 Cubana, 2 Poona, 1 Agoueve, 1 Grumpensis, 1 Havana, 1 Putten, 1 Telekebir, 1 Worthington |
| H (4) | 03-6026 2008.2 | 1 Carrau, 1 Madelia, 1 Soahanina, 1 Sundsvall |
| I (9) | 03-6026 2008.2 | 3 Gaminara, 2 Hvittingfoss, 1 Saphra, 1 IIIb 16:$z_{10}$:e,n,x,z, 1 IV 16:$z_4$,$z_{23}$:−, 1 IV 16:$z_4$,$z_{32}$:− |
| K (8) | 03-6026 2008.2 | 4 Cerro, 1 IIIa 18:g,$z_{51}$:−, 1 IIIa 18:$z_4$,$z_{23}$:−, 1 IIIa 18:$z_4$,$z_{32}$:−, 1 IIIb 18:l,v:z |
| L (9) | 03-6026 2008.2 | 4 Minnesota, 1 IIIa 21:g,$z_{51}$:−, 1 IIIb 21:l,v:z, 1 IIIa 21:$z_{29}$:−, 1 IV 21:$z_{36}$:−, 1 IV 21:$z_4$,$z_{23}$:− |
| O (10) | 03-6026 2008.2 | 3 Adelaide, 1 Alachua, 1 Ealing, 1 IIIa 35:g,$z_{51}$:−, 1 IIIb 35:l,v:e,n,x,z−, 1 IIIb 35:l,v:z, 1 IIIb 35:l,v:$z_{35}$, 1 IIIb35:r:e,n,x,z |
| Vi (10) | 04-6026 2008.2 | 10 Typhi |

sions were prepared by emulsifying the growth on the BHI slant with 3 ml of 0.85% saline, which is equivalent to the manufacturer's recommendation of suspending bacterial growth in an amount 3 to 5 times the amount of a match head in 0.5 ml of saline. Ten microliters of the suspension for DS antisera or 20 μl for MDL antisera was transferred to a partitioned glass slide containing 1 drop of the respective somatic factor, and the slide was rocked back and forth for 1 minute. For all diphasic organisms, flagellar phase induction was performed to ensure that the factor being tested for was present. Flagellar antigens for tube agglutination were prepared by adding 10 ml of 0.6% formalin-treated saline to the BHI broth, which sat for 1 h prior to testing. Three drops of DS antisera or 0.01 ml (0.04 ml for absorbed factors) of MDL antisera was added to 10- by 75-mm glass tubes to which 0.5 ml (DS antisera) or 1.0 ml (MDL antisera) of broth suspension was then added. Tubes were incubated for 1 h in a 50°C water bath. Antigens for the DS flagellar slide agglutination assay were prepared as described above for the slide agglutination assay. Ten microliters of the suspension was transferred to a partitioned glass slide containing 1 drop of DS flagellar antiserum and rocked back and forth for 1 minute. A flagellar slide agglutination assay was not performed with MDL sera. For Vi antigen testing, strains of *Salmonella enterica* serotype Typhi were grown on BHI slants overnight at 35°C and then emulsified in 3 ml of 0.85% saline, and the suspension was split for testing in the DS or MDL protocol. For the DS protocol, 0.2 ml of suspension was added to 2 ml of 0.85% saline, heated for 15 min at 121°C, and centrifuged

TABLE 2. Organisms used for homologous testing of flagellar antisera

| Factor (no. of strains) | Lot no. | No. of strains of the indicated homologous *Salmonella* serotype(s) and/or subgroup(s) |
|---|---|---|
| a (13) | 05-6026 2008.2 | 8 Paratyphi A, 2 Lomalinda, 1 Baildon, 1 Luciana, 1 Miami |
| b (9) | 04-6026 2008.2 | 3 Paratyphi B, 2 Ohio, 2 Minnesota, 1 Fluntern, 1 Hvittingfoss |
| c (7) | 04-6026 2008.2 | 5 Choleraesuis, 1 Decatur, 1 Paratyphi C |
| d (10) | 05-6026 2008.2 | 2 Gaminara, 2 Muenchen, 2 Schwartzengrund, 2 Typhi, 1 Putten, 1 Telekebir |
| eh (10) | 05-6026 2008.2 | 2 Anatum, 2 Braenderup, 2 Newport, 2 Saintpaul, 1 Bardo, 1 Sandiego |
| i (10) | 04-6026 2008.2 | 5 Typhimurium, 2 Aberdeen, 2 Kentucky, 1 Lagos |
| k (8) | 05-6026 2008.2 | 3 Blockley, 3 Thompson, 1 Abaetetuba, 1 Singapore |
| r (10) | 04-6026 2008.2 | 3 Heidelberg, 3 Infantis, 2 Virchow, 1 Bovismorbificans, 1 Welterveden |
| y (10) | 05-6026 2008.2 | 2 Bareilly, 1 Amager, 1 Brunei, 1 Benin, 1 Carrau, 1 Coeln, 1 Hartford, 1 Madelia, 1 Pomona |
| z (10) | 04-6026 2008.2 | 3 Poona, 3 Worthington, 2 Kiambu, 1 Soahanina, 1 Sundsvall |
| z6 (10) | 05-6036 2008.3 | 5 Weltevreden, 4 Kentucky, 1 II 30:l,z28:z6 |
| z10 (10) | 04-6026 2008.2 | 3 Hadar, 3 Mbandaka, 2 Istanbul, 1 Glostrup, 1 Haifa |
| z29 (10) | 04-6026 2008.2 | 4 Tennessee, 2 Cubana, 1 Agoueve, 1 Ekpoui, 1 IIIa 21:z29:−, 1 IV 17:z29:− |
| l (10) | 04-6026 2008.2 | 2 Javiana, 1 Brandenberg, 1 Litchfield, 1 Napoli, 1 Ohio, 1 Panama, 1 Putten, 1 Uganda, 1 Worthington |
| v (10) | 04-6036 2008.3 | 2 Brandenberg, 2 Litchfield, 2 Potsdam, 1 Bredeney, 1 Give, 1 London, 1 Panama |
| w (9) | 05-6036 2008.3 | 4 Worthington, 1 Bamboye, 1 Kedougou, 1 Meleagridis, 1 Ohio, 1 Putten |
| z13 (10) | 05-6036 2008.3 | 8 Uganda, 1 Napoli, 1 IIIb 61:l,z13:1,5 |
| z28 (10) | 05-6036 2008.3 | 9 Javiana, 1 II 30:l,z28:z6 |
| 1 (10) | 05-6026 2008.2 | 2 Typhimurium (1 variant Copenhagen), 1 Anatum, 1 Give, 1 Heidelberg, 1 Newport, 1 Poona, 1 Schwartzengrund, 1 Thompson, 1 Uganda |
| 2 (10) | 06-6036 2008.3 | 2 Heidelberg, 2 Newport, 2 Typhimurium (1 variant Copenhagen), 1 Amager, 1 Litchfield, 1 Muenchen, 1 Saintpaul |
| 5 (10) | 06-6036 2008.3 | 2 Thompson, 2 Uganda, 1 Blockley, 1 Bovismorbificans, 1 Fluntern, 1 Javiana, 1 Kiambu, 1 Lagos |
| 6 (10) | 05-6036 2008.3 | 4 Poona, 3 Anatum, 3 London |
| 7 (10) | 04-6036 2008.3 | 2 Gaminara, 2 Give, 2 Schwartzengrund, 1 Benin, 1 Madelia, 1 Pomona, 1 Strasbourg |
| g (13) | 04-6026 2008.2 | 4 Oranienburg, 2 Enteritidis, 1 Agona, 1 Berta, 1 Derby, 1 Dublin, 1 Ealing, 1 Montevideo, 1 Senftenberg |
| f (10) | 04-6027 2008.2 | 3 Berta, 2 Havana, 2 Rissen, 1 Adelaide, 1 Agona, 1 Derby |
| m (10) | 06-6027 2008.2 | 2 Oranienberg, 1 Agbeni, 1 Ealing, 1 Emek, 1 Enteritidis, 1 Kintambo, 1 Monchaui, 1 Montevideo, 1 Nitra |
| p (10) | 05-6027 2008.2 | 10 Dublin |
| q (2) | 07-6027 2008.2 | 2 Blegdam |
| s (10) | 04-6027 2008.2 | 3 Agona, 3 Ealing, 3 Montevideo, 1 Emek |
| t (10) | 05-6027 2008.2 | 4 Oranienberg, 3 Senftenberg, 1 Berta, 1 Kintambo, 1 Monchaui |
| u (2) | 05-6027 2008.2 | 2 Rostock |
| z4 (13) | 05-6026 2008.2 | 3 Albany, 2 Cerro, 2 IIIa 53:$z_4$$z_{23}$:−, 1 Alachua, 1 Chameleon, 1 Corvallis, 1 IIIa 18:$z_4$$z_{23}$:−, 1 IIIa 41:$z_4$$z_{23}$:−, 1 IV 16:$z_4$$z_{32}$:− |
| z23 (18) | 04-6036 2008.3 | 6 Cerro, 2 IIIa 18:$z_4$$z_{23}$:−, 2 IV 50:$z_4$$z_{23}$:−, 1 Ajiobo, 1 Alachua, 1 Bere, 1 Corvallis, 1 IIIa 41:$z_4$$z_{23}$:−, 1 IIIa 63:$z_4$$z_{23}$:−, 1 IV: 6,7:$z_4$$z_{23}$:−, 1 IV 11:$z_4$$z_{23}$:− |
| z24 (10) | 05-6036 2008.3 | 7 Albany, I IIIa 35:$z_4$$z_{24}$:−, 1 IIIa 53:$z_4$$z_{24}$:−, 1 IV 6,7:$z_4$$z_{24}$:− |
| z32 (6) | 05-6036 2008.3 | 5 IV 16:$z_4$$z_{32}$:−, 1 Chameleon |
| en (13) | 05-6026 2008.2 | 4 Branderup, 1 Hadar, 1 Hvittingfoss, 1 Istanbul, 1 Lomalinda, 1 Luciana, 1 Mbandaka, 1 Potsdam, 1 Singapore, 1 Sandiego |
| x (10) | 04-6036 2008.3 | 1 Gatuni, 1 Hadar, 1 Hartford, 1 Hvittingfoss, 1 Istanbul, 1 Johannesburg, 1 Lomalinda, 1 Soahanina, 1 Sundsvall, 1 Singapore |
| z15 (10) | 05-6036 2008.3 | 3 Braenderup, 2 Sandiego, 1 Luciana, 1 Mbandaka, 1 Potsdam, 1 Telekebir, 1 Yaba |

TABLE 3. Results of somatic testing with DS antisera

| Group | Homologous reactions | | | | Heterologous reactions | |
|---|---|---|---|---|---|---|
| | No. tested | No. with reaction: | | | No. tested | No. positive |
| | | 3+/4+ | 2+ | ≤1+ | | |
| A | 10 | 10 | 0 | 0 | 5 | 0 |
| B | 20 | 20 | 0 | 0 | 5 | 0 |
| C1 | 10 | 10 | 0 | 0 | 5 | 0 |
| C2 | 10 | 10 | 0 | 0 | 5 | 0 |
| D | | | | | | |
| Total | 15 | 6 | 3 | 6 | 10 | 0 |
| Lot 04-6026 | 10 | 2 | 2 | 6 | 5 | 0 |
| Lot 06-6091 | 5 | 4 | 1 | 0 | 5 | 0 |
| E1 | 10 | 0 | 7 | 3 | 5 | 0 |
| E4 | 7 | 7 | 0 | 0 | 5 | 0 |
| F | 7 | 7 | 0 | 0 | 5 | 0 |
| G | 10 | 10 | 0 | 0 | 5 | 0 |
| H | 4 | 4 | 0 | 0 | 5 | 0 |
| I | 9 | 9 | 0 | 0 | 5 | 0 |
| K | 8 | 8 | 0 | 0 | 5 | 0 |
| L | 9 | 9 | 0 | 0 | 5 | 0 |
| O | 10 | 10 | 0 | 0 | 5 | 0 |
| Vi | 10 | 10 | 0 | 0 | 2 | 0 |
| Total | 149 | 130 | 10 | 9 | 77 | 0 |
| Polyvalent O | 44 | 41 | 3[a] | 0 | 14 | 1[b] |
| Polyvalent O1 | 36 | 30 | 2[b] | 4[b] | 15 | 0 |

[a] All three were group B strains.
[b] All strains belonged in group F; one strain showed a strong cross-reaction in polyvalent O antiserum, and four strains failed to react in polyvalent O1 antiserum.

at 900 × *g* for 20 min and the supernatant was discarded; the pellet was resuspended in 0.2 ml 0.85% saline. MDL antigens were prepared by heating 0.5 ml of suspension for approximately 5 min in a simmering water bath that had reached the boiling point. All antisera were tested with a 0.85% saline control.

**Interpretation of agglutination tests.** Results of all agglutination tests were observed using a slit lamp. Agglutination reactions were scored from negative to 4+ (4+, 100% of cells clumped with a clear background; 3+, 75% of cells clumped with slightly cloudy background; 2+, 50% of cells clumped with moderately cloudy background, etc). Under MDL's criteria, reactions with a score of ≥3+ are considered homologous, however, DS uses scores of ≥2+ as homologous end points, and this was used in evaluating their antisera. When conflicting results between DS and MDL antisera were noted, a new antigenic preparation was made and the organism(s) was retested with both antisera. In some cases, additional strains were tested to help resolve discrepancies.

## RESULTS

**Somatic antisera.** Of the 149 strains tested with somatic antisera, 140 gave an acceptable end point reaction for DS antisera of 2+ or better, for a sensitivity of 94% (Table 3). Only two sera, groups D and E1, gave unacceptable reactions. For group D lot 04-6026, 6 of the initial 10 isolates tested gave 1+ or negative reactions, as did an additional 5 strains (data not included). However, a second lot (06-6091) of group D requested from the manufacturer gave satisfactory reactions (100% specificity and sensitivity) with all strains tested. For group E1, three strains failed to react and the other seven strains gave 2+ reactions. No agglutination occurred with any of the 77 heterologous strains used to check for cross-reactions with somatic sera.

TABLE 4. Flagellar antiserum test results using a tube agglutination assay

| Factor | Homologous reactions | | | | Heterologous reactions | |
|---|---|---|---|---|---|---|
| | No. tested | No. with reaction: | | | No. tested | No. positive |
| | | 3+/4+ | 2+ | ≤1+ | | |
| a | 13 | 13 | | | 7 | 0 |
| b | 9 | 9 | | | 5 | 0 |
| c | 7 | 7 | | | 7 | 0 |
| d | 10 | 9 | | 1 | 5 | 0 |
| eh | 10 | 10 | | | 5 | 0 |
| i | 10 | 10 | | | 5 | 0 |
| k | 8 | 8 | | | 5 | 0 |
| r | 10 | 10 | | | 5 | 0 |
| y | 10 | 10 | | | 5 | 0 |
| z | 10 | 10 | | | 5 | 0 |
| $z_6$ | 10 | 10 | | | 5 | 0 |
| $z_{10}$ | 10 | 10 | | | 5 | 0 |
| $z_{29}$ | 10 | 10 | | | 5 | 0 |
| l | 10 | 10 | | | 5 | 0 |
| v | 10 | 10 | | | 5 | 0 |
| w | 9 | 9 | | | 5 | 0 |
| $z_{13}$ | 10 | 9 | | 1 | 5 | 0 |
| $z_{28}$ | 10 | 10 | | | 5 | 0 |
| 1 | 10 | 10 | | | 9 | 0 |
| 2 | 10 | 10 | | | 5 | 0 |
| 5 | 10 | 10 | | | 5 | 0 |
| 6 | 10 | 10 | | | 5 | 0 |
| 7 | 10 | 10 | | | 4 | 0 |
| g | 13 | 13 | | | 5 | 0 |
| f | 10 | 10 | | | 5 | 0 |
| m | 10 | 10 | | | 4 | 0 |
| p | 10 | 10 | | | 5 | 0 |
| q | 2 | 2 | | | 5 | 0 |
| s | 10 | 10 | | | 3 | 0 |
| t | 10 | 10 | | | 5 | 0 |
| u | 2 | 2 | | | 5 | 0 |
| $z_4$ | 13 | 13 | | | 5 | 0 |
| $z_{23}$ | 18 | 17 | 1 | | 6 | 0 |
| $z_{24}$ | 10 | 10 | | | 8 | 0 |
| $z_{32}$ | 6 | 6 | | | 8 | 0 |
| en | 13 | 12 | | 1 | 5 | 0 |
| x | 10 | 10 | | | 5 | 0 |
| $z_{15}$ | 10 | 9 | 1 | | 5 | 0 |
| Total | 373 | 368 | 2 | 3 | 201 | 0 |

The sensitivity for homologous reactions for polyvalent O serum was 100%; however, one group F strain crossed strongly with the polyvalent O serum, for a specificity of 92.9%. Polyvalent O1 serum did not pick up four strains of group F (sensitivity, 88.9%), but no cross-reactions were noted with heterologous strains.

**Flagellar antisera.** Using the tube agglutination assay, the sensitivity was 99.2%, with one unacceptable reaction each for factors d, $z_{13}$, and en (Table 4). Specificity was 100%, with no cross-reactions noted with any flagellar serum in the tube agglutination assay. Results for flagellar antisera using a slide agglutination method were not as satisfactory (Table 5). Twenty-two strains gave unacceptable reactions (≤1+) in 13 antisera, for a sensitivity of 88.9%. Antisera were also less specific (91%) with this assay method, with 12 strains giving cross-reactions in six sera. MDL antisera were not used in slide agglutination assays as their titers are determined for use in tube agglutination assays. No MDL or DS antisera reacted in the saline control.

TABLE 5. Flagellar antisera test results using a slide agglutination assay

| Factor | Homologous reactions | | | | Heterologous reactions | |
|---|---|---|---|---|---|---|
| | No. tested | No. with reaction: | | | No. tested | No. positive |
| | | 3+/4+ | 2+ | ≤1+ | | |
| a | 4 | 2 | 1 | 1 | 3 | 0 |
| b | 5 | 5 | | | 3 | 0 |
| c | 5 | 2 | 2 | 1 | 3 | 0 |
| d | 5 | 2 | 2 | 1 | 3 | 0 |
| eh | 5 | 5 | | | 3 | 0 |
| i | 5 | 5 | | | 3 | 0 |
| k | 5 | 4 | 1 | | 3 | 0 |
| r | 5 | 4 | 1 | | 3 | 0 |
| y | 5 | 4 | | 1 | 3 | 1 |
| z | 5 | 3 | 2 | | 3 | 0 |
| $z_6$ | 5 | 4 | 1 | | 3 | 0 |
| $z_{10}$ | 5 | 3 | 1 | 1 | 3 | 0 |
| $z_{29}$ | 5 | 5 | | | 3 | 0 |
| l | 5 | 3 | | 2 | 3 | 0 |
| v | 5 | 4 | | 1 | 3 | 1 |
| w | 5 | 2 | 1 | 2 | 3 | 0 |
| $z_{13}$ | 5 | 4 | | 1 | 3 | 0 |
| $z_{28}$ | 5 | 3 | 2 | | 3 | 0 |
| 1 | 5 | 5 | | | 1 | 0 |
| 2 | 5 | 1 | 4 | | 3 | 0 |
| 5 | 5 | 4 | 1 | | 3 | 0 |
| 6 | 5 | | | 5 | 3 | 0 |
| 7 | 5 | 1 | 3 | 1 | 3 | 0 |
| g | 5 | 4 | 1 | | 3 | 0 |
| f | 5 | 5 | | | 3 | 0 |
| m | 5 | 5 | | | 3 | 0 |
| p | 5 | 5 | | | 3 | 0 |
| q | 2 | 2 | | | 3 | 0 |
| s | 5 | 3 | 2 | | 3 | 0 |
| t | 4 | 4 | | | 3 | 0 |
| u | 2 | 2 | | | 3 | 0 |
| $z_4$ | 10 | 6 | 1 | 3 | 7 | 0 |
| $z_{23}$ | 10 | 7 | 1 | 2 | 8 | 0 |
| $z_{24}$ | 10 | 8 | 2 | | 8 | 5 |
| $z_{32}$ | 6 | 6 | | | 8 | 5 |
| en | 5 | 3 | 2 | | 2 | 0 |
| x | 5 | 5 | | | 2 | 0 |
| $z_{15}$ | 5 | 4 | 1 | | 2 | 0 |
| Total | 198 | 144 | 32 | 22 | 128 | 12 |

## DISCUSSION

Overall, DS antisera performed very well. All somatic antisera except polyvalent O1 and somatic D and E1 sera were 100% sensitive and specific, and a second lot of group D antisera obtained from DS performed similarly. Flagellar antisera, when used in a tube agglutination assay, were 99% sensitive and 100% specific; however, the same sera used in a slide agglutination assay were less sensitive (89%) and specific (91%) in our hands. Unacceptable homologous reactions (≤1+) occurred in 13 of the 38 antisera tested; however, 10 of the 12 cross-reactions noted in the slide agglutination assay occurred with $z_4$ complex sera, usually $z_4z_{23}$ organisms crossing in absorbed $z_{24}$ and $z_{32}$ sera, indicating that residual $z_4$ antibody may remain in these lots. We recommend that if a weak reaction is encountered it be confirmed with a tube agglutination assay. In the tube assay legitimate weak reactions would be stronger and therefore be confirmed, while cross-reactions should drop out.

We also noticed two unusual antibody combinations that, although they are minor, could cause some confusion. DS includes flagellar factor $z_6$ in its 1 complex serum, causing organisms without the 1 complex antigen to react in this serum. This is redundant and somewhat confusing, since DS also produces a $z_6$ serum. Also, DS's m antiserum contains both the m antigen from the mt complex and the m antigen from the gm complex, which could lead to misidentification of a serotype. For example, if an organism reacts in the g complex serum and is then tested with appropriate absorbed g complex sera (factor f, m, p, q, s, t, or u) and the t reaction is weak or negative, an mt organism could be identified as gm because the microbiologist would not know whether the reaction is caused by the m from the mt complex or by the m from the gm complex.

We strongly recommend that DS provide clients with an unambiguous description or photo representation of homologous end points for their sera in their product insert. The manufacturer's insert advises that only strong agglutination observed within 1 minute should be regarded as positive, but no further definition is provided. The representative from DS, when on-site in our laboratory, read what is considered a 2+ somatic reaction by MDL standards (not an acceptable homologous reaction in our laboratory) as a strong reaction. Had the representative not been on-site, we would have considered all 2+ reactions to be unacceptable, although the manufacturer determines the titers of their antisera by intending a 2+ reaction to be a homologous end point.

Overall, DS antisera performed very well. An accepted performance standard of 90% accuracy was met or greatly exceeded for all groups of antisera tested (somatic, 96%; polyvalent O, 98%; polyvalent O1, 92%; flagellar [tube], 99%; and flagellar [slide], 90%]. DS has the added advantage of offering an extensive number of complex and absorbed factors needed to serotype a wide variety of *Salmonella* strains, including those in subgroups II, III, and IV.

## ACKNOWLEDGMENTS

This study was funded in part by Denka Seiken Co., Ltd., Tokyo, Japan.

We thank the Biologics Unit for their consistent production of high-quality reagents for use throughout the MDL.

## REFERENCES

1. **Frenzen, P. D., T. L. Riggs, J. C. Buzby, T. Breur, T. Roberts, D. Voestch, S. Reddy, and the FoodNet Working Group.** 1999. *Salmonella* cost estimate updated using FoodNet data. Food Rev. **22:**10–15. http://www.ers.usda.gov/publications/foodreview/May1999.
2. **Janda, J. M., and S. L. Abbott.** 2006. The enterobacteria, p. 81–103. ASM Press, Washington, DC.
3. **Le Minor, L., and R. Rohde.** 1989. Guidelines for the preparation of *Salmonella* antisera. WHO Collaborating Center for Reference and Research on *Salmonella*, Institut Pasteur, Paris, France.
4. **Outbreak Response and Surveillance Team, Centers for Disease Control and Prevention.** Accessed March 2007. Outbreak surveillance data. Centers for Disease Control and Prevention, Atlanta, GA. http://www. cdc.gov/foodborneoutbreak/.
5. **Pegues, D. A., M. E. Ohl, and S. I. Miller.** 2002. *Salmonella* including *S.* Typhi, p. 669–697. *In* M. J. Blaser, P. D. Smith, J. I. Ravdin, H. B. Greenberg, and R. L. Guerrant (ed.), Infections of the gastrointestinal tract. Lippincott, Williams & Wilkins, Philadelphia, PA.
6. **Voetsch, T., J. Van Gilder, F. J. Angulo, M. M. Farlye, S. Shallow, R. Marcus, P. R. Cieslak, V. C. Deneen, R. V. Tauxe, et al.** 2004. FoodNet estimate of the burden of illness caused by nontyphoidal *Salmonella* infections in the United States. Clin. Infect. Dis. **38**(Suppl. 3)**:**S127–S134.