Serotyping of *Serratia marcescens*: Evaluation of Le Minor's H-Immobilization Test and Description of Three New Flagellar H Antigens

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The H-immobilization test of Le Minor for determining flagellar (H) antigens was evaluated and compared with tube and slide H-agglutination tests. The test proved specific and easy to perform, and titration end points were clearly discernible. The degree of serological cross-reactivity between H antigen reference strains of *Serratia marcescens* was low. Consequently, this test was adopted for routine serological analysis of H antigens, using unabsorbed rabbit immune anti-H sera. As a result of using this procedure, three new provisional H antigens, designated H14, H15, and H16, are proposed.

Within the last 15 years, *Serratia marcescens*, an opportunistic pathogenic microorganism, has assumed an ever-increasing role with respect to outbreaks of nosocomial cross-infections. From 1957 onward, Ewing, Davis, and co-workers successively defined the somatic (O) and flagellar (H) antigens of *S. marcescens* (1-3) for purposes of taxonomy and epidemiological investigations within the clinical setting. Currently, 15 O and 13 H antigens of this microorganism are recognized (2). With appropriate monospecific anti-O and anti-H rabbit immune sera, it was possible to serotype approximately 95% of clinical isolates of *S. marcescens* from a variety of clinical sources and geographic localities (2). Sedlak (12) examined 91 isolates of *S. marcescens*, which were assigned to 10 O serogroups. The value of serotyping as an epidemiological tool was also underscored by Wilfert et al. (22). Other investigators introduced bacteriocin and phage typing as additional means to facilitate the epidemiological work-up of incriminated nosocomially significant strains of *S. marcescens* (4-6, 8, 10, 14, 15, 18, 20, 21); with these latter procedures, it was possible to type roughly 90% of the isolates.

Recently we encountered a multiple-drug-resistant strain of *S. marcescens* that, up until August 1976, had caused infections in a total of 19 patients from four different university hospitals at Erlangen. The nonconjugative strain proved to be of bacteriocin type 18 (19). However, on several occasions, patients with longstanding *S. marcescens* infection due to this particular strain yielded phenotypic variants

(17) that had been bacteriocin type 4 or 9 but that on repeated subcultures had reverted to bacteriocin type 18. Therefore, it was of interest to serotype this problem strain, including phenotypic variants, as well as previously encountered, nosocomially significant isolates of *S. marcescens*, some of which had proved to be bacteriocin nontypable (14). During the course of the serological investigations, we wished to compare a number of techniques, among these Le Minor's H-immobilization test (unpublished data), to expedite and, if possible, to simplify the serotyping procedures. Relevant data with regard to the serological analysis of flagellar (H) antigens of *S. marcescens* are presented below, including the description of three new H antigens.

MATERIALS AND METHODS

Bacteria. The 24 reference somatic (O) and flagellar (H) antigen strains of *S. marcescens* listed in Table 1 were a gift from Betty R. Davis, Enterobacteriology Unit, Center for Disease Control, Atlanta, Ga. *S. marcescens* strain S326 n.t. (serotype O13:H14), bacteriocin nontypable, had been isolated in 1971 from a postmortem wound specimen of the index case of a small outbreak of nosocomial cross-infections in the North Carolina Baptist Hospital, Winston-Salem. The group A bacteriocin (phage tail) control indicator strain *S. marcescens* S1i (serotype O6:H15) had been isolated in the same institution in 1969 (15, 16, 21); the source of this strain, however, is unknown. *S. marcescens* strain LF-München (serotype O13:H16), a rapidly lactose-fermenting isolate that also proved to be bacteriocin nontypable, was recovered in May 1976 from a tracheal aspirate of a newborn infant in the Children's Hospital, University of Munich, Munich, Germany.

Media. Brain heart infusion broth, tryptic soy

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TABLE 1. Compilation of *S. marcescens* reference O and H antigen strains

O antigen no.	<i>S. marcescens</i> strain no.	Serotype	H antigen no.	<i>S. marcescens</i> strain no.	Serotype
Reference O antigens			Reference H antigens		
O1	866-57	O1:H5	H1	863-57	O3:H1
O2	868-57	O2:H1	H2	836-57	O5:H2
O3	863-57	O3:H1	H3	862-57	O6:H3
O4	864-57	O4:H4	H4	864-57	O4:H4
O5	867-57	O5:H1	H5	866-57	O1:H5
O6	862-57	O6:H3	H6	680-57	O5:H6
O7	843-57	O7:H10	H7	814-57	O4:H7
O8	1604-57	O8:H3	H8	877-57	O6:H8
O9	4534-60	O9:H11	H9	1783-57	O14:H9
O10	1287-59	O10:H8	H10	2420-57	O6:H10
O11	1914-63	O11:nonmotile	H11	827-57	O5:H11
O12	6320-58	O12:H9	H12	874-57	O14:H12
O13	3607-60	O13:H4	H13	2436-57	O5:H13
O14	4444-60	O14:H12	H14	S326 n.t.	O13:H14
O15	4523-60	O15:H8	H15	Sli	O6:H15
			H16	LF München	O13:H16

broth (TSB) and agar (TSA), Difco agar, and MacConkey agar with added crystal violet were purchased from Difco laboratories, Detroit, Mich. The microorganisms were maintained on TSA slants at 4°C and in a mixture of 1 volume of brain heart infusion broth plus 1 volume of heat-inactivated bovine serum (Behringwerke AG, Marburg, Germany) at -65°C. Semisolid (ss) TSA U-tubes (10 ml of medium per tube) were prepared by addition of 0.3% (wt/vol) Difco agar to TSB (= 0.3% semisolid ssTSA); these tubes served for the determination of motility of all H antigen reference strains and all isolates to be typed. For the H-immobilization test (see below), TSB was rendered semisolid by the addition of 0.4% (wt/vol) Difco agar to TSB (= 0.4% ssTSA).

Reagents. Analytical-grade formalin (35%) and crystallized phenol were purchased from E. Merck, Darmstadt, Germany. A 0.6% solution of formaldehyde and a 0.5% solution of phenol in aqueous 0.154 M NaCl, respectively, were prepared according to Edwards and Ewing (2). Evipan-natrium (methylcyclohexenyl-methyl-barbituric acid, sodium salt) was a gift from Farbenfabriken Bayer AG, Leverkusen, Germany. Phosphate-buffered saline (PBS), pH 7.5, was prepared and autoclaved (121°C, 15 min) according to Schmidt (11).

Preparation of formalinized/phenolized *S. marcescens* H cells. In accordance with the technique of B. R. Davis (personal communication) and that of Edwards and Ewing (2), the *S. marcescens* strains were successively passed through six 0.3% ssTSA U-tubes, after which 1 loopful of each motile strain was inoculated in the late afternoon hours into 20 ml of TSB; the cultures were incubated at 35°C overnight, and the next morning either 20 ml of 0.6% formaldehyde or 20 ml of 0.5% phenolized saline was added. The cells were held at 35°C for an additional 24 h. Subcultures to plates of MacConkey agar with added crystal violet served to monitor effective killing of

the cells. Properly inactivated H cell suspensions served either as immunogens for rabbits or as agglutinogens for serological tests. The formalinized H cells were stored at 4°C for no longer than 6 weeks.

Immunization of rabbits and processing of rabbit immune anti-H sera. Based on Edwards and Ewing (2) and on the recommendations of B. R. Davis (personal communication), rabbits (2.5 kg of body weight; Firma Willy Schmid, Holzgerlingen, Germany) received a series of five intravenous injections of formalinized H cell suspensions, given at 5-day intervals; the volumes of the intravenous inocula were 0.5, 1.0, 2.0, 4.0, and 4.0 ml, respectively. On day 7 after the last injection, the animals were anesthetized by the intravenous inoculation of 1.0 ml of Evipan-natrium, after which the animals were exsanguinated aseptically by cardiac puncture. The rabbit blood was held at room temperature for 1 h, after which the blood clots were loosened, held at 4°C for an additional 2 h, and separated by centrifugation at $2,500 \times g$ (4°C) for 20 min. The sera were dispensed in 2-ml aliquots into sterile, screw-capped vials and stored at -65°C. As required, sera were heat-inactivated at 56°C for 30 min in a water bath.

Serological tests. (i) Tube H-agglutination tests. Tube H-agglutination tests were performed according to the technique of Edwards and Ewing (2). The H antigens (formalinized H cells) had been prepared as described above. Heat-inactivated rabbit immune anti-H sera were diluted 1:10, 1:20, and so on, until 1:10,240, in PBS, pH 7.5. Small Widal tubes (11 by 80 mm) received 0.5 ml of serum dilution plus 0.5 ml of H antigen; control tubes were inoculated with 0.5 ml of PBS plus 0.5 ml of H antigen. The tubes were incubated in a water bath for 2 h at 50°C, after which they were examined for flocculent, loose agglutinates. Titers were recorded as the reciprocal of the highest dilution of serum that had yielded macroscopic evidence of H-agglutination.

(ii) Slide H-agglutination tests. Slide H-aggluti-

nation tests were performed with the same serum dilutions in PBS (pH 7.5) and the same H antigens (formalized H cells) as in tube H-agglutination tests. One drop (0.05 ml) of serum dilution (or control PBS) plus 1 drop of H antigen were combined on one-quarter sections of ordinary microscope glass slides. The slides were gently tilted back and forth manually for a total of 3 min, after which they were read and interpreted (9). As a rule, the terminal and one or two subterminal reactive serum dilutions resulted in faintly visible agglutination, which had to be read with a $\times 3$ magnifying lens against an obliquely illuminated dark background. Titers were defined as above.

(iii) **Tube O-agglutination tests.** Tube O-agglutination tests were carried out in accordance with the technique of Felix and Bensted (7), except for the following minor modifications. For the preparation of O cells, each *S. marcescens* isolate was subcultured to a chocolate agar plate, which was incubated overnight at 35°C. On the next morning, 10 smooth-appearing colonies of each isolate were picked and transferred into 4 ml of TSB; after incubation at 35°C for 5 h, two large (14 cm in diameter) TSA plates were inoculated with 0.2 ml of TSB growth, respectively. The plates were incubated at 35°C overnight. The next morning, the growths were harvested with a total of 20 ml of sterile 0.154 M NaCl. The cells were centrifuged at $12,062 \times g$ (10,000 rpm) and 4°C for 10 min (Sorvall RC-5 centrifuge, SS34 rotor), after which the supernatant fluids were discarded. The cell sediments were resuspended in 20 ml of 0.154 M NaCl and boiled in a water bath for 30 min. The cells were washed once more in 0.154 M NaCl, resuspended in 20 ml of saline, and subsequently stored at 4°C for no longer than 3 months. The turbidity of the O cell suspensions corresponded approximately to that of McFarland barium sulfate standard no. 10 (3×10^9 colony-forming units/ml). The O-agglutination tests were performed by adding 0.5 ml of diluted (1:2 in PBS) stock O cells to 0.5 ml of a rabbit immune serum dilution in small Widal tubes. The master serum dilutions were 1:0, 1:10, 1:20, and so on, until 1:1,280, in PBS, pH 7.5. Control tubes received 0.5 ml of PBS plus 0.5 ml of O cells. The tubes were incubated in a water bath at 37°C for 2 h, after which the racks were transferred and held at 4°C overnight (ca. 16 to 18 h). The tubes were then held at room temperature for an additional 2 h, after which they were inspected macroscopically for granules and aggregates floating in the clear fluid, which resisted very gentle shaking (9). The serum titers were recorded as the reciprocal values of the highest dilutions of sera that yielded O-agglutination.

(iv) **Slide O-agglutination tests.** Comparative tests consisted of adding 0.05 ml of diluted (1:2) stock O cells (boiled for 30 min; see above) to 0.05 ml of serum dilution (or PBS for control purposes) on standard microscope glass slides that had been divided into four sections (9). For the determination of the serogroup (O antigen) of clinical isolates of *S. marcescens*, undiluted stock O cells were used. The slides were gently tilted back and forth manually for

2 min at room temperature, after which they were read with the naked eye and recorded. Again, serum titers were defined as the reciprocal values of the highest serum dilutions that resulted in macroscopically evident O-agglutination.

(v) **H-immobilization test of Le Minor.** Following the suggestion of B. R. Davis to evaluate L. Le Minor's flagellar immobilization test for *S. marcescens* (unpublished data), and after having obtained Dr. Le Minor's consent (L. Le Minor, personal communication), the following protocol was adopted for the routine performance of the test subsequent to a series of preliminary experiments. Each isolate of *S. marcescens* to be typed was passed once through a 0.3% ssTSA U-tube (check for motility). Depending on the number of isolates or sera to be examined, appropriate numbers of metal-capped tubes (13 by 100 mm) received 3 ml of 0.4% ssTSA (the height of the medium column measured about 3.4 cm); after autoclaving (121°C, 15 min), the tubes were transferred to a water bath and held at 56°C for about 1 h. Heat-inactivated, unabsorbed rabbit immune anti-H sera were diluted in PBS, pH 7.5, as follows: 1:0, 1:4, 1:8, and so on, until 1:512. Then, 0.05 ml of each serum dilution was delivered aseptically with 0.1- or 0.2-ml pipettes to the appropriately labeled tubes, which had been removed from the 56°C water bath immediately prior to addition of serum. Motility control tubes were used as such. The tubes were shaken gently on a laboratory shaker to ensure even distribution of the antibodies. The tubes were then transferred to and held at 4°C for 20 min; this time was sufficient for adequate gelation to occur. After solidification, the tubes, including control tubes, were inoculated with *S. marcescens* candidate strains to a depth of no more than 2 mm. The needles were flamed between each tube. The tubes were incubated at 35°C overnight, after which they were read and interpreted. It should be stressed that after the serum dilutions had been added to the ssTSA tubes, the sera had been further diluted 60-fold; thus, the final serum dilutions were 1:60, 1:240, 1:480, and so on, until 1:30,720. The titer of sera was defined as the highest dilution permitting the motile isolate (relative to the control tube) to migrate downward from the point of inoculation for one-half of the total height of semisolid medium (ca. 1.7 cm). This end point empirically was found to correlate well with H-slide agglutination titers. H antigen determinations of clinical isolates of *S. marcescens* were performed as follows. After one U-tube passage, the motile isolates were screened with a 1:4 dilution (final dilution, 1:240) of each rabbit immune anti-H serum, respectively. Those sera that had immobilized the isolate were then titrated in a manner analogous to the above-mentioned procedure.

Bacteriocin typing. Isolates were typed as based on susceptibility to one or more of 10 selected group A (phage tail) bacteriocins of *S. marcescens* (13, 21).

RESULTS

A series of preliminary experiments served to determine a number of potential variables with

regard to the three H-typing procedures used, i.e., the tube and slide H-agglutination and the H-immobilization tests. During the preparation of H cells of *S. marcescens*, it was observed that phenolized saline (0.25% final concentration of phenol) failed to kill the majority of strains, whereas formalinized saline (0.3% final concentration of formaldehyde) reliably rendered all *S. marcescens* strains nonviable. Therefore, immunogenic and agglutinogenic H cells of *S. marcescens* were prepared by formalinization exclusively. It should be added, though, that phenolized and formalinized H cells yielded identical titers in slide H-agglutination tests. It was noted that formalinized H cells that had been stored at 4°C for up to 6 weeks still proved adequately agglutinogenic, whereas storage for 8 weeks or longer at 4°C resulted in weakly agglutinogenic cells. Therefore, formalinized H cell suspensions were discarded after storage in excess of 6 weeks. In this context, 30-min-boiled O cells of the majority of *S. marcescens* strains became slightly rough after storage at 4°C for more than 3 months, as evidenced by trace auto-agglutinability in PBS, pH 7.5. Thus, 30-min-boiled stock O cells were discarded prior to the 3-month expiration date. Formalinized H cell suspensions of selected *S. marcescens* strains that had been concentrated 10- and 20-fold (Sorvall RC-5 centrifuge, SS34 rotor; 12,062 $\times g$, 10 min, 4°C) yielded macroscopically very clearly discernible titration end points in slide H-agglutination tests, whereas cells that had been concentrated up to fivefold required the aid of a magnifying lens for the terminal and one or two subterminal serum dilutions. For example, rabbit immune serum no. 115, which had been made against *S. marcescens* strain CDC O5:H2, had a titer of 1:5,120 against standard (1 $\times$) and 5 $\times$ concentrated homologous formalinized H2 cells and a titer of 1:2,560 against 10 $\times$ and 20 $\times$ concentrated H2 cells, respectively. Boiling of formalinized or live cells of selected *S. marcescens* reference CDC H antigen strains for 30 min abolished H-agglutinability. The tube and slide O- and H-agglutination tests yielded comparable titers with regard to rabbit immune and patient sera; titers differed by no more than one dilution step. For example, patient G.S., a female patient with a chronically infected hip endoprosthesis of over 1 year's duration due to the multiple-drug-resistant strain of *S. marcescens* (bacteriocin type 18; serotype O6/O14:H12), had anti-H antibody titers of 1:1,280 by both tests immediately prior to institution of curative amikacin chemotherapy; likewise, her O titer against antigen O14 was

1:80 as measured with the tube O- and the slide O-agglutination procedures, respectively. It should be stressed that the slide H-agglutination test was not suitable for checkerboard titrations of rabbit immune anti-H sera, as numerous cross-reactions at low serum dilutions became evident, confirming the experiences of Edwards and Ewing (2). Furthermore, CDC *S. marcescens* reference strain O4:H7 gave rise to "flaky" growth in TSB, which persisted after addition of formaldehyde, rendering the interpretation of titration end points by both tube and slide H-agglutination tests extremely difficult; with the H-immobilization tests, however, clear-cut end points were obtained.

Finally, Dr. Le Minor's H-immobilization test was evaluated in a further series of preliminary experiments. This test was found to yield specific and reproducible results. Isolates of *S. marcescens* required only one passage through 0.3% ssTSA U-tubes prior to performance of the test. Rabbit immune anti-H sera with titers as high as 1:320 against the homologous O antigen of the immunogenic *S. marcescens* strain (Table 2) failed to inhibit the motility of the test strains in 0.4% ssTSA. Properly immunized rabbits developed immobilizing titers of $\geq 1:3,840$ (Table 3), and, furthermore, the titers obtained with the H-immobilization test correlated well with the results of the slide H-agglutination tests; titers differed no more than fourfold (Table 3). In addition, the degree of serological cross-reactivity detected among the 13 *S. marcescens* H antigen reference strains was low (Table 4); the same was true for *S. marcescens* isolates S326 n.t. (bacteriocin nontypable), Sli (formerly bacteriocin type 1, now type 16), and LF-München (bacteriocin nontypable), whose flagella were found to represent new H antigens, designated H14, H15, and H16, respectively (Table 4). The only major cross-reaction observed was the reciprocal one between *S. marcescens* strains CDC O6:H8 and CDC O14:H9 (Table 4). For instance, our clinical *S. marcescens* isolate no. S86 (bacteriocin type 14), which proved to be of serogroup O13, yielded the following typing results with the H-immobilization test. Rabbit immune serum no. 126, anti-CDC O6:H8, gave a titer of 1:1,920, whereas serum no. 127, anti-CDC O14:H9, immobilized the strain up to a dilution of 1:15,360, an eightfold difference, which was interpreted as significant; therefore, the H antigen of this isolate was recorded as H9. Most important, the results obtained with the checkerboard titrations of the rabbit immune anti-H sera permitted the use of unabsorbed sera (Table 4). As a

consequence, the H-typing procedure for clinical isolates of *S. marcescens* evolved into a relatively simple task. As stated in Materials and Methods, the isolates were passed first through one U-tube of 0.3% ssTSA (check for motility). Next, the isolates were exposed to rabbit anti-H immune sera diluted 1:4 (final dilution, 1:240) in 0.4% ssTSA. Finally, only those sera that had immobilized the isolates in the screen test were titrated fully against the candidate strains.

DISCUSSION

In our hands, Le Minor's H-immobilization test proved the simplest procedure for the serological analysis of flagellar antigens of *S. marcescens*; this test yielded clearly discernible end points. Furthermore, the degree of serological cross-reactivity between certain H antigens of *S. marcescens* was similar to the results Edwards and Ewing (2) had obtained with tube H-agglutination tests and to those obtained by B. R. Davis (personal communication) with commercially available rabbit immune sera. The results from the checkerboard titrations of all 16 anti-H sera led to a simplified approach for the routine H-typing of clinical isolates of *S. marcescens* with the aid of unabsorbed anti-H

TABLE 2. Titration of rabbit immune anti-H sera against homologous O cells of *S. marcescens*

Rabbit immune serum no.	H antigen reference strain no.	Titers obtained against homologous O cells ^a
122	O3:H1	320
116	O5:H2	160
118	O6:H3	40
120	O4:H4	160
123	O1:H5	160
124	O5:H6	20
125	O4:H7	160
126	O6:H8	320
127	O14:H9	40
128	O6:H10	320
129	O5:H11	40
130	O14:H12	160
131	O5:H13	80
961	S326 n.t., O13:H14	160
177	S1i, O6:H15	160
180	LF-München, O13:H16	80

^a Titrations were carried out by using the slide O-agglutination test (30-min-boiled O cells). Titers listed are the reciprocal values of the highest serum dilutions that yielded macroscopically evident O-agglutination.

TABLE 3. Titers of anti-H sera compared by slide H-agglutination and H-immobilization tests

Rabbit immune serum no.	H antigen reference strain no.	Titers obtained with:	
		Slide H-agglutination test	H-immobilization test
122	O3:H1	30,720 ^a	30,720 ^b
116	O5:H2	15,360	3,840
118	O6:H3	7,680	7,680
120	O4:H4	15,360	7,680
123	O1:H5	30,720	30,720
124	O5:H6	15,360	30,720
125	O4:H7	30,720	30,720
126	O6:H8	15,360	15,360
127	O14:H9	15,360	15,360
128	O6:H10	30,720	30,720
129	O5:H11	30,720	7,680
130	O14:H12	15,360	7,680
131	O5:H13	7,680	3,840

^a Titers listed are the reciprocal values of the highest dilutions of sera that yielded macroscopically visible H-agglutination; the serum master dilutions had been modified to give final dilutions identical to those of the H-immobilization test.

^b Titers shown are the reciprocal values of the highest dilutions of sera that prevented inocula of motile *S. marcescens* strains from migrating beyond one-half the length of the semisolid medium column.

sera, a considerable saving in effort and expense. In addition, with the antecedent screening procedure, i.e., the use of 1:4 diluted (1:240 final dilution) immune serum, the number of minor cross-reactions was reduced (Table 4), and consequently the number of required full serum titrations could be limited.

As for the three new H antigens of *S. marcescens* described in the present study, undoubtedly there exist additional H antigens of this microorganism. Therefore, the H antigen scheme presented here should be considered a tentative one.

Our experiences with serogrouping (determination of O antigens) of clinical *S. marcescens* isolates, particularly with respect to the major and extremely bothersome cross-reactions of antigens O6 and O14, will be the subject of a separate communication.

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TABLE 4. Checker-board titrations of rabbit immune anti-*H* sera against all *S. marcescens* *H* antigens using the *H*-immobilization test

Titrated against <i>H</i> antigen <i>S. marcescens</i> reference strain no.	Rabbit immune serum no.															
	122	116	118	120	123	124	125	126	127	128	129	130	131	961	177	180
H1	30,720 ^a	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12	H13	H14	H15	H16
O3:H1	—	— ^b	—	—	—	—	—	—	—	—	—	—	—	—	—	—
O5:H2	—	3,840	—	—	—	—	—	—	—	—	60	—	—	—	—	—
O6:H3	—	—	7,680	—	—	—	—	—	—	960	—	60	—	—	—	—
O4:H4	—	60	—	7,680	—	—	—	—	—	—	—	240	—	—	—	—
O1:H5	—	—	—	—	30,720	—	—	60	—	—	—	—	—	—	—	—
O5:H6	—	—	—	—	—	30,720	—	—	—	240	—	—	960	—	—	—
O4:H7	—	—	—	—	—	—	30,720	—	—	—	—	—	—	—	480	—
O6:H8	—	—	—	—	—	—	—	15,360	3,840	60	—	—	—	—	—	—
O14:H9	—	—	—	—	—	—	—	1,920	15,360	—	60	—	60	—	—	—
O6:H10	—	—	480	—	—	—	—	60	60	30,720	—	—	60	—	—	—
O5:H11	—	60	—	—	—	—	—	—	—	7,680	—	—	60	—	—	—
O14:H12	—	—	—	480	—	—	—	—	—	—	—	7,680	—	—	—	—
O5:H13	—	—	—	—	—	—	—	60	—	—	—	—	3,840	—	—	—
S326 n.t. (H14)	—	—	—	—	—	—	—	—	—	—	—	—	—	3,840	—	—
Sli (H15)	—	—	—	—	—	—	960	—	—	—	—	—	60	—	30,720	—
LF-München (H16)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	15,360

^a Titers listed are the reciprocal values of the highest dilutions of sera that limited the migration of motile strains to one-half of the length of the semisolid medium column.

^b —, Titers of less than 1:60; i.e., undiluted immune serum failed to immobilize the tested motile strain.

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