Serological Classification of *Pseudomonas aeruginosa* by a Slide Agglutination Test

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Serological classification of *Pseudomonas aeruginosa* by the slide agglutination test with live organisms was studied, based on the O antigen schema adopted by the international expert panel sponsored by the Subcommittee on *Pseudomonas* and Related Organisms of the International Committee on Systematic Bacteriology. The typing results obtained by the slide test with well-absorbed O sera were identical to those obtained by the conventional tube agglutination test with autoclaved organisms. Most O antigens occur singly; but O2, O5, and O16 occur in four combinations. Antigens O13 and O14 are closely related, as are O7 and O8, and it would be convenient to classify organisms possessing these antigens collectively as O7,8 and O13,14.

Before 1970 a variety of serological classification systems for *Pseudomonas aeruginosa* was used in different countries, such as those reported by Habs (4), Verder and Evans (15), Meitert (9), and Lányi (8). To establish an internationally acceptable serotyping schema, an international expert panel was organized in 1970 under the auspices of the Subcommittee on *Pseudomonas* and Related Organisms of the International Committee on Systematic Bacteriology. The panel agreed to adopt 12 heat-stable somatic antigens from Habs' schema and 5 more antigens from other typing systems; these were designated by arabic numerals 1 through 17 (P. V. Liu, Chairman; personal communication).

Many workers performed the slide agglutination test with live organisms (2, 6, 8-10, 13, 16, 17) for the serotyping of *P. aeruginosa* because of its practical advantage over the conventional tube agglutination test with autoclaved organisms. However, only Lányi has reported detailed procedures for the slide test (8), and he used his own serotyping system. Homma (5) and Kodama and Ishimoto (7), who studied serotyping of *P. aeruginosa* extensively with the slide test, used their own classification system.

We have investigated the performance of the slide O agglutination test under the expert panel schema. Some basic findings which may contribute to a better application of the new schema to *Pseudomonas* serotyping are reported here.

MATERIALS AND METHODS

Bacteria. Seventeen strains of *P. aeruginosa*, selected by the expert panel as representing specific heat-stable somatic antigens, were received from P. V. Liu of the University of Louisville, Louisville, Ky.

(Table 1). The first 12 are the original Habs' strains, which Liu received from M. Véron, Institut Pasteur, Paris. These reference strains were used to prepare rabbit antisera and absorb cross-agglutinins. For convenience, I shall refer to these strains by their O antigens, rather than by their original designations. To evaluate the prepared typing sera, we used 98 strains of *P. aeruginosa* isolated from patients in Albany Medical Center Hospital in 1964-65, lyophilized and maintained since then in our laboratory. These strains are designated by B followed by four-digit numbers.

Some strains representative of Verder and Evans' system (15) and Véron's subtype strains for types 2 and 5 (16) were also examined. The former were sent to this laboratory by N. B. McCullough, National Institute of Allergy and Infectious Diseases, Bethesda, Md.; the latter were sent by M. Véron.

Preparation of immune sera. Bacteria grown on Trypticase soy agar (Baltimore Biological Laboratory) for 18 to 20 h at 30°C were suspended in saline, heated in a boiling-water bath for 2.5 h, washed with sterile saline three times, and used to immunize rabbits after the density was properly adjusted. O antisera prepared by the procedure of Verder and Evans (15) were not potent enough to be used for the slide test. Satisfactory sera were obtained by injecting a dense bacterial suspension in a shorter period of time, as described by Mikkelsen (11, 12). A suspension containing 5×10^{10} bacteria per ml was given intravenously in increasing doses of 1.0, 2.0, and 4.0 ml at 4-day intervals. If the rabbit had lost more than 10% of its initial weight by the second and third injections, the dosage was reduced. Three to four days after the third injection, the rabbit was bled out. Sera produced by this method generally gave strong reactions at 1:10 dilution or higher in the slide test. The disadvantage, as mentioned by Mikkelsen (11), was occasional loss of a rabbit due to the heavy dosage.

A third method, tried later, was the use of the chrome vaccine of Ando et al. (1). To a saline suspen-

TABLE 1. *Reference strains of P. aeruginosa used for antiserum production*

Anti-gen	Original designation	Origin
O1	5933 (Véron)	Habs, group 1
O2	5934 (Véron)	Habs, group 2
O3	5935 (Véron)	Habs, group 3
O4	5936 (Véron)	Habs, group 4
O5	5937 (Véron)	Habs, group 5
O6	5939 (Véron)	Habs, group 6
O7	5938 (Véron)	Habs, group 7
O8	5940 (Véron)	Habs, group 8
O9	5941 (Véron)	Habs, group 9
O10	5943 (Véron)	Habs, group 10
O11	5944 (Véron)	Habs, group 11
O12	5945 (Véron)	Habs, group 12
O13	6092 (Véron)	Sandvik, group II
O14	1M-1 (Verder & Evans)	Verder & Evans, group V
O15	170022 (Lányi)	Lányi, group 12
O16	170003 (Lányi)	Lányi, group 3
O17	Type 10 (Meitert)	Meitert, type 10

sion containing 5×10^{10} bacteria per ml was added 1% thiomerosal solution at a final concentration of 1:5,000. Then a 1% solution of chromium potassium sulfate which had been filtered through a 450-nm membrane filter (Millipore Corp.) was added at 1/10 the volume of the bacterial suspension. The mixture was kept at 37°C for 3 to 4 days. Bacteria collected by centrifugation were washed once with saline and resuspended to the original volume in saline containing 0.01% thiomerosal. Rabbits tolerated well this detoxified vaccine. Of the various immunization schedules tested, two 5-ml intravenous injections 5 days apart, followed by bleeding out after 5 days, evoked quick and high responses. The titers of O sera obtained were comparable to those obtained by the method of Mikkelsen.

Absorption of cross-reacting agglutinins. Bacteria grown for 20 h on Trypticase soy agar at 30°C were suspended in saline, heated in a boiling-water bath for 1 h, washed with saline three times, and used for absorption. The pellet from the last centrifugation was mixed thoroughly with undiluted or diluted serum. Depending on the amount of cross-agglutinin, the ratio by volume of the pellet to the serum (adjusted for the dilution factor) varied from 1:1 to 1:4.

Tube and slide O agglutination tests. For both the tube and slide O agglutination tests, the O antigens consisted of cultures grown on Trypticase soy agar at 30°C for 20 h; for the tube test, they were also heated at 120°C for 1 h. Otherwise, the tube test was performed by the method of Verder and Evans (15).

For the slide test, the surface of a glass slide was divided into 8 to 10 compartments with a wax pencil. The pattern used by Finkelstein and Mukerjee (3) was practical for quick mixing and conserving the reagents. One drop of a properly diluted antiserum was placed in each compartment. A small amount of bacterial growth was placed near the drop and mixed evenly with the antiserum. The slide was rocked by hand continuously, and the reaction was read at the end of 1 min. An arbitrary scale from +++++ (a strong agglu-

tion appearing within a few seconds) to + (weak, but definitive agglutination by the end of 1 min) was used. As observed by Wahba (17), loose, viscous aggregations occasionally appeared quickly but did not progress further, giving an appearance different from full agglutination. They were disregarded because in runs with autoclaved cultures these reactions often disappeared and left negative results.

RESULTS

Cross-reactions in the tube and slide agglutination tests. Unabsorbed O sera, particularly those produced by the method of Mikkelsen (11, 12) or with the chrome vaccine (1), showed cross-reactions against all the autoclaved reference strains in the tube test due to the presence of a common component, as pointed out by Lányi (8). For instance, an anti-O3 serum prepared by the method of Mikkelsen showed various degrees of reaction against all the autoclaved antigens (Table 2). After this serum was absorbed with the O9 strain at a pellet-to-serum ratio of 1:4, all the cross-reactions disappeared, resulting in the production of a specific anti-O3 serum. Absorption of this serum with only the O13 strain at a pellet-to-serum ratio of 1:1, however, could not produce a specific serum in the tube test.

When tested with live organisms on slides, the unabsorbed serum did not react with many strains at even a 1:5 dilution. In particular, an antigen like the O1 strain, which reacted strongly with the unabsorbed serum in the tube test, caused no reaction in the slide test. Some of the antisera, such as anti-O11, anti-O12, and anti-O15, showed no cross-reaction at all on slides and could be used as specific O sera without absorption. In contrast to the tube test, absorption of the anti-O3 serum with the O9 or O13 strain produced a specific O serum for the slide test.

As long as potent antisera were employed, there were no difficulties in carrying out the slide test with live organisms. The following observations were all made by the slide test.

Occurrence of multiple antigens in a single strain. Cross-reactions between O2 and O5 organisms, between O7 and O8 organisms, and between their counterparts in other classification systems, such as those of Verder and Evans (15) and Lányi (8), have been frequently discussed (2, 4, 6, 8, 12, 15-17). The complexity of O2 and O5 antigens was analyzed by Véron (16), who introduced classification by substrains into groups O2 and O5. Since a close relationship between O2, O5, and O16 organisms has been pointed out by Homma (5) and Terada et al. (14), we analyzed the antigenic makeup of these three strains.

TABLE 2. Agglutinin titers of unabsorbed and absorbed anti-O3 sera, tested with autoclaved (tube) and live (slide) *P. aeruginosa*

Strain	Titer of anti-O3, K2400 serum					
	Tube test			Slide test		
	Unabsorbed	Absorbed with O9	Absorbed with O13	Unabsorbed	Absorbed with O9	Absorbed with O13
O1	1,280	— ^a	40	— ^b	—	—
O2	320	—	—	—	—	—
O3	2,560	1,280	2,560	320	160	160
O4	160	—	—	20	—	—
O5	160	—	—	—	—	—
O6	640	—	20	5	—	—
O7	160	—	—	—	—	—
O8	80	—	—	—	—	—
O9	5,120	—	160	160	—	—
O10	640	—	20	10	—	—
O11	80	—	—	—	—	—
O12	80	—	—	—	—	—
O13	640	—	—	10	—	—
O14	160	—	—	20	—	—
O15	80	—	—	—	—	—
O16	80	—	—	—	—	—
O17	5,120	—	40	320	—	—

^a Negative at 1:20 dilution.^b Negative at 1:5 dilution.

An anti-O2 serum, in the unabsorbed state, showed reactions against the O2, O5, and O16 strains and also against the O9 and O17 strains in the slide test (Table 3). The cross-reaction against the O9 and O17 strains was frequently observed in other typing sera and was easily removed by absorbing sera with the O9 strain. This serum, after absorption with the O9 and O16 strains, retained only the agglutinin against the homologous O2 antigen. Likewise, an anti-O5 serum, which reacted with the O2, O5, and O16 strains in the unabsorbed state, reacted only against the homologous O5 antigen after absorption with the O16 strain.

In contrast, an anti-O16 serum, which reacted with the O2, O5, and O16 strains in the unabsorbed state, completely lost the reactivity against the O2 and O5 strains and almost completely against the O16 strain after absorption with the O2 strain. Absorption of this serum with the O5 strain removed all reactivity against the O2 and O5 strains, leaving low reactivity against the O16 strain.

These results demonstrate clearly that O16 antigen is common to the O2, O5, and O16 strains, but it is a single major antigen in the O16 strain. Besides O16 antigen, the reference strains for O2 and O5 possess their own specific O antigens. Factor sera for O2 and O5 can be prepared by proper absorption (Table 3). An unabsorbed anti-O16 serum can be used as a factor serum for O16.

With this set of factor sera it is possible to determine the antigenic formulas of isolates whose antigens are related to O2, O5, and O16. In the dozen isolates we have tested thus far, these three antigens occur in four combinations (see also Table 8). Our stock cultures happen to include Verder and Evans' group I and X strains (15) and Véron's substrains for O2 and O5 (16). Their antigenic formulas were easily determined (Table 3).

Other striking cross-reactions constantly seen in the reference strains were between the O7 and O8 strains and between the O13 and O14 strains. Reciprocal absorption of each antiserum reduced the homologous titer sharply in the slide test, particularly with the anti-O7 and anti-O13 sera (Table 4). For practical purposes it would be advisable to classify these related organisms collectively and express their antigenic formulas as O7,8 and O13,14.

Occurrence of cross-reacting agglutinins which are not detected by the heterologous reference strains. If an antiserum shows a strong reaction against the homologous strain and no reaction against any of the 16 heterologous reference strains at a proper serum dilution, it can generally be used as a specific O antiserum. But this is not always the case. An example is an anti-O9 serum (K500) which, after being absorbed with the reference O13 strain, seemed a good candidate for specific O9 antiserum in the slide test (Table 5). When this antiserum was

TABLE 3. Antigenic analyses of *P. aeruginosa* strains related to antigens O2, O5, and O16 by the slide agglutination test

Source and designation of strain	Anti-O2 (J2512) 1:5		Anti-O5 (K594) 1:10		Anti-O16 (J5990) 1:5			Saline	Antigenic formula
	Unab-sorbed	Absorbed with O9 & O16	Unab-sorbed	Absorbed with O16	Unab-sorbed	Absorbed with O2	Absorbed with O5		
Reference (Liu)									
5933:O1	—	—	—	—	—	—	—	—	
5934:O2	+++	+++	++	—	++	—	—	—	O2,16
5935:O3	—	—	—	—	—	—	—	—	
5936:O4	—	—	—	—	—	—	—	—	
5937:O5	+++	—	++++	+++	+++	—	—	—	O5,16
5939:O6	—	—	—	—	—	—	—	—	
5938:O7	—	—	—	—	—	—	—	—	
5940:O8	—	—	—	—	—	—	—	—	
5941:O9	++	—	—	—	—	—	—	—	
5943:O10	—	—	—	—	—	—	—	—	
5944:O11	—	—	—	—	—	—	—	—	
5945:O12	—	—	—	—	—	—	—	—	
6092:O13	—	—	—	—	—	—	—	—	
1M-1:O14	—	—	—	—	—	—	—	—	
170022:O15	—	—	—	—	—	—	—	—	
170003:O16	+++	—	++++	—	+++	±	+	—	O16
Meitert 10:O17	++	—	—	—	—	—	—	—	
Isolates									
B1480		—		+++	++			—	O15,16
B1759		—		—	++			—	O16
B1763		—		—	+++			—	O16
B1766		—		+++	+++			—	O5,16
B1795		+++		—	+++			—	O2,16
B1807		+++		+++	+++			—	O2,5,16
B1843		++		+++	+++			—	O2,5,16
B1852		—		+++	+++			—	O5,16
B1899		—		+++	+++			—	O5,16
Verder and Evans									
2243:1a,1b,1c		+++		—	+++			—	O2,16
359:1a,1c,1d		—		—	+++			—	O16
G2312:10,1a		—		++	++			—	O5,16
Véron									
623,O:2b		++		+++	+++			—	O2,5,16
624,O:2ab		+++		—	+++			—	O2,16
625,O:5d		—		+++	+++			—	O5,16
626,O:5cd		—		+++	+++			—	O5,16

used to examine strains derived from patient isolates, however, some showed moderate to strong reactions. Three O11 strains (B1774, B1845, and B1846) reacted to anti-O9 as well as anti-O11 serum. When the anti-O9 serum was absorbed with both the O13 strain and B1846, these isolates showed no reaction. Similarly, the cross-agglutinin of an anti-O7,8 serum to some isolates was easily eliminated by absorption with a cross-reacting isolate (Table 7).

Pattern of wide-range cross-reactions. Many of the anti-O sera cross-reacted against heterologous reference strains in the slide test. As expected, the extent and degree of cross-reactions varied greatly from one antiserum to

another. To formulate absorption schedules for preparing the specific anti-O sera, it was first necessary to examine each antiserum for reactions against all the reference strains. In this process we observed a definite pattern. Cross-reactions among the O3, O6, O9, O10, O13, O14, and O17 strains occurred frequently; an antiserum to any of these strains may not cross-react with all the others, but any cross-reactions which occur are limited to these strains (Table 6). The O1, O4, and O11 strains cross-reacted less frequently. An antiserum (NW116) obtained by immunizing rabbits with a mixture of equal amounts of O6 and O13 antigens exhibited strong to moderate reactions to all seven strains

TABLE 4. Agglutinin titers of anti-O7 and -O8 sera and anti-O13 and -O14 sera of *P. aeruginosa* in the slide test, before and after reciprocal absorptions

Strain	Anti-O7 (NW127)		Anti-O8 (K3901)		Anti-O13 (J228)		Anti-O14 (K3929)		Saline
	Unab-sorbed	Absorbed with O8	Unab-sorbed	Absorbed with O7 & O9	Unab-sorbed	Absorbed with O9 & O14	Unab-sorbed	Absorbed with O13	
O1	— ^a	—	—	—	—	—	—	—	—
O2	—	—	—	—	—	—	—	—	—
O3	—	—	—	—	—	—	—	—	—
O4	—	—	—	—	—	—	—	—	—
O5	—	—	—	—	—	—	—	—	—
O6	—	—	—	—	5	—	—	—	—
O7	320	20	40	—	—	—	—	—	—
O8	80	—	160	40	—	—	—	—	—
O9	—	—	—	—	80	—	—	—	—
O10	—	—	—	—	—	—	5	—	—
O11	—	—	—	—	—	—	—	—	—
O12	—	—	—	—	—	—	—	—	—
O13	—	—	—	—	320	10	40	—	—
O14	—	—	—	—	320	—	320	40	—
O15	—	—	—	—	—	—	—	—	—
O16	—	—	—	—	—	—	—	—	—
O17	—	—	10	—	40	—	—	—	—

^a Negative at 1:5.TABLE 5. Presence of a common antigen in the O9 strain and some isolates of *P. aeruginosa* as revealed by the slide agglutination test

Strain	Anti-O9, K500 (1:10)		Saline
	Absorbed with O13	Absorbed with O13 & B1846	
Reference			
O1	—	—	—
O2	—	—	—
O3	—	—	—
O4	—	—	—
O5	—	—	—
O6	—	—	—
O7	—	—	—
O8	—	—	—
O9	++++	++++	—
O10	—	—	—
O11	—	—	—
O12	—	—	—
O13	—	—	—
O14	—	—	—
O15	—	—	—
O16	—	—	—
O17	—	—	—
Isolates			
B1763 (NT ^a)	++++	—	—
B1769 (NT)	++++	—	—
B1774 (O11)	++	—	—
B1845 (O11)	++	—	—
B1846 (O11)	+++	—	—

^a NT, Nontypable.

listed above. Few cross-reactions were seen with the remaining strains.

Use of polyvalent sera for routine exam-

ination. In routine examination of isolates with the slide test, it is advisable to use polyvalent sera for the initial screening. However, when monovalent sera were pooled, the cumulative effect of minor agglutinin components, which had been neglected in each individual serum, often produced noticeable cross-reactions, as had been pointed out by Lányi (8). Sometimes it was difficult to absorb out this kind of cross-reacting agglutinin. A pool (polyvalent serum III) of antisera against O3, O6, O9, O10, O13, O14, and O17 often solved this problem, minimizing the necessity for absorption of the pooled sera.

Anti-O2,5,16 and anti-O7,8, which occasionally showed a few cross-reactions with other reference strains, comprise another group (polyvalent serum II). Anti-O2,5,16 can best be produced by immunizing rabbits with an O2,5,16 strain (such as B1807, Table 3). Anti-O7,8 can be produced by immunization with either the O7 or O8 strain; it should react strongly against both the O7 and O8 strains.

The remaining antisera, anti-O1, -O4, -O11, -O12, and -O15, comprise another group (polyvalent serum I).

Polyvalent, bivalent, and monovalent sera to be used for routine serotyping are listed in Table 7. Cross-reacting strains encountered in all the antisera prepared thus far are also given. An individual serum may not exhibit cross-reactions against all the strains in the list. Although the selection of absorbing strains is largely dependent on the pattern of cross-reactions in the individual antiserum, the strains which have so far

TABLE 6. *Distribution of cross-reacting antigen(s) among some reference strains of P. aeruginosa as revealed by the slide agglutination test^a*

Strain	Anti-O3 (K2400)	Anti-O6 (K5425)	Anti-O9 (K5001)	Anti-O10 (K617)	Anti-O13 (J228)	Anti-O17 (K584)	Anti-O6,13 (NW116)	Saline
O1	-	-	-	-	-	-	-	-
O2	-	-	-	-	-	-	-	-
O3	++++	-	+	-	-	-	++	-
O4	++	-	-	-	-	-	±	-
O5	-	-	-	-	-	-	-	-
O6	-	++++	-	-	±	-	++++	-
O7	-	-	-	-	-	-	-	-
O8	-	-	-	-	-	-	-	-
O9	++++	-	++++	+++	++	++	+++	-
O10	+	++	-	++++	-	-	++++	-
O11	-	-	-	-	-	-	-	-
O12	-	-	-	-	-	-	-	-
O13	++	+	-	-	++++	-	++++	-
O14	+	++++	-	+	++++	-	++++	-
O15	-	-	-	-	-	-	-	-
O16	-	-	-	-	-	-	-	-
O17	+++	+	++	++++	++	++++	+++	-

^a All antisera were diluted to 1:10.TABLE 7. *Preparation of absorbed antisera for the routine serotyping of P. aeruginosa*

Antiserum	Immunizing strains	Strains against which cross-reactions may occur	Strains which may be used for absorption
Polyvalent I			
Anti-O1,4,11 ^a	O1, O4 and O11	O9, 13, 14, 17	O9 + O14
Anti-O12, 15 ^a	O12 and O15	O3, 4, 14, 17	
Polyvalent II			
Anti-O2, 5, 16	B1807(O2, 5, 16)	O9, 17	O9 + B1380(O11)
Anti-O7, 8	O7 or O8	O6, 9, 11, 17	
Polyvalent III			
Anti-O3, 9 ^a	O3 and O9	O7, 8, 11, 13, 14, 17	O4 + B1846(O11)
Anti-O6, 13 ^a	O6 and O13	O3, 4, 9, 10, 14, 17	
Anti-O10, 17 ^a	O10 and O17	O3, 6, 9, 13, 14	
Anti-O1	O1	O3, 6, 9, 17	O9
Anti-O2	O2	O5, 9, 16, 17	O9 + O16
Anti-O3	O3	O4, 6, 9, 10, 13, 14, 17	O9 or O13 or O6 + O9
Anti-O4	O4	O3, 6, 9, 11, 13, 14, 15, 17	O9 + O11 + O13
Anti-O5	O5	O2, 16	O16
Anti-O6	O6	O3, 9, 10, 13, 14, 17	O10 + O13
Anti-O7, 8	O7 or O8	O6, 9, 11, 17	B1380(O11)
Anti-O9	O9	O3, 4, 6, 13, 14, 17	O13 + B1846(O11)
Anti-O10	O10	O3, 6, 9, 13, 14, 17	O9
Anti-O11	O11	O3, 4, 17 ^b	None
Anti-O12	O12	O3, 4 ^b	None
Anti-O13, 14	O13 or O14	O3, 4, 6, 9, 10, 17	O9
Anti-O15	O15	O14, 17 ^b	None
Anti-O16	O16	O2, 5	None
Anti-O17	O17	O9	O9

^a Rabbits were immunized with a mixture of cultures.^b Cross-reactions were low-grade, and no absorption was necessary.

been most successful for absorption are also listed.

Classification of isolated strains. Strains

isolated from patients in 1964-65 were examined by the slide test. Our results (Table 8) resemble those of C. O. Brokopp, R. Gomes-Lus, and J. J.

Farmer III (Abstr. Annu. Meet. Am. Soc. Microbiol. 1977, C105, p. 53), who typed recent isolates by using the schema of the expert panel.

DISCUSSION

Two major principles helped to shape the proposed international schema. First, Habs' schema (4) has priority over any other system currently available. This is one reason why it has been adopted by the expert panel. Second, the panel decided to define the somatic antigens, not serotypes or serogroups. They did anticipate the occurrence of strains with multiple antigens, but such strains can be easily identified by antigenic formulas (P. V. Liu, personal communication). There is no need for creation of a new type or group or for changes in designation, such as Homma's recently proposed system with an alphabetic designation (5).

Our findings confirm that no major change is necessary in the panel's schema at this time. What is needed is experience in applying the schema to actual serotyping practice.

Since Habs (4) reported that heating organisms at 120°C for 2 h increases agglutinability, autoclaved organisms have been utilized for the tube test in many laboratories, but this time-consuming procedure has been an obstacle to wide acceptance of serotyping. Moreover, our results (Table 2), as well as those reported by Lányi (8), clearly indicate that autoclaving uncovers an antigen which is common to all *P. aeruginosa* strains and is in a cryptic state in living organisms. When autoclaved organisms are used in the agglutination test, the agglutinin

to the common antigen must be absorbed from antisera. This procedure is not necessary when live organisms are used. Since using nonautoclaved organisms in the tube test is more dangerous and far more time-consuming than the slide test with live organisms, we recommend the slide test for routine laboratory use.

Besides the common antigen, there are other cross-reacting antigens which are distributed among a few heterologous strains. Antigens of this type participating in the tube test may not always participate in the slide test, and vice versa. It is imperative that any set of antisera for serotyping be checked as to whether it can be used for the slide test, for the tube test, or for both.

To prepare specific O antisera, it was sometimes necessary to absorb the antisera with cross-reacting isolates. At the same time, properly absorbed O factor sera can reveal the existence of multiple antigens in a single strain. Antigens O2, O5, and O16 were found to occur in four combinations. Homma (5) and Terada et al. (14) proposed a single group for all strains associated with these related antigens, but the use of antigenic formulas seems more logical. Since these antigens are among those most frequently encountered in many countries (2, 4, 8, 12, 15-17), a finer fingerprinting may help the epidemiological survey.

Antigens O13 and O14 are closely related, as are O7 and O8. We suggest that these antigens be expressed collectively as O7,8 and O13,14, rather than creating new groups for them as proposed by Homma (5) and Terada et al. (14). It might be possible to analyze the patterns of these antigens, as we did with O2, O5, and O16, if enough isolates become available.

When carefully absorbed sera with high potencies are used for serotyping of isolates, the slide test with live organisms and the tube test with autoclaved organisms have thus far given identical results. The isolates which could not be typed by the slide test remained untyped by the tube test. The simple, reliable slide technique should be adopted as the routine procedure in clinical laboratories. Pyocine typing or phage typing is valuable as a supplementary procedure when a finer fingerprinting of strains is needed.

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TABLE 8. Classification of 98 strains of *P. aeruginosa* isolated from patients in 1964-65 according to the proposed international schema

Antigenic formula	No. of strains	%
O1	13	13.3
O2,16	1	1.0
O2,5,16	3	3.1
O3	5	5.1
O4	15	15.3
O5,16	6	6.1
O6	24	24.5
O7,8	0	0
O9	3	3.1
O10	1	1.0
O11	17	17.3
O12	0	0
O13,14	0	0
O15	0	0
O16	2	2.0
O17	0	0
Nontypable	8	8.2

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