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Serovar Determination and Molecular Taxonomic Correlation in *Mycobacterium avium*, *Mycobacterium intracellulare*, and *Mycobacterium scrofulaceum*: a Cooperative Study of the International Working Group on Mycobacterial Taxonomy

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A cooperative study was conducted by the International Working Group on Mycobacterial Taxonomy to correlate the agglutination serovar designations of *Mycobacterium avium*, *Mycobacterium intracellulare*, and *Mycobacterium scrofulaceum* strains with the species ascriptions of these organisms according to molecular criteria and cultural properties and to assess the reproducibility of serovar determinations for a set of 63 reference strains of these species. Among the molecular criteria, the level of agreement between results obtained with nucleic acid probes and T-catalase serology results was 94% for strains of *M. avium* and *M. intracellulare*. Nucleic acid probes were not available for *M. scrofulaceum*, but none of the 10 strains ascribed to this species on the basis of catalase serology data reacted with a nucleic acid probe for *M. avium* or *M. intracellulare*. Ascription to a species on the basis of mycolic acid high-performance liquid chromatography patterns was in agreement with catalase serology results in 86% of the cases examined. Most strains belonging to serovars 1 through 6 and 8 through 11 were identified by molecular criteria as *M. avium*, most strains belonging to serovars 7, 12 through 20, 23, and 25 were identified as *M. intracellulare*, and most strains belonging to serovars 41 through 43 were identified as *M. scrofulaceum*, in agreement with common current practice. Evidence for assigning serovar 27 to *M. scrofulaceum* was obtained. However, two strains of a given serovar may, on occasion, be placed in different species. The dominant species assignments for strains belonging to serovars 21, 24, 26, and 28 remain unresolved. Data from laboratories which used panels of sera that corresponded to all of the serovars represented in the study were in agreement with the consensus results in 84% of the instances. Laboratories in which a limited panel of sera was used were far more likely to report results that did not agree with the consensus results.

The dominant focus of most of the previous cooperative studies of the International Working Group on Mycobacterial Taxonomy (IWGMT) has been on the numerical taxonomy of the genus *Mycobacterium* (8, 10, 12, 14, 18, 21, 24–26), although some studies have been directed toward evaluation of the actual laboratory methods employed in mycobacterial systematics (22, 23). Systematic methods can fall into two categories. One category is based on physiologic or biochemical responses to a number of generally available chemical agents, and the tests can be performed independently in any laboratory. The other category is based on interaction with reference materials derived directly or indirectly from standard organisms; this type of method is specific to the group under study and requires the availability of reference materials, such as antibodies or nucleic acid probes. The previously published methodologic reports of the IWGMT addressed the first of these categories and included information about pigmentation, biochemical reactions, and resistance to inhibitors (22, 23). The second category of tests includes seroagglutination, which provides important infrasubspecific information on strains of such species as *Mycobacterium avium*, *Mycobacterium intracellulare*, and *Mycobacterium scrofulaceum*. The members of these three species produce similar polar glycopeptidolipid surface antigens that are responsible for very specific seroagglutination of whole cells (2). Standardized antisera are presently not generally available for agglutination typing of these organisms; those wishing to perform the tests must select individual strains that are representative of more than 30 serovars and use them to immunize rabbits. This can lead to interlaboratory discrepancies in the characterization of strains. The study reported here was designed to help evaluate the strains of *M. avium*, *M. intracellulare*, and *M. scrofulaceum* that serve as reference standards for each of the known agglutinating serovars and the comparability of results from laboratories in which different sera or minor modifications of techniques were used. Another purpose was to further test the species distributions of the infrasubspecific serovars with molecular reference standards, such as nucleic acid and antibody probes and high-performance liquid chromatography (HPLC) patterns of mycolic acids.

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MATERIALS AND METHODS

Strains. Serovars 1 through 28, usually considered to belong in either *M. avium* or *M. intracellulare*, and serovars 41 through 43, usually considered to belong in *M. scrofulaceum*, were represented in this study. A total of 63 strains from the reference collection of the National Jewish Center for Immunology and Respiratory Disease, Denver, Colo., were coded and distributed as unknown strains to all participants. As in all IWGMT studies, the code was not broken until the test results had been returned to the coordinator, and no new results were added to the data base once the code had been broken.

Tests. Workers in seven of the laboratories performed seroagglutination tests by using methods pioneered by Schaefer (16), with minor individual modifications as noted below. Unless otherwise noted, each laboratory produced its own rabbit antisera to strains selected as representative for each of the serovars. Enzyme-linked immunosorbent assays (ELISA) and chromatographic analyses were also performed in one laboratory to detect the individual glycopeptidolipid antigens responsible for specific seroagglutination. In addition to the testing of cells for serovar identification, extracts of the strains were subjected to DNA-RNA probe (Gen-Probe, Inc., San Diego, Calif.) (15) and catalase antibody probe (20) analyses, and their HPLC patterns were determined (4), to help assign the strains to their correct species.

(i) **Laboratory 1.** In laboratory 1 the strains were typed by the tube agglutination method described by Good and Beam (7). Sera against serovars 1 through 28 and 41 through 43 were used. Unless otherwise noted, agglutination reactions were confirmed by cross-absorption tests.

(ii) **Laboratory 2.** In laboratory 2 only sera against serovars 1 through 11 were used. The sera were not cross-absorbed, and the method of Schaefer was used (16).

(iii) **Laboratory 3.** In laboratory 3 strains were tested with sera against serovars 1 through 28 and 41 through 43 by using the tube agglutination method of Schaefer (16). The serum designated Cole by workers in this laboratory corresponds to the serum of previously proposed *M. scrofulaceum* serovar 44 (9), which was later recognized to be synonymous with serovar 27 (30), and the designation serovar 27 is used in this report. Serovars 41, 42, and 43 were reported as serovars scrofulaceum, Lunning, and Gause, respectively, according to the convention that preceded the current numerical coding of serovars (30) and were translated to current code designations by the coordinator.

(iv) **Laboratory 4.** In laboratory 4 a slide agglutination technique was used; only sera against serovars 1 through 20 were used.

(v) **Laboratory 5.** In laboratory 5 strains were tested against serovars 1 through 28 and 41 through 43 by using a microtube agglutination test (13).

(vi) **Laboratory 6.** In laboratory 6 only sera against serovars 1, 2, 4 through 11, 13 through 23, 25 through 27, and 41 were used, and agglutination tests were performed by using the microtube modification (13). The sera were not produced in this laboratory, but were obtained from the laboratory of the National Jewish Center for Immunology and Respiratory Disease. Serovar 41 was reported as serovar scrofulaceum, according to the convention that preceded the current numerical coding of serovars (30).

(vii) **Laboratory 7.** Workers in laboratory 7 assumed the responsibility for assembling, coding, and distributing the 63 strains to be tested. The composite results of seroagglutination tests, an ELISA performed with murine monoclonal and rabbit polyclonal antibodies, thin-layer chromatography tests, and, in some cases, gas chromatography tests (5, 17) were used by workers in this laboratory to confirm the concordance of the data obtained with the original strain serovar designations.

(viii) **Laboratory 8.** In laboratory 8 T-catalase intrinsic enzyme dot blot serologic testing (20) was performed with sonic extracts of the test strains and cross-absorbed sera specific for four species (*M. avium*, *M. intracellulare*, *M. scrofulaceum*, and *Mycobacterium tuberculosis*), as well as a dot of nonabsorbed polyvalent antibody to confirm the presence of T-catalase in each extract.

(ix) **Laboratory 9.** In laboratory 9 sonic extracts of all strains were examined with DNA probes specific for rRNAs of *M. avium* and *M. intracellulare* (Gen-Probe).

(x) **Laboratory 10.** In laboratory 10 extracts of all strains were examined by HPLC, and selected mycolic acid peaks were used to establish the identities of strains (4).

(xi) **Laboratory 11.** In laboratory 11 the cultures were examined for conventional phenotypic cultural properties, including pigment, Tween hydrolysis, catalase, growth temperature, nitrate reduction, and 3-day aryl sulfatase activity (22, 23).

RESULTS

The codes, original designations, and nominal serovars of all of the reference strains distributed for this study are listed in Table 1. Strain 6 failed to grow and was excluded from consideration.

The results of all tests except the tests for the cultural properties (i.e., pigmentation and physiologic and biochemical activities) are shown by strain and by laboratory in Table 2. The sequence in which the strains are listed is based on the consensus serovar results but distributed among each of the species categories on the basis of the nucleic acid, catalase antibody, and HPLC data. The confirmatory result obtained by the distributing laboratory, laboratory 7, is shown for each strain, along with the seroagglutination results from laboratories 1, 3, and 5 and the consensus serovar results. The consensus results represent agreement of the reports from two or more of the four laboratories that used a panel of sera that included all of the serovars in the study (laboratories 1, 3, 5, and 7). The results from laboratories 2, 4, and 6, which were based on an incomplete panel of sera, are also shown.

The cultural properties were in general agreement with the species assignments based on molecular analyses. With few exceptions, *M. avium* and *M. intracellulare* strains grew into buff to pale yellow colonies and generated less than 45 mm of foam in the semiquantitative catalase test; the strains identified as *M. scrofulaceum* were scotochromogenic, exhibiting a bright yellow to orange pigment, and generated 45 mm or more of foam in the catalase test. Of special interest is the observation that 15 (75%) of the *M. avium* strains grew at 45°C, whereas none of the strains identified as *M. intracellulare* or *M. scrofulaceum* were able to grow at that temperature. None of the strains in the study gave significant reactions in the nitrate reduction test. Data for strains which gave discrepant results in any of the tests or with which species crossover of serovars was seen are presented in Table 3.

As shown in Table 2, the Gen-Probe and T-catalase serologic data were in complete accord for the first 20 strains identified as *M. avium* and corresponded to serovars 1 through 6, 8 through 11, and 21; five of these 20 strains were

TABLE 1. Source designations and nominal serovars of reference strains coded and distributed for this study

Strain	Source designation	Nominal serovar	Strain	Source designation	Nominal serovar
1	11907-300	1	33	P-49	7
2	Melnick	18	34	Wood Duck	12
3	P-39	14	35	Harrison	27
4	EW 10407	42	36	72-888	25
5	157 Manten	7	37	CDC 1217	23
7	1195 CDC	25	38	128 Germany	3
8	W 552	19	39	McKenzie 13723	26
9	14816-1424	11	40	ATCC 15987	16
10	23393	23	41	TMC 1419	20
11	2993	21	42	10409	22
12	TMC 1461	10	43	9055 Matthews	28
13	2729 Cardiff	41	44	Brooks	43
14	Darden	19	45	TMC 1473	15
15	4443-1237	5	46	M-150	43
16	34540	6	47	Edgar Boone	14
17	B-92	1	48	Yandle	16
18	6450-204	9	49	1602-1965	10
19	14141-1395	2	50	Hillberry 1244-9	26
20	4990 O'Connor	18	51	12645	24
21	ATCC 23435	8	52	P-54	17
22	Bridge	41	53	Simpson	15
23	25546-759	5	54	TMC 1463	4
24	6845	28	55	6194	2
25	AT 545 Findley	20	56	Leonard-158	7
26	6195	3	57	Anderson	43
27	Lane 3081	27	58	5154 O'Connor	22
28	1784-286	9	59	P-42	12
29	SJB #2	8	60	16741 Cardiff	2
30	TMC 1466	13	61	Lunning CDC 1198	42
31	TMC 1462	11	62	13528-1079	4
32	ATCC 25122	13	63	TMC 1406	16

identified as *M. intracellulare* on the basis of the HPLC patterns, but the interpretation of HPLC curves at the time that this study was done was based on restricted peak comparisons (4). Recent computer-based programs for pattern recognition have led to more accurate identification by HPLC (3). Typical phenotypic culture patterns were observed for the *M. avium* strains, with a few exceptions; three strains gave catalase reactions which generated more than 45 mm of foam (Table 3), and one strain was reported to give a positive reaction in the Tween hydrolysis test.

Only three discrepancies were observed between the Gen-Probe nucleic acid and the T-catalase serologic results among the next 28 strains in Table 2; these 28 strains were identified as *M. intracellulare* and included all available members of serovars 12 through 20, 23, and 25, as well as one or more members of serovars 7, 26, and 43. Of special interest is the group of three strains belonging to serovar 23, which reacted like *M. intracellulare* with the T-catalase probe but failed to react or reacted like *M. avium* with the DNA probe. This group is discussed further below along with the molecular nonconsensus strains listed in Table 2. A total of 26 of the strains in the *M. intracellulare* group were identified as members of that species by HPLC; strains 7 and 36, both serovar 25 strains, were reported to be a mixed culture or to have the *M. scrofulaceum* HPLC pattern, respectively. Three of the strains in the *M. intracellulare* group differed in one or more cultural properties from the typical pattern for this species; one strain produced orange pigment but had low catalase activity, one generated more than 45 mm of foam in the semiquantitative catalase test but was not pigmented, and the third produced a typical *M. scrofulaceum* phenotypic pattern of scotochromogenicity and high catalase activity (Table 3).

Ten strains were recognized as *M. scrofulaceum* on the basis of both T-catalase serologic test and HPLC patterns. There is no Gen-Probe kit for this species; thus, all 10 of these strains were correctly recorded as not identifiable with the available DNA probes. Six of the seven strains belonging to traditional (30) scrofulaceum serovars 41 through 43 were identified as *M. scrofulaceum*; one serovar 43 strain (strain 57) was identified by molecular methods and on the basis of cultural phenotypic properties as *M. intracellulare* (Table 2). One serovar 7 strain (strain 33) was identified by molecular and cultural methods as *M. scrofulaceum*, in contrast to two other strains of this serovar that were identified as *M. intracellulare*, as expected.

As noted previously, Goslee and colleagues (9) proposed a fourth serovar in the *M. scrofulaceum* group and designated it serovar 44. Wolinsky later recognized this serovar to be synonymous with "the recently established serovar 27 of the *M. avium* complex," citing a communication from D. Dawson, and withdrew the proposal of serovar 44 (29). We found no published phenotypic description of the original strains attributed to serovar 27, but one of us (D.D.) advises that the original representatives of both serovars 26 and 27 exhibited phenotypic properties characteristic of *M. scrofulaceum* and that neither was ever designated a member of the *M. avium* complex. Despite this, Schaefer assigned series 20 numerical designations, which by convention are reserved for the *M. avium* complex, to these two serovars instead of numbers in series 40, which are used for serovars of *M. scrofulaceum* (7). Both of the strains recognized as serovar 27 strains and

TABLE 2. Species as determined by molecular methods and serovars as determined by agglutination of 62 coded strains of *M. avium*, *M. intracellulare*, *M. scrofulaceum*, and related mycobacteria

Strain	Species identification as determined by molecular methods				Serovar(s) as determined by agglutination							
	Catalase	Gen-Probe	HPLC	Consensus	Laboratory 7	Laboratory 1	Laboratory 3	Laboratory 5	Consensus[a]	Laboratory 2	Laboratory 4	Laboratory 6
1	*M. avium*	*M. avium*	*M. avium*	*M. avium*	1	SA[b]	NRX[c]	1?, SA	NC[d]	1(2, 3)[e]	1	1
17	*M. avium*	*M. avium*	*M. intracellulare*	*M. avium*	1	1[f]	17	SA	1	1	3	SA
19	*M. avium*	*M. avium*	*M. avium*	*M. avium*	2	2	2	2?	2	2	3	SA
55	*M. avium*	*M. avium*	*M. intracellulare*	*M. avium*	2	1[f]	24, 25	2	2	SA	SA	8
60	*M. avium*	*M. avium*	*M. avium*	*M. avium*	2	2	NRX	2?	2	NRX	3	SA
26	*M. avium*	*M. avium*	*M. intracellulare*	*M. avium*	NT[g]	3	3, 2	3	3	2	3	NT
38	*M. avium*	*M. avium*	*M. avium*	*M. avium*	3, 23, 25	3	SA	3?	3	7	SA	NRX
54	*M. avium*	*M. avium*	*M. avium*	*M. avium*	4	4	4	4	4	NRX	4	19
62	*M. avium*	*M. avium*	*M. avium*	*M. avium*	4	4	4	4	4	SA	4	NRX
15	*M. avium*	*M. avium*	*M. intracellulare*	*M. avium*	5	5	5	NRX	5	NRX	5	4
23	*M. avium*	*M. avium*	*M. avium*	*M. avium*	5	5	5	NRX	5	NRX	5	SA
16	*M. avium*	*M. avium*	*M. avium*	*M. avium*	6, 8	SA	6	SA	6	8(2, 3, 6)	6	6
29	*M. avium*	*M. avium*	*M. avium*	*M. avium*	8	8	8	8	8	8	8	NRX
18	*M. avium*	*M. avium*	*M. intracellulare*	*M. avium*	9	9	9	9	9	10	9	NRX
28	*M. avium*	*M. avium*	*M. avium*	*M. avium*	9	9	9	9?	9	SA	9	SA
12	*M. avium*	*M. avium*	*M. avium*	*M. avium*	10	10	10	10	10	8, 1	10	NRX
49	*M. avium*	*M. avium*	*M. avium*	*M. avium*	10	10	10	10	10	NRX	4	NRX
9	*M. avium*	*M. avium*	*M. avium*	*M. avium*	11	11, 10	11, 10	11	11	NRX	11	SA
31	*M. avium*	*M. avium*	*M. avium*	*M. avium*	11	SA	11, 10	11	11	NRX	11	NT
11	*M. avium*	*M. avium*	*M. avium*	*M. avium*	21	21	21, 8	21, 8	21	8, 1	NRX	4
5	*M. intracellulare*	*M. intracellulare*	*M. intracellulare*	*M. intracellulare*	7	7, 13[f]	7	7	7	NRX	7	7
56	*M. intracellulare*	*M. intracellulare*	*M. intracellulare*	*M. intracellulare*	7	7	7, 13	7	7	7	7	NRX
24	*M. intracellulare*	*M. intracellulare*	*M. intracellulare*	*M. intracellulare*	12	12, 28	12	12	12	1, 2	12	NRX
34	*M. intracellulare*	*M. intracellulare*	*M. intracellulare*	*M. intracellulare*	12	12	7, 13	12	12	NRX	12	NRX
59	*M. intracellulare*	*M. intracellulare*	*M. intracellulare*	*M. intracellulare*	12	12, 28	12	12	12	(7)	12, 13	NRX
30	*M. intracellulare*	*M. intracellulare*	*M. intracellulare*	*M. intracellulare*	13	13	13	13	13	7	13	SA
32	*M. intracellulare*	*M. intracellulare*	*M. intracellulare*	*M. intracellulare*	13	13	13	13	13	7	12	13
3	*M. intracellulare*	*M. intracellulare*	*M. intracellulare*	*M. intracellulare*	14	SA	14	14	14	11	14	NRX
47	*M. intracellulare*	*M. intracellulare*	*M. intracellulare*	*M. intracellulare*	14	14	NRX	14	14	11	14	14
21	*M. intracellulare*	*M. intracellulare*	*M. intracellulare*	*M. intracellulare*	15	15, 42	19	15	15	NRX	15	NRX
45	*M. intracellulare*	*M. intracellulare*	*M. intracellulare*	*M. intracellulare*	15	15, 42	NRX	15	15	SA	15	9
53	*M. intracellulare*	*M. intracellulare*	*M. intracellulare*	*M. intracellulare*	15	15, 42	NRX	15	15	SA	15	NRX
40	*M. intracellulare*	*M. intracellulare*	*M. intracellulare*	*M. intracellulare*	16	16	16	16	16	NRX	16	NRX
48	*M. intracellulare*	*M. intracellulare*	*M. intracellulare*	*M. intracellulare*	16	16	16	16	16	NRX	16	NRX
63	*M. intracellulare*	*M. intracellulare*	*M. intracellulare*	*M. intracellulare*	16	16	16	16	16	6, 7, 8, 11	16	NRX
52	*M. intracellulare*	*M. intracellulare*	*M. intracellulare*	*M. intracellulare*	17	17	17	17	17	(7)	17	16
20	*M. intracellulare*	*M. intracellulare*	*M. intracellulare*	*M. intracellulare*	9	18	18	18	18	NRX	18	2
8	*M. intracellulare*	*M. intracellulare*	*M. intracellulare*	*M. intracellulare*	19	19	19	19?	19	NRX	NRX	NRX
14	*M. intracellulare*	*M. intracellulare*	*M. intracellulare*	*M. intracellulare*	19	19	19	19	19	1, 2(11)	1	19

Continued on following page

TABLE 2—*Continued*

Strain	Species identification as determined by molecular methods				Serovar(s) as determined by agglutination							
	Catalase	Gen-Probe	HPLC	Consensus	Laboratory 7	Laboratory 1	Laboratory 3	Laboratory 5	Consensus[a]	Laboratory 2	Laboratory 4	Laboratory 6
41	*M. intracellulare*	*M. intracellulare*	*M. intracellulare*	*M. intracellulare*	20	1[f]	20	NT	20	NT	SA	SA
25	*M. intracellulare*	*M. intracellulare*	*M. intracellulare*	*M. intracellulare*	21	20	14	NRX	NC	11(5)	SA	5
10	*M. intracellulare*	*M. avium*	*M. intracellulare*	*M. intracellulare*	23	23	9	23	23	8, 1	7	23
37	*M. intracellulare*	NRX	*M. intracellulare*	*M. intracellulare*	23	23	23	23	23	7	7	23
51	*M. intracellulare*	NRX	*M. intracellulare*	*M. intracellulare*	23	23, 24	23	23	23	7	7	23
7	*M. intracellulare*	*M. intracellulare*	Mixed culture?	*M. intracellulare*	25	25	SA	25	25	1, 2	15	SA
36	*M. intracellulare*	*M. intracellulare*	*M. scrofulaceum*	*M. intracellulare*	25	25	NRX	25	25	7(8)	SA	SA
50	*M. intracellulare*	*M. intracellulare*	*M. intracellulare*	*M. intracellulare*	26	26	26	26	26	NRX	SA	NRX
57	*M. intracellulare*	*M. intracellulare*	*M. intracellulare*	*M. intracellulare*	43	43	NRX	43	43	1	NRX	NRX
33	*M. scrofulaceum*	NRX	*M. scrofulaceum*	*M. scrofulaceum*	7	7, 1, 12, 13	12	7	7	7	7	7
39	*M. scrofulaceum*	NRX	*M. scrofulaceum*	*M. scrofulaceum*	26	26	26	26	26	NRX	NRX	26
27	*M. scrofulaceum*	NRX	*M. scrofulaceum*	*M. scrofulaceum*	27	NRX	27	27	27	NRX	NRX	NRX
35	*M. scrofulaceum*	NRX	*M. scrofulaceum*	*M. scrofulaceum*	27	27[f]	27	27	27	NRX	NRX	27
13	*M. scrofulaceum*	NRX	*M. scrofulaceum*	*M. scrofulaceum*	41	41	20, 24, 25	41	41	NRX	20	41
22	*M. scrofulaceum*	NRX	*M. scrofulaceum*	*M. scrofulaceum*	41	41	8	41	41	NRX	20	41
4	*M. scrofulaceum*	NRX	*M. scrofulaceum*	*M. scrofulaceum*	42	42, 1[f]	42	42	42	NRX	NRX	SA
61	*M. scrofulaceum*	NRX	*M. intracellulare*	*M. scrofulaceum*	42	1[f]	NRX	42	42	NRX	SA	NRX
44	*M. scrofulaceum*	NRX	*M. scrofulaceum*	*M. scrofulaceum*	43	43	43	43	43	NRX	NRX	8
46	*M. scrofulaceum*	NRX	*M. scrofulaceum*	*M. scrofulaceum*	43	43	43	43	43	NRX	NRX	8
2	NT	NRX	*M. intracellulare*	NC	18	18	18	18	18	NT	18	SA
42	*M. scrofulaceum*	NRX	*M. avium*	NC	22	22	22	22	22	7	7	13
58	*M. intracellulare*	NRX	?	NC	22	22	22	22	22	NRX	7	22
43	*M. intracellulare*	NRX	*M. scrofulaceum*	NC	28	28	28, 24	28	23	NT	NRX	2

[a] Consensus serovar results are based on the data from laboratories 1, 3, 5, and 7.
[b] SA, spontaneous agglutination.
[c] NRX, no reaction with any of the reference antisera or probes tested.
[d] NC, no consensus.
[e] Parentheses indicate weak reactions (≤2+).
[f] Not confirmed by cross-absorption.
[g] NT, test not performed.

one of the two strains recognized as serovar 26 strains in this study were characterized as *M. scrofulaceum*. It is also of interest that Saito and colleagues previously examined the two source strains of serovars 26 and 27 with cross-absorbed antibody to their alpha antigens and reported that all four strains react as *M. scrofulaceum* strains (15).

For four strains (strains 2, 42, 43, and 58), no consensus on ascription to a species on the basis of molecular data could be reached. In addition, two strains (strains 37 and 51) were assigned to *M. intracellulare* on the basis of T-catalase serology and HPLC results, but they could not be identified by the Gen-Probe kits. Similar problems were encountered in ascribing members of an *M. avium* complex-like cluster of strains (cluster 4) to a species in a recent IWGMT numerical taxonomic report (27).

When the observed serovar assignments were compared

TABLE 3. Characteristics of strains whose identification is ambiguous

Strain	Species as determined by molecular methods			Consensus serovar	Pigment	Catalase reaction (mm of foam)	Growth at 45°C[a]	Tween hydrolysis
	Catalase	Gen-Probe	HPLC					
55	*M. avium*	*M. avium*	*M. intracellulare*	2	None	<30	0	+
54	*M. avium*	*M. avium*	*M. avium*	4	None	>45	1	−
16	*M. avium*	*M. avium*	*M. avium*	6	Pale yellow	>45	1	−
5	*M. intracellulare*	*M. intracellulare*	*M. intracellulare*	7	Orange	34	0	−
33	*M. scrofulaceum*	NRX[b]	*M. scrofulaceum*	7	Yellow	>45	0	−
29	*M. avium*	*M. avium*	*M. avium*	8	None	>45	1	−
3	*M. intracellulare*	*M. intracellulare*	*M. intracellulare*	14	Orange	>45	0	−
2	NT[c]	NRX	*M. intracellulare*	18	None	<30	0	−
8	*M. intracellulare*	*M. intracellulare*	*M. intracellulare*	19	None	>45	0	−
42	*M. scrofulaceum*	NRX	*M. avium*	22	Orange	40	0	−
58	*M. intracellulare*	NRX	?	22	None	?	?	?
37	*M. intracellulare*	NRX	*M. intracellulare*	23	None	<30	0	−
51	*M. intracellulare*	NRX	*M. intracellulare*	23	None	>45	0	−
39	*M. scrofulaceum*	NRX	*M. scrofulaceum*	26	Orange	>45	0	−
43	*M. intracellulare*	NRX	*M. scrofulaceum*	28	None	>45	0	−

[a] 0, no growth; 1, growth; ?, questionable growth.
[b] NRX, no reaction with any of the probes tested.
[c] NT, test not performed.

with the nominal serovar assignments of the original 62 strains tested, discrepancies were seen with strains 21, 24, 25, and 51. In three of these cases, the results of the confirmatory analysis in laboratory 7 were in agreement with the consensus results of laboratories 1, 3, and 5, suggesting that there was a labelling error in the reference stock collection; no consensus emerged from the results obtained with strain 25. Workers in laboratory 7, who used a combination of seroagglutination, ELISA, and chromatography, had only one disagreement with the consensus serovar results for the 61 results which they submitted, for an agreement rate of 98.5%. For laboratory 1, the only laboratory that routinely confirmed agglutination results with absorption tests, the rate of agreement with the consensus results was 88.7%; four of the seven failures were associated with spontaneous agglutination of the cell suspensions, which interfered with the reading of results. For laboratory 3 the rate of agreement with the consensus results was 71%; only one of the disagreements was due to spontaneous agglutination, and the remainder were equally divided between lack of agglutination with any of the test sera and agglutination with a serum that did not conform to the consensus serovar. For laboratory 5 the rate of agreement with consensus results was 93.4%; two disagreements were associated with spontaneous agglutination, and the other two were associated with failure to agglutinate with any of the sera.

Laboratories 2, 4, and 6 each lacked antisera to a number of the serovars represented in this study and would have been expected to report many strains as not typeable in their systems. In addition to reporting a large number of strains as unidentifiable because of missing sera, these laboratories also tended to ascribe a disproportionate number of these strains to the wrong serovars, presumably because of unrecognized cross-reactions.

DISCUSSION

The data in Table 2 support the redistribution of serovars among the species *M. intracellulare* and *M. avium* that was suggested previously by the results of independent DNA hybridization tests (1), Gen-Probe studies (15), and T-catalase serologic studies (19, 20). They also illustrate the caution that must be exercised in assuming that a member of a particular serovar automatically must belong to a certain species. In this study in at least four instances (serovars 7, 18, 26, and 43 and possibly serovar 22), two or more strains belonging to a particular serovar were placed in two different species according to the semantide-based species assignment criteria used. However, despite these few exceptions, the common ascription of serovars 1 to 6 and 8 to 11 to *M. avium* and serovars 7 and 12 to 20 to *M. intracellulare* is supported by the data presented here. Furthermore, these data provide a basis for ascribing members of serovar 21 to *M. avium* and members of serovars 26 and 27 to *M. scrofulaceum*; the last two ascriptions are supported by similar conclusions of Saito and his colleagues, which were based on an examination of these strains with antibody specific for the alpha antigen of *M. scrofulaceum* (15).

The strains of serovars 22 and 23 that appeared to fall into the *M. avium* complex on the basis of molecular and cultural criteria, but failed to react with the DNA probe for either *M. avium* or *M. intracellulare*, appear to correspond to organisms belonging to cluster 4 as described in a recent open-ended study report of the IWGMT (28). Similar strains have been reported to react with the SNAP X probe (Syngene, Inc., San Diego, Calif.) (11). The T-catalase serologic results, which reflect mRNAs, and the results of recent rRNA analyses performed by Ferguson and colleagues (6) suggest that these organisms may be closely related to *M. intracellulare*. These and similar strains are under more intensive investigation in a current semantide-based IWGMT study.

In laboratories 1, 3, and 5, which relied on agglutination of the coded strains with a full panel of sera, the mean level of agreement with the consensus serovar results was 84%; 7 of the 30 discrepancies were associated with spontaneous agglutination. The use of an ELISA or chromatography of extracts might have permitted determination of the serovars for those strains (5, 17). The highest proportion of reactions of individual strains with more than one serum occurred in laboratory 1, and the specificity of those reactions was supported by the results of absorption tests.

The high proportion of strains that were ascribed to nonconsensus serovars in laboratories 2, 4, and 6, which did not use a full panel of sera for all of the serovars represented in this study, may have been associated with the use of antiserum concentrations that were too high, allowing cross-reactions to occur. Sera that are prepared by inoculation of rabbits with whole cells of serovar reference strains contain some antibody to the common mycobacterial antigens that lie at the bacterial surface, underneath the loose coat of the glycopeptidolipid antigen responsible for serovar-specific agglutination (29). These results illustrate the value of using preabsorbed sera and confirming agglutination results by absorption with the test strain, especially when less than a full panel of sera is used.

The results of this study validate the utility of most of the existing reference serovar strains but identify possible problems with strains 21 (= ATCC 23435), 24 (= 6845), 25 (= AT 545 Findley), and 51 (= 12645).

REFERENCES

1. **Baess, I.** 1983. Deoxyribonucleic acid relationships between different serovars of *Mycobacterium avium*, *Mycobacterium intracellulare* and *Mycobacterium scrofulaceum*. Acta Pathol. Microbiol. Immunol. Scand. **91:**201–203.
2. **Brennan, P. J., H. Mayer, G. O. Aspinall, and J. E. Nam Shin.** 1981. Structures of the glycopeptidolipid antigens from serovars in the *Mycobacterium avium/Mycobacterium intracellulare/Mycobacterium scrofulaceum* serocomplex. Eur. J. Biochem. **115:** 7–15.
3. **Butler, W. R., K. C. Jost, Jr., and J. O. Kilburn.** 1991. Identification of mycobacteria by high-performance liquid chromatography. J. Clin. Microbiol. **29:**2468–2472.
4. **Butler, W. R., and J. O. Kilburn.** 1988. Identification of major slowly growing pathogenic mycobacteria and *Mycobacterium gordonae* by high-performance liquid chromatography of their mycolic acids. J. Clin. Microbiol. **26:**50–53.
5. **Denner, J. C., A. Y. Tsang, D. Chatterjee, and P. J. Brennan.** 1992. Comprehensive approach to identification of serovars of *Mycobacterium avium* complex. J. Clin. Microbiol. **30:**473–478.
6. **Ferguson, C., P. Hammond, R. Lankford, D. McAllister, and V. Jonas.** 1991. Phenotypic discordance within species of the genus *Mycobacterium* revealed by application of DNA probes, abstr. 30. Progr. Abstr. 91st Annu. Meet. Am. Soc. Microbiol. 1991. American Society for Microbiology, Washington, D.C.
7. **Good, R. C., and R. E. Beam.** 1984. Seroagglutination, p. 105–122. *In* G. P. Kubica and L. G. Wayne (ed.), The mycobacteria: a sourcebook. Marcel Dekker, Inc., New York.
8. **Goodfellow, M., A. Lind, H. Mordarska, S. Pattyn, and M. Tsukamura.** 1974. A co-operative numerical analysis of cultures considered to belong to the "rhodochrous" taxon. J. Gen. Microbiol. **85:**291–302.
9. **Goslee, S., T. K. Rynearson, and E. Wolinsky.** 1976. Additional serotypes of *Mycobacterium scrofulaceum*, *Mycobacterium gordonae*, *Mycobacterium marinum*, and *Mycobacterium xenopi* determined by agglutination. Int. J. Syst. Bacteriol. **26:** 136–142.
10. **Kubica, G. P., I. Baess, R. E. Gordon, P. A. Jenkins, J. B. G. Kwapinski, C. McDurmont, S. R. Pattyn, H. Saito, V. A. Silcox, J. L. Stanford, K. Takeya, and M. Tsukamura.** 1972. A cooperative numerical analysis of rapidly growing mycobacteria. J. Gen. Microbiol. **73:**55–70.
11. **Lim, S. D., J. Todd, J. Lopez, E. Ford, and J. M. Janda.** 1991. Genotypic identification of pathogenic *Mycobacterium* species by using a nonradioactive oligonucleotide probe. J. Clin. Microbiol. **29:**1276–1278.
12. **Meissner, G., K. H. Schroder, G. E. Amadio, W. Anz, S. Chaparas, H. W. B. Engel, P. A. Jenkins, W. Kappler, H. H. Kleeberg, E. Kubala, M. Kubín, D. Lauterbach, A. Lind, M. Magnusson, Z. D. Mikova, S. R. Pattyn, W. B. Schaefer, J. L. Stanford, M. Tsukamura, L. G. Wayne, I. Willers, and E. Wolinsky.** 1974. A cooperative numerical analysis of nonscotochromogenic and nonphotochromogenic slowly growing mycobacteria. J. Gen. Microbiol. **83:**207–235.
13. **Reznikov, M., and J. H. Leggo.** 1972. Modification of Schaefer's procedure for serotyping of organisms of the *Mycobacterium avium-M. intracellulare-M. scrofulaceum* complex. Appl. Microbiol. **23:**819–823.
14. **Saito, H., R. E. Gordon, I. Juhlin, W. Käppler, J. B. G. Kwapinski, C. McDurmont, S. R. Pattyn, E. H. Runyon, J. L. Stanford, I. Tarnok, H. Tasaka, M. Tsukamura, and J. Weiszfeiler.** 1977. Cooperative numerical analysis of rapidly growing mycobacteria. Int. J. Syst. Bacteriol. **27:**75–85.
15. **Saito, H., H. Tomioka, K. Sato, H. Tasaka, and D. J. Dawson.** 1990. Identification of various serovar strains of *Mycobacterium avium* complex by using DNA probes specific for *Mycobacterium avium* and *Mycobacterium intracellulare*. J. Clin. Microbiol. **28:**1694–1697.
16. **Schaefer, W. B.** 1965. Serologic identification and classification of atypical mycobacteria by their agglutination. Am. Rev. Respir. Dis. **92**(Suppl.)**:**85–93.
17. **Tsang, A. Y., J. C. Denner, P. J. Brennan, and J. K. McClatchy.** 1992. Clinical and epidemiological importance of typing of *Mycobacterium avium* complex isolates. J. Clin. Microbiol. **30:**479–484.
18. **Wayne, L. G., L. Andrade, S. Froman, W. Kappler, E. Kubala, G. Meissner, and M. Tsukamura.** 1979. A co-operative numerical analysis of *Mycobacterium gastri*, *Mycobacterium kansasii*, and *Mycobacterium marinum*. J. Gen. Microbiol. **109:**319–327.
19. **Wayne, L. G., and G. A. Diaz.** 1986. Differentiation between T-catalases derived from *Mycobacterium avium* and *Mycobacterium intracellulare* by a solid-phase immunosorbent assay. Int. J. Syst. Bacteriol. **36:**363–367.
20. **Wayne, L. G., and G. A. Diaz.** 1987. Intrinsic catalase dot blot immunoassay for identification of *Mycobacterium tuberculosis*, *Mycobacterium avium*, and *Mycobacterium intracellulare*. J. Clin. Microbiol. **25:**1687–1690.
21. **Wayne, L. G., T. M. Dietz, C. Gernez-Rieux, P. A. Jenkins, W. Käppler, G. P. Kubica, J. B. G. Kwapinski, G. Meissner, S. R. Pattyn, E. H. Runyon, K. H. Schröder, V. A. Silcox, M. Tsukamura, and E. Wolinsky.** 1971. A cooperative numerical taxonomic analysis of scotochromogenic slowly growing mycobacteria. J. Gen. Microbiol. **66:**255–271.
22. **Wayne, L. G., H. C. Engbaek, H. W. B. Engel, S. Froman, W. Gross, J. Hawkins, W. Käppler, A. G. Karlson, H. H. Kleeberg, I. Krasnow, G. P. Kubica, C. McDurmont, E. E. Nel, S. R. Pattyn, K. H. Schröder, S. Showalter, I. Tarnok, M. Tsukamura, B. Vergmann, and E. Wolinsky.** 1974. Highly reproducible techniques for use in systematic bacteriology in the genus *Mycobacterium*: tests for pigment, urease, resistance to sodium chloride, hydrolysis of Tween 80, and β-galactosidase. Int. J. Syst. Bacteriol. **24:**412–419.
23. **Wayne, L. G., H. W. B. Engel, C. Grassi, W. Gross, J. Hawkins, P. A. Jenkins, W. Käppler, H. H. Kleeberg, I. Krasnow, E. E. Nel, S. R. Pattyn, P. A. Richards, S. Showalter, M. Slosarek, I. Szabo, I. Tarnok, M. Tsukamura, B. Vergmann, and E. Wolinsky.** 1976. Highly reproducible techniques for use in systematic bacteriology in the genus *Mycobacterium*. II. Tests for niacin and catalase and for resistance to isoniazid, thiophene 2-carboxylic acid hydrazide, hydroxylamine, and *p*-nitrobenzoate. Int. J. Syst. Bacteriol. **26:**311–318.
24. **Wayne, L. G., R. C. Good, M. I. Krichevsky, R. E. Beam, Z. Blacklock, S. D. Chaparas, D. Dawson, S. Froman, W. Gross, J. Hawkins, P. A. Jenkins, I. Juhlin, W. Käppler, H. H. Kleeberg, I. Krasnow, M. J. Lefford, E. Mankiewicz, C. McDurmont, G. Meissner, P. Morgan, E. E. Nel, S. R. Pattyn, F. Portaels, P. A. Richards, S. Rüsch, K. H. Schröder, V. A. Silcox, I. Szabo, M. Tsukamura, and B. Vergmann.** 1981. First report of the cooperative, open-ended study of slowly growing mycobacteria by the International Working Group on Mycobacterial Taxonomy. Int. J. Syst. Bacteriol. **31:**1–20.
25. **Wayne, L. G., R. C. Good, M. I. Krichevsky, R. E. Beam, Z. Blacklock, H. L. David, D. Dawson, W. Gross, J. Hawkins, P. A. Jenkins, I. Juhlin, W. Käppler, H. H. Kleeberg, I. Krasnow, M. J. Lefford, E. Mankiewicz, C. McDurmont, E. E. Nel, F.**

Portaels, P. A. Richards, S. Rüsch, K. H. Schröder, V. A. Silcox, I. Szabo, M. Tsukamura, L. Van den Breen, and B. Vergmann. 1983. Second report of the cooperative, open-ended study of slowly growing mycobacteria by the International Working Group on Mycobacterial Taxonomy. Int. J. Syst. Bacteriol. **33**:265–274.

26. **Wayne, L. G., R. C. Good, M. I. Krichevsky, Z. Blacklock, H. L. David, D. Dawson, W. Gross, J. Hawkins, P. A. Jenkins, I. Juhlin, W. Käppler, H. H. Kleeberg, V. Levy-Frebault, C. McDurmont, E. E. Nel, F. Portaels, S. Rüsch-Gerdes, K. H. Schröder, V. A. Silcox, I. Szabo, M. Tsukamura, L. Van Den Breen, B. Vergmann, and M. A. Yakrus.** 1989. Third report of the cooperative, open-ended study of slowly growing mycobacteria by the International Working Group on Mycobacterial Taxonomy. Int. J. Syst. Bacteriol. **39**:267–278.
27. **Wayne, L. G., R. C. Good, M. I. Krichevsky, Z. Blacklock, H. L. David, D. Dawson, W. Gross, J. Hawkins, V. V. Levy-Frebault, C. McManus, F. Portaels, S. Rüsch-Gerdes, K. H. Schröder, V. A. Silcox, M. Tsukamura, L. Van Den Breen, and M. A. Yakrus.** 1991. Fourth report of the cooperative open-ended study of slowly growing mycobacteria by the International Working Group on Mycobacterial Taxonomy. Int. J. Syst. Bacteriol. **41**:463–472.
28. **Wayne, L. G., D. Hollander, B. Anderson, H. A. Sramek, C. M. Vadheim, and J. I. Rotter.** 1992. Immunoglobulin A (IgA) and IgG serum antibodies to mycobacterial antigens in Crohn's disease patients and their relatives. J. Clin. Microbiol. **30**:2013–2018.
29. **Wolinsky, E.** 1979. Emendation of proposed additional serotypes of mycobacteria determined by agglutination. Int. J. Syst. Bacteriol. **29**:59.
30. **Wolinsky, E., and W. B. Schaefer.** 1973. Proposed numbering scheme for mycobacterial serotypes. Int. J. Syst. Bacteriol. **23**:182–183.