Development of an agglutination procedure for use in the identification of *Mycobacterium paratuberculosis*

by

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Signatures have been redacted for privacy

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I. INTRODUCTION

Paratuberculosis or Johne's disease is a chronic disease of the intestinal mucosa characterized by clinical symptoms of emaciation and diarrhea. The disease is most often seen in ruminants, particularly cattle and was first described by Johne and Frothingham (18) in 1895. The disease however, is also found in pigs (46) goats (23, 53) and sheep (23, 53).

The etiologic agent, *Mycobacterium paratuberculosis* was first cultivated by Twort and Ingram (60) in 1912 using a medium supplemented with dead tubercle bacilli.

Doyle (6) reported in 1959 an annual mortality of 2 to 10 percent. In a more recent report, Kopecky (20) reported that paratuberculosis in cattle was becoming more widely distributed in the United States. It was found in 33 states in 1949 and was found in 46 states in 1971.

The pathology of the disease, diagnostic procedures and laboratory isolation have been described in numerous scientific publications (34, 53, 35).

The isolation of this somewhat fastidious organism has been improved by the incorporation of mycobactin into the growth medium (34).

Serological procedures such as complement fixation and hemagglutination tests have been used in the diagnosis of

this disease with varying degrees of success. In addition, antigens have been extracted from the causative organism as a paratuberculin and have been injected both intradermally and intravenously as a test for allergy. These tests when compared with the standard tuberculin test for tuberculosis have been only partially successful (42, 52).

The primary criteria for laboratory identification of M. paratuberculosis have been mycobactin dependence, slow growth and typical colony characteristics.

Biochemical tests (37) have been used as an aid in the identification of M. paratuberculosis but do not have widespread acceptance.

An additional problem encountered in culturing M. paratuberculosis is the extended period of incubation required before colonies of the organism appear on growth media. Incubation times from 6 to 20 weeks are not uncommon for primary isolation of this organism. Often the colonies appear sparsely or mixed with contaminants thus requiring subculture and still further incubation time.

Considering the lack of knowledge on serological studies of the M. paratuberculosis organism itself coupled with the very successful use of serological identification (48, 49, 50, 51) of M. avium it seemed likely that such procedures might be applicable in identifying M. paratuberculosis. Success in serologically differentiating M. paratuberculosis

from other mycobacteria would, in some cases, speed up identification but may also have epidemiologic value in tracing infected herds. Research in evaluating a serological agglutination test is described here.

II. REVIEW OF THE LITERATURE

A. Description of the Disease

Paratuberculosis is considered to be primarily, a disease of the ruminant digestive tract and is caused by a small acid fast rod-shaped organism. According to Larsen (23) the organism is believed to multiply in the digestive tract and may be transmitted to other sites via the macrophages. Feces, containing M. paratuberculosis is thought to be the primary source of infection. Gilmour (11) found that in sheep a minimum of 10^3 organisms are necessary to initiate infection. Other sources of dissemination may be the genito-urinary tract, particularly the testes, bulbourethral gland, seminal vesicles and semen (25).

Pearson and McClellan (41) have documented uterine infections by this organism while Lawrence (29) has reported isolation of M. paratuberculosis from a fetus indicating that calves may be born with the disease.

Time elapsing between exposure and onset of clinical symptoms may vary from less than one year to several years. Some animals may eliminate the organisms completely while others may carry them through life without developing clinical symptoms of the disease.

The first clinical signs of disease are gradual weight

loss, rough coat and decreased milk production usually accompanied by diarrhea that does not respond to treatment (23). Merkal et al. (38) have speculated that antigen-antibody reactions in the intestinal tract may result in release of diarrhea-producing substances.

Signs of clinical disease may be precipitated (23) by heavy milk production, pregnancy or nutritional deficiencies. Infection usually occurs at an early age leading to a protracted disease and eventually death (53, 62).

Gross pathologic changes typical of paratuberculosis include swelling and thickening of the intestinal submucosa and formation of broad transverse ridges which give the gut wall a corrugated appearance (53).

Microscopically, lesions of paratuberculosis are characterized (23) by hyperplasia of the macrophages that are most numerous in the tunica propia of the intestine. Most bacilli are contained in macrophages and giant cells. Adjacent lymph nodes are also observed to contain macrophages in which are found acid-fast bacilli.

B. Allergy

Allergic manifestations to growth metabolites of _M. paratuberculosis_ have been found in animals infected with or exposed to _M. paratuberculosis_. Bang (1) in 1908 discovered that paratuberculous animals would demonstrate a

positive allergic reaction to tuberculin prepared from M. avium. He found that injection of avian tuberculin subcutaneously would result in a rise in body temperature 9 to 19 hours following injection.

Production of a specific paratuberculin (Johnin) from the Johne's bacillus itself was first attempted by Twort and Ingram (60) in 1912. The attempts were largely unsuccessful because of the small numbers of cells obtained in culture.

Dunkin (7) in 1929 grew the organism on the surface of a liquid medium and was the first to produce paratuberculin in quantity. The production of purified protein derivatives (PPD) of Johnin was made possible by improved culture media (62) and has been evaluated by Larsen et al. (26).

The intradermal test (52, 15) for detecting sensitivity of an animal to tuberculosis is performed by injecting 0.1 to 0.2 ml. of tuberculin intradermally and observing the site for 72 hours. A positive reaction is indicated by a local swelling with erythema with or without necrosis at the injection site.

Johnin was injected intravenously by Larsen and Kopecky (24) who observed a positive test in 80% of the cattle showing clinical signs of paratuberculosis. The test is performed by injecting 3.0 ml. of Johnin intravenously

and observing the animal's temperature curve at 0, 4, 6, and 8 hours after injection. If the temperature rises 1.5°F over normal body temperature the test is considered positive. Limitations of the test are (1) the environmental temperature should not be above 86°F and (2) the animal's temperature should be less than 102.5°F.

The efficacy of using paratuberculin or avian tuberculin diagnosing paratuberculosis has been evaluated by several investigators. Pearson and McClellan (42) examined 192 culturally negative animals and found 43% to give reactions to johnin or avian tuberculin or both. Among 38 culturally positive cattle 92% were positive to avian tuberculin or johin or both.

A problem with skin testing was described by Sigurdsson (52) who has made the observation that the disease is usually well advanced before sensitivity occurs. Larsen et al. (27) has found a large percentage of infected cattle never become reactors. Because of these limitations, diagnosis of paratuberculosis has for the most part occurred in the laboratory using cultural methods and serodiagnostic tests.

C. Serological Methods

1. Complement-fixation test

Probably the most extensively used serological test procedure used in the diagnosis of paratuberculosis has been the complement-fixation test. The procedure was first

attempted by Twort (59) in 1912. The test has been used and evaluated by a number of other investigators since that time.

Ringdal (45) claimed the complement-fixation test to be the only reliable method of diagnosis at an early stage of disease. Of 127 animals tested in which acid-fast organisms were found by microscopic examination of the rectal mucosa and/or feces, 98.4% also showed a positive complement-fixation reaction. Of 48 animals in which positive fecal cultures of M. paratuberculosis were isolated, 43 had a positive complement-fixation reaction while 5 were negative to the complement-fixation test.

Rankin (43) found that in animals experimentally infected intravenously with M. paratuberculosis a minimum of 3 months was required before complement-fixing antibodies appeared. He also found that on two farms where the disease was thought not to exist 10 to 20 per cent of the adult cattle gave positive complement-fixation reactions.

Sigurdsson (52) thought that the complement-fixation test would only be of value if a species-specific antigen could be found to counteract the false positive effects caused by contact of the animal with other mycobacteria.

2. Hemagglutination test

The second major procedure used in the diagnosis of paratuberculosis has been the hemagglutination test. Sheep

erythrocytes were found by Middlebrook and Dubos (40) to absorb specific components of mycobacterial extracts which would render them agglutinable by sera containing appropriate antibody.

The test procedure has been applied and evaluated by Larsen et al. (28) in paratuberculosis infected herds. In a herd of 368 cattle, 164 had hemagglutination titers of 1:32 or above. In this herd, only 45 were found at slaughter to harbor M. paratuberculosis of which 22 had developed signs of clinical disease. Larsen concludes in his paper that because the test procedure lacks specificity and does not correlate with actual clinical disease, it is not of major diagnostic value.

3. Fluorescent antibody procedures

Coons et al. (5) and associates in 1941 introduced the concept and procedure of labeling antibodies with a fluorescent material. Since that time the fluorescent antibody procedure has found wide application in many areas of clinical microbiology.

Jones et al. (19) at the Communicable Disease Center applied the fluorescent antibody technique to the study of mycobacteria and suggested that it was useful but their results were not conclusive.

The recent work of Martins et al. (30) and colleagues in 1973 gave better results. Antisera were made to 11 strains

of various species of mycobacteria killed with ultraviolet light. Specificity of the resulting conjugates was improved with the exception of one strain which showed cross-agglutination with all other conjugates.

Gilmour (12) using conjugated rabbit antiserum was able to distinguish between M. avium and M. paratuberculosis using fluorescent antibody procedures. However, the application of fluorescent antibody procedures for the diagnosis of mycobacterial infections have not gained wide acceptance.

4. Agglutination tests

The development of a practical direct whole cell agglutination procedure for the study of Mycobacterium avium and related strains can be attributed to Dr. W. B. Schaefer (48, 49). Schaefer's work was primarily directed to the possibility of differentiating between pathogenic and nonpathogenic strains of M. avium. Thirty-seven strains of M. avium isolated from chickens were found to be composed of 2 major serological subgroups. Type I and Type II (later designated serotypes 1 and 2). Type II was 3-4 times more prevalent in swine and chickens than Type I. Serotype 2 isolations were in addition found to be more pathogenic. Originally, Schaefer (51) found approximately 20 different serotypes of M. avium. However, at this time the taxonomic position of M. avium relative to M. intracellulare had not

been fully resolved. Recently, Meissner (32) and co-workers proposed that the M. avium-M. intracellulare complex be composed of one taxon namely, M. avium. In addition, Wolinsky and Schaefer (61) proposed that the cumbersome system of naming serotypes by proper names be changed whereby all serotypes be given an arabic numeral. At present, there are 20 known serotypes of M. avium appropriately labeled "M. avium Serotypes 1 through 20."

Several modifications of Schaefer's original procedure have been made in an effort to simplify and improve the test methods.

Engel and Berwald (8) modified the tube agglutination procedure for use on glass plates. By their method, they reported the reduction of incubation time from 3 hours to 1 minute. They reported good correlation of results with Schaefer's tube test. The procedure however, is dependent upon obtaining well-dispersed and stable antigen preparations. Mycobacteria often demonstrate autoagglutination tendencies and hence have seldom been used in agglutination procedures.

Other investigators have reduced the size of the tube and volume of reactants to effect economy and simplicity in the test. Reznikov and Leggo (44) working with this simplified system tested 76 strains of M. avium. With few

exceptions good correlation with Schaefer's tube test was observed by this method.

Recently Thoen et al. (57) and associates described a method for serotyping M. avium using plastic micro-titration plates instead of tubes. In addition, the method in contrast to previous procedures utilizes antigen grown in liquid culture. The use of the liquid-grown antigen minimizes the problem of autoagglutination so often evident with antigens grown on agar surfaces. The procedure also utilizes autoclaving to kill the cells and avoids the long waiting time required by the conventional buffer-phenol system.

Ninety-one isolates representing all 20 serotypes of M. avium were tested by the microagglutination procedure and compared to the tube method which was run simultaneously. Using this method 84 of 91 isolates tested were successfully identified.

Since serological identification of the mycobacteria has been developed as a valid and useful technique only in the last decade, information on the comparison of M. paratuberculosis strains by these procedures has been limited.

The most recent investigation attempting to serologically identify M. paratuberculosis was performed by Jarnagin et al. (16) using a modification of the microagglutination technique of Thoen et al. referred to above. These investigators tested 30 isolates of M. paratuberculosis

using the plastic well plate. In addition, the microtitration plate method was compared with the conventional tube procedure of Schaefer. The *M. paratuberculosis* isolates were tested against antisera produced in rabbits immunized to known strains of *M. paratuberculosis*. In addition, cross-agglutination studies were performed using various isolates of *M. avium* serotypes 1, 2, 4 and 8. Good correlation was found between the two methods and no cross-agglutination was observed between the *M. avium* and *M. paratuberculosis* strains. Details of this investigation will be presented as a portion of this thesis.

D. Cultural Methods

The first investigators to cultivate *M. paratuberculosis in vitro* were Twort and Ingram (60) in 1912. They first isolated the organism on an inspissated glycerine-egg medium. Their medium contained killed *M. tuberculosis* which they found contained an unknown growth factor required for cultivation of the fastidious Johne's bacillus. Further experimentation indicated that *M. phlei* was superior to all other mycobacteria tested in providing this growth factor.

This growth factor, later known as mycobactin was isolated and purified by Francis et al. (9). Mycobactin is soluble lipid solvents, but not in water. It also has a chelation

property of binding to iron, copper and other heavy metals.

Snow (54, 55) found that mycobactin has a molecular weight of 870. Mycobactin was also found to contain an alkali-labile ester bond which when hydrolyzed results in the liberation of two chemical entities: cobactin ($C_{12}H_{22}O_4N_2$) and mycobactic acid ($C_{35}H_{55}O_7N_3$) neither of which alone can support the growth of M. paratuberculosis.

Merkal (35) has observed that cultural examination has been the most uniformly successful method of detecting M. paratuberculosis infection in cattle herds. Cultural examination is dependent on the control of contaminating microorganisms commonly found in the intestinal tract of cattle. Various materials have been used as decontaminating agents (33, 3) but benzalkonium chloride has been found to be the least toxic (39) to M. paratuberculosis. Addition of amphotericin B to the growth medium will also control contaminating fungi (36).

Cultural examination has the disadvantage that the infected animal must be shedding approximately 100 organisms per gram of feces in order to be detected (35). This is however offset by the poorer alternative of detection using allergic tests which may result in false positive observations.

III. MATERIALS AND METHODS

A. Processing of Fecal Specimens

The procedure used in the isolation of *M. paratuberculosis* from fecal specimens was a modification of the procedure as described by Merkal (34).

One gram of fecal material was added to 40 ml of sterile distilled water contained in a 50 ml centrifuge tube. The fecal material was shaken on an Eberbach shaker for 30 minutes at room temperature. The larger particles were allowed to settle for 30 minutes. The uppermost 5.0 ml of the fecal suspension was transferred to a 50 ml centrifuge tube containing 50.0 ml of 0.3% benzalkonium chloride (Zephiran). The tube was inverted several times to assure uniform distribution and allowed to stand undisturbed for 24 hours at room temperature.

One tenth of a milliliter of undisturbed sediment was added to each of 3 tubes of Herrold's egg yolk agar medium (13, 34) containing mycobactin[1] (2 mg per 1000 ml of medium). An additional 0.1 ml aliquot of fecal suspension was added to a tube of Herrold's egg yolk agar medium containing no mycobactin as a control.

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[1]Available from Veterinary Services Laboratories, USDA Ames, Iowa.

The inoculated media were incubated for 1 week at 37°C in a slanted position to assure uniform distribution of the inoculum on the medium surface. At the end of the one week's incubation, the tubes were placed in a vertical position and the plastic caps loosened slightly to allow excess moisture to evaporate. Incubation was continued for an additional 19 weeks with observations for colony appearance being made weekly. Smears were made from colonies suspected of being M. paratuberculosis and were stained for acid-fast characteristics by the Ziehl-Neelsen procedure (22).

B. Processing of Tissue Specimens

Tissue specimens, such as lymph nodes, sections of intestine or ileocecal valves were rinsed twice in Butterfield's buffer (22) before processing. If ileocecal valves or intestinal mucosa were to be examined approximately 4.0 gm of mucosa were scraped from the suspect tissue with a scalpel and placed in a sterile blendor jar containing 50.0 ml of 2.5% trypsin solution. The mixture was adjusted to neutrality using 4.0% sodium hydroxide and stirred for 30 minutes on a magnetic mixer. The digested material was then filtered through gauze to remove large pieces of undigested tissue. The filtrate was centrifuged at 1650 RCF for 30 minutes. The supernatant was poured off and discarded.

The sediment was re-suspended in 40 ml of 0.1% Zephiran[1] and allowed to stand undisturbed at room temperature for 24 hours. The sediment which resulted was inoculated onto modified Herrold's egg yolk agar using the procedure described above for fecal specimens.

Lymph nodes were processed by rinsing twice in 1:1000 sodium hypochlorite solution followed by trimming of excess fat from the specimen. The washed and trimmed tissues were transferred to a Waring Blendor jar containing 50.0 ml of sterile nutrient broth with 0.4% phenol red indicator. The tissues were macerated for 2 minutes in the blender after which 5.0 ml of tissue suspension was added to 5.0 ml of 0.5N NaOH contained in a 20 x 125 mm screw cap tube. The tube was shaken thoroughly to mix the contents and allowed to stand at room temperature for 10 minutes. The digestive action of the alkali was stopped by the dropwise addition of sufficient 6N HCl to change the indicator to yellow.

The tubes were then centrifuged at 1650 RCF for 30 minutes. The resulting sediment was inoculated onto modified Herrold's egg yolk agar.

In addition, two tubes each of Stonebrink's (22), Middlebrook's 7H10 (22) and Lowenstein-Jensen media (22) were inoculated with 0.1 ml aliquots of tissue suspension.

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[1]Available as Zephiran chloride (benzalkonium chloride) from Winthrop Laboratories, New York, New York 10016.

These media were incubated at 37°C for 8 weeks with observations for isolation of mycobacteria other than _M. paratuberculosis_ being made at weekly intervals. Tubes of Herrold's egg yolk medium with added mycobactin were held for an additional 12 weeks to allow adequate incubation time for _M. paratuberculosis_.

Those cultures showing growth of acid-fast organisms were processed in two ways for identification.

C. Identification of _M. paratuberculosis_ Isolates

Colonies that appeared from the 5th to the 20th week post-inoculation on mycobactin-containing media as punctiform, moist, white, glistening colonies were tentatively identified as _M. paratuberculosis_. These colonies were sub-cultured on two slants each of Herrold's egg yolk agar containing mycobactin and 2 slants of Herrold's agar without mycobactin. _M. paratuberculosis_ should grow only on the tubes containing mycobactin. Caution should be observed since the characteristic of mycobactin dependency can be lost on repeated subculture.

Microscopically, cells of _M. paratuberculosis_ are Gram positive and acid-fast. The cells average 0.5um in width to 1.0 um in length. The cells may or may not appear in clumps.

Supplementary tests as used for differentiating other mycobacteria have been evaluated by Merkal and Thurston (37) and have been found to be inconclusive for identifying *M. paratuberculosis*.

D. Identification of *M. avium* Isolates

Isolates of *M. avium* were identified by their slow growth rate, failure to hydrolyze Tween 80, and by their resistance to the following compounds: thiophene-2-carboxylic acid hydrazide (2, 22), 10 ug/ml; streptomycin (17, 22), 2.0ug/ml; isonicotinic acid hydrazide (21, 22), 10 ug/ml; neotetrazolium chloride (10, 22), 25 ug/ml; and rifampin (31, 22), 0.025 ug/ml.

E. Selection of Cultures for Use as Antigens

M. paratuberculosis isolates 35123-2728 and 703114-862 were selected for use as antigens in the production of reference antisera in rabbits. Selection was primarily based upon records kept at Veterinary Services Laboratories (VSL). These two isolates were selected on the basis that they were isolates from cattle herds with a history of known endemic paratuberculosis and also that they originated from different geographic origins.

Antigens were selected from different geographical areas to determine if differences in agglutination specificity occur between isolates from these localities.

The isolates of M. paratuberculosis to be studied and compared were isolated from various animal sources and localities in the continental United States (See Table 10).

Cultures of M. avium to be used for production of antiserum were selected from the culture repository of Veterinary Services Laboratories, APHIS-USDA, Ames, Iowa isolated from routine clinical specimens. In addition, some cultures and antisera were obtained from the laboratory of the late Dr. W. B. Schaefer at the National Jewish Hospital in Denver, Colorado. The M. avium cultures are tabulated in Tables 11 and 12.

F. Production of Antiserum

Antiserum in which specific agglutinating activity against M. paratuberculosis could be demonstrated was produced in rabbits by a modification of the method of Schaefer (48). The special growth requirements necessitated that the organisms be cultivated on Herrold's egg yolk agar with added mycobactin.

One 3mm loopful of a 6-week old culture of M. paratuberculosis was suspended in 2.0 ml of sterile distilled water contained in a 16 x 125 mm screw cap tube. The contents of this tube were uniformly distributed onto the surface of two 100mm glass petri plates of Herrold's egg yolk agar with mycobactin. The seeded plates were incubated for 4-6 weeks at 37°C until luxuriant growth was obtained.

The resulting culture growth was removed by scraping the surface of the agar with a rubber "policeman" into 25.0 ml of phosphate buffered saline (pH 7.0) with 0.5% phenol. The cell suspension was allowed to remain for 5 days at room temperature to kill the cells. At the termination of the 5-day killing period, the cell suspension was adjusted to 0.40 optical density on a Bausch and Lomb 340 spectrophotometer at a wavelength of 525 nm.

One and one half milliliters of bacterial suspension was injected intravenously twice weekly, into each of two 500g New Zealand White rabbits. The injection schedule was continued for 4 weeks after which the rabbits were allowed to rest 6 days before being bled. The animals were bled by cardiac puncture and the serum removed by standard techniques (4). The serum was preserved by addition of 0.1 ml of 1.0% 8-quinolinol sulphate per 10 ml of serum (50).

Antiserum against M. avium was produced by the method of Schaefer (48).

The resulting M. paratuberculosis and M. avium antisera were titrated against their respective homologous strains to determine agglutinin content. The maximum dilution in which cell agglutination occurred was determined to be the titer of the serum and was used as the working

dilution in all experiments. The working dilutions of all antisera are shown in Table 13.

G. Production of Cell Suspensions for Agglutination Tests

Cell suspensions of M. paratuberculosis and M. avium were prepared for use in all agglutination tests by culture on Herrold's egg yolk agar with added mycobactin. Cultural procedures were identical to those described above for immunizing antigen.

Two 3 mm loopfuls of cultured cells were removed (Figures 1 and 2) from the agar surface and suspended in 2.0 ml of Middlebrook's 7H9 Broth Base[1] without added enrichments but containing 0.001% Tween 80 in a 20 X 125 mm screw cap tube (Figure 3). The suspension of the cells in a small amount of Tween-containing 7H9 broth diluent promotes uniform dispersal of cells. Eight milliliters of 7H9 broth with Tween 80 were added to bring the volume to 10.0ml (Figure 4).

The cell suspension was autoclaved at 121°C for 12 minutes to eliminate the safety hazards of using live mycobacterial cells of unknown type (57). The cell suspension

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[1]Available from Difco Laboratories, Detroit, Michigan, Catalog #0713-01.

was allowed to cool for 30 minutes and additional Middlebrook 7H9 broth base with Tween 80 was added to bring the volume to 25.0 ml.

Autoagglutination can be a problem with some M. paratuberculosis and M. avium strains; therefore the killed cell suspensions were allowed to remain at room temperature overnight to allow non-dispersed particulate matter to sediment. The remaining cell suspension was standardized to 0.45 O.D on a Bausch and Lomb Spectronic 20[1] spectrophotometer using a wavelength of 525 nm. Antigens were coded by an independent observer to minimize bias in interpreting results.

H. The Tube Agglutination Method

The tube procedure for determining agglutinating activity was a modification of that described by Schaefer (48).

One-half milliliter aliquots of each antiserum were pipetted into separate, dry 11 X 100mm serological test tubes.[2]

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[1]Available from Bausch and Lamb Optical, Inc., Rochester, New York.

[2]Available from Bellco Glass Co., Vineland, New Jersey, Catalog No. 1711.

Figure 1.

Removal of mycobacterial cells by use of an inoculating loop prior to suspension in Tween-containing diluent

Figure 2.

Suspension of mycobacterial cells on the sides of the test tube to enhance uniform suspension of cells

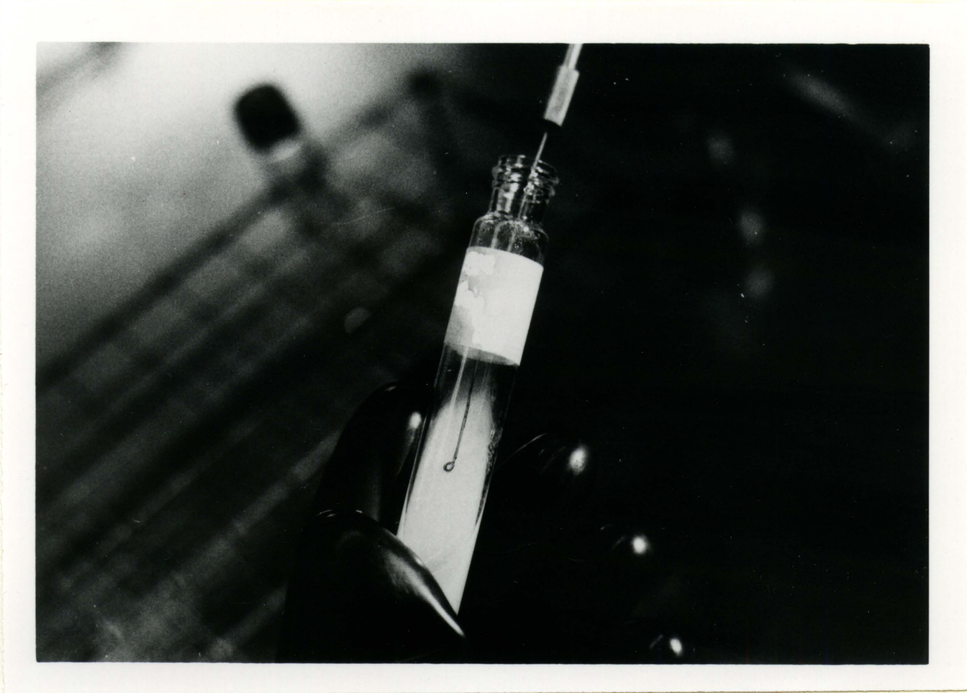


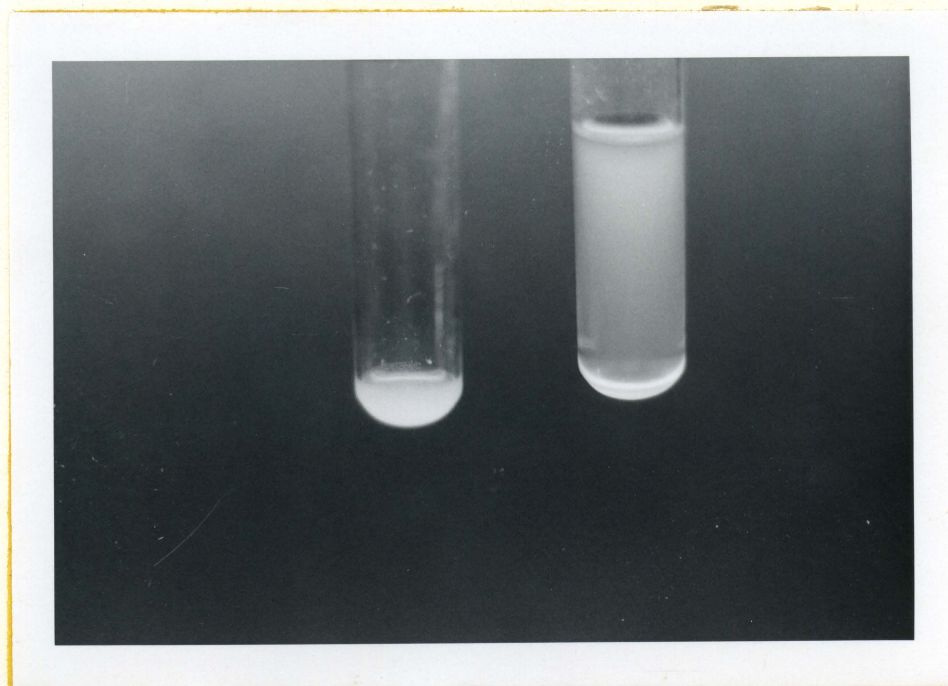
Figure 3.

Addition of Tween-containing diluent

Figure 4.

Suspended mycobacterial cells prior to addition of diluent (Left) Cell preparation with added diluent ready for autoclaving (Right)

7H-9 BROTH



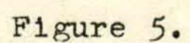


Figure 5.

Addition of antiserum to plate wells

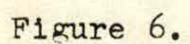
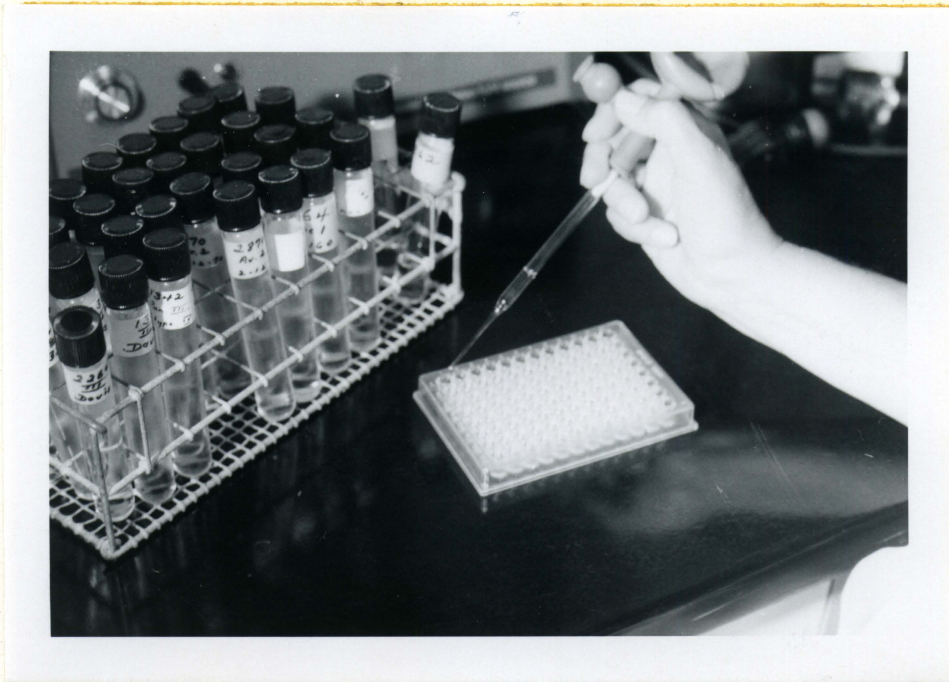


Figure 6.

Addition of cell suspension (antigen) to plate wells



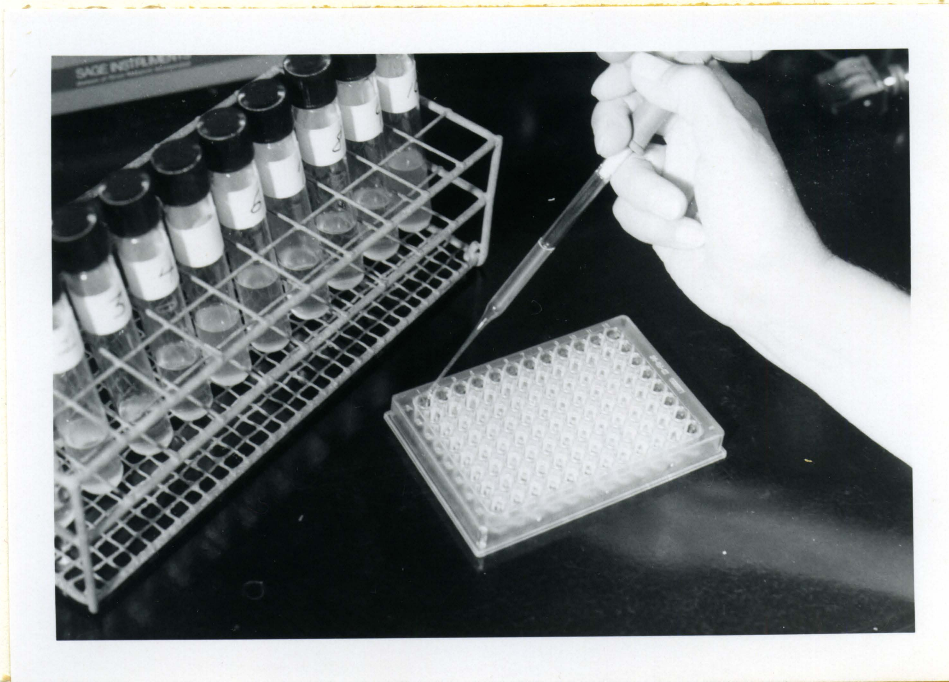


Figure 7.

Mixing of antigen-antiserum on a Thomas-Boerner shaker

Figure 8.

The plates are covered with a plastic cover prior to incubation

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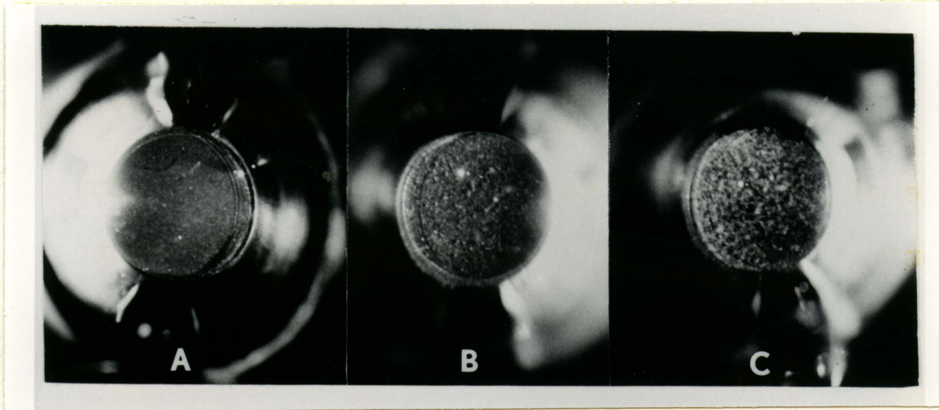
Figure 9.

Example of microagglutination reactions at 6 hours

A. Control   B. +2 reaction   C. +4 reaction

Figure 10.

The light source-microscope combination used in observing the microagglutination reactions


A
B
C

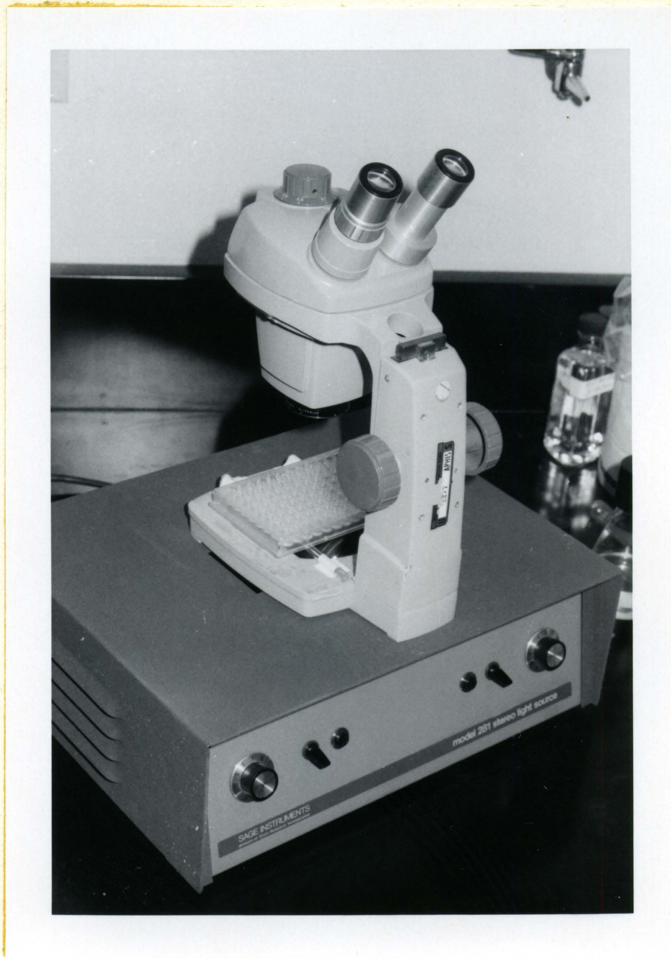

model 281 stereo light source
SAGE INSTRUMENTS

Figure 11.

Aluminum test tube rack used for incubating and observing the tube agglutination tests

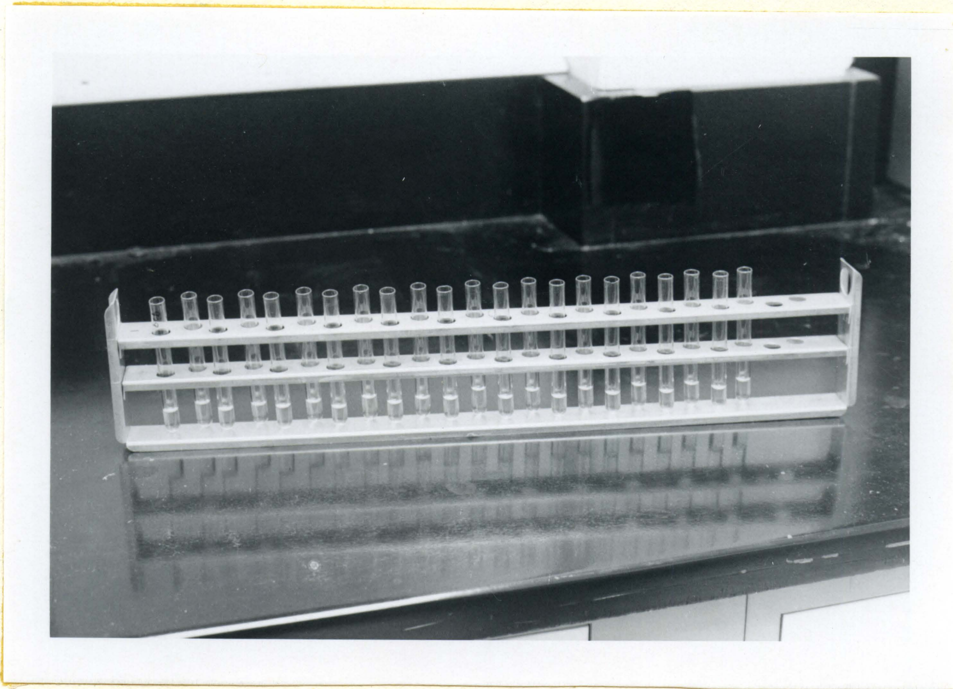


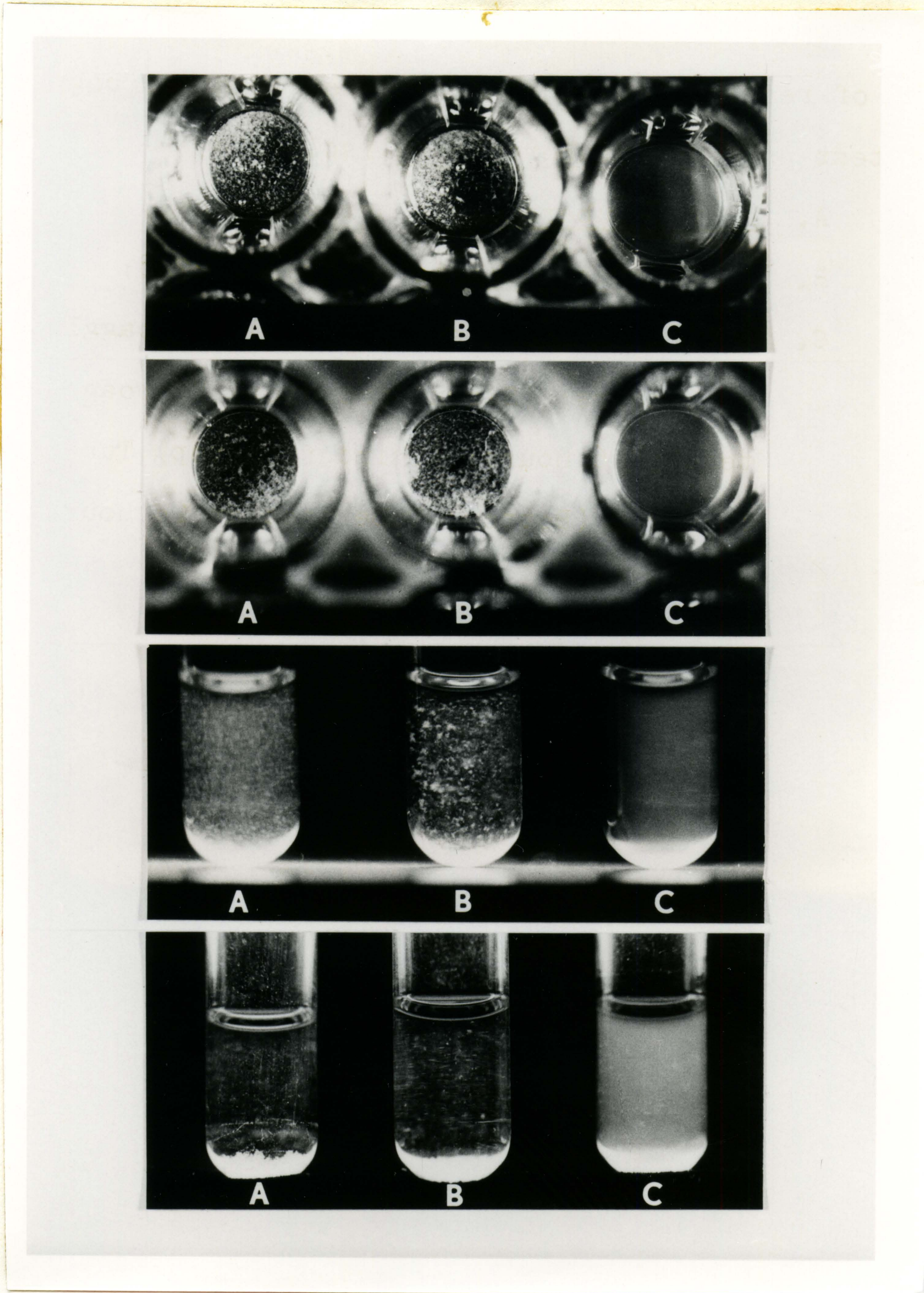
Figure 12.

Example of results between the tube test and microagglutination test on *M. paratuberculosis* isolate #928

A. *M. paratuberculosis* antiserum 862;

B. *M. paratuberculosis* antiserum 2728;

C. Control (no antiserum) (Top) Microagglutination at 3 hours; (Second from top) Microagglutination test at 6 hours; (Third from top) Tube test at 6 hours; (Bottom) Tube test at 18 hours.


A
B
C
A
B
C
A
B
C
A
B
C

The tubes were contained in a 24-hole, single row aluminum test tube rack especially constructed for reading mycobacterial agglutination tests (Figure 11). An additional tube containing 0.5 ml of phenolized phosphate buffered saline was prepared as a control. To each tube was added 0.5ml of the standardized cell suspension of the mycobacterial isolate to be tested. The rack of tubes containing the antigen and antiserum was shaken vigorously by hand for 1 minute and then incubated at 37°C.

Observations for agglutinating activity were made at 6 and 18 hours using a 3X Edroy magnifying headset.[1] Readings were made on a scale from +1 showing small clumps of agglutinated cells distributed throughout an opalescent suspending fluid to +4 in which large clumps of agglutinated cells appeared throughout a transparent suspending fluid at 6 hours but were completely sedimented at 18 hours. Intermediate reactions between the two extremes were observed and noted.

As in the case of the Schaefer procedure (48), both reference antisera must show agglutinating activity at both reading times for the tested isolate to be designated a particular serotype.

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[1]Available from Edroy Products Co., 480 Lexington Avenue, New York, New York.

I. The Microagglutination Method

The sparse growth often manifested in primary cultures of M. paratuberculosis may result in a limited amount of cell suspension being obtained.

The microagglutination procedure for serotyping mycobacteria may be useful as less reagents are required and was found by Thoen et al. (57) to be comparable with the Schaefer tube test. This procedure was therefore included to evaluate the efficacy of its use with M. paratuberculosis.

Linbro FS-96-TC microtitration plates[1] were prepared by cleaning the wells of each plate in a jet of compressed air. Antisera prepared against two representatives of each of the twenty M. avium serotypes and the two M. paratuberculosis isolates were dispensed into separate wells in amounts of 0.08 ml (Figure 5). Following addition of antiserum to each well, 0.08ml of standardized cell suspension was added (Figure 6). A control well containing cell suspension and diluent but no antiserum was also prepared.

The plates were shaken for 45 seconds on a Thomas-Boerner shaking apparatus (Figure 7) in order to mix the antiserum and cell suspension. The plates were covered (Figure 8) with a Linbro plastic cover (Model 53)[1] and

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[1]Available from Linbro Chemical Co., New Haven, Connecticut.

and incubated at 37°C in a moist chamber. Observations for agglutination were made after 3 and 6 hours incubation using a Bausch and Lomb dissection microscope at a magnification of 7X (Figure 9). A Sage model 281 stereo light source was used for illumination (Figure 10).

As in the tube test, agglutination must be observed to both antisera of a particular serotype at both reading times in order to designate a mycobacterial isolate as a particular serotype.

J. Developmental Methods and Organization

The development and evaluation of the agglutination procedures for M. paratuberculosis were organized in two parts. A description of these groups as to isolates and antisera studied are tabulated in Table 1.

Table 1. Summary of isolates and antisera studied in Parts I and II

No. M. Paratuberculosis isolates studied		M. paratb. Antisera Used	Number of M. avium Isolates Studied	M. avium Serotypes represented
Part I	30	#862 #2728	56	1,2,4,8
Part II	13	#862 #2728	43	3,5,6,7,9,10 11,12,13,14 15,16,17,18 19,20

Serotypes 1,2,4, and 8 in Part I were examined as a group since these represent 90% of all M. avium isolates obtained from clinical specimens at VSL and hence are of greatest veterinary interest (58).

Isolates in Part II constituted the remaining but infrequently found serotypes hence the numbers that could be studied were limited.

The M. paratuberculosis antisera and isolates were tested by both the tube and microagglutination methods (Figure 12). In addition, they were examined for cross-agglutinating activity to all M. avium isolates. Conversely, isolates of M. paratuberculosis were examined using both procedures for cross-agglutination with M. avium antisera.

Experiments were duplicated and the results examined for statistical validity by the method of chi square.

IV. RESULTS

A. Part I Observations

Observations made on the Part I study comparing *M. paratuberculosis* and *M. avium* serotypes 1,2,4 and 8 revealed a high degree of specificity with no cross-agglutination (Tables 2 and 14). Twenty-nine of 30 isolates of *M. paratuberculosis* reacted solely with the *M. paratuberculosis* antisera in both the tube and microagglutination tests.

The degree of reaction exhibited between *M. paratuberculosis* antisera and the isolates studied was varied (Table 3). In both the tube and microagglutination procedures, the strongest agglutination reactions were elicited by *M. paratuberculosis* antiserum 2728. The maximum +4 reaction was demonstrated by 26.6% of the *M. paratuberculosis* isolates tested in the microagglutination test and 23.3% of isolates tested by the tube procedure. An average of 44% of the isolates tested in both the tube and microagglutination methods using antiserum 862 demonstrated an intermediate +2 reaction while 36.6% of the isolates gave a +2 reaction with 2728.

B. Part II Observations

The results of agglutination tests performed on isolates in Part II are tabulated in Tables 4, 5, 15 and 16. An

analysis of the results will reveal that in the microagglutination test, antiserum 2728 also demonstrates more marked agglutination reactions than antiserum 862. In the tube procedure 12 of 13 isolates demonstrated +4 reactions when tested with both antisera. In the microagglutination test using antiserum 862, a +3 reaction was elicited by a 38.4% of the isolates.

Table 2. Results of agglutination tests on isolates examined in Part I

Antigen	No. Isolates Tested	Test[a]	Antiserum: *M. avium* serotypes 1	2	4	8	*M. paratuberc.*
M. paratuberc.	30	T	0	0	0	0	29[b]
		M	0	0	0	0	29
M. avium sero. 1	15	T	15	0	0	0	0
		M	15	0	0	0	0
M. avium sero. 2	15	T	0	15	0	0	0
		M	0	15	0	0	0
M. avium sero. 4	13	T	0	0	13	0	0
		M	0	0	13	0	0
M. avium sero. 8	13	T	0	0	0	13	0
		M	0	0	0	13	0

[a]T = Tube test; M = Microagglutination test.

[b]Number of isolates showing reaction.

Table 3. Comparison of reactions using the tube and microagglutination procedures on isolates of M. paratuberculosis studied in Part I

Antiserum	Maximum[a] Degree of Reaction	Procedure: Microagglutination No. Showing Reaction	Microagglutination % of Total	Tube No. Showing Reaction	Tube % of Total
F862	0	1	3.3	1	3.3
2728	0	1	3.3	1	3.3
F862	+1	2	6.6	1	3.3
2728	+1	0	0.0	0	0.0
F862	+2	13	43.3	14	46.6
2728	+2	11	36.6	11	36.6
F862	+3	10	33.3	9	30.0
2728	+3	10	33.3	11	36.6
F862	+4	4	13.3	5	16.6
2728	+4	8	26.6	7	23.3

[a]0 No reaction
+1 Very small clumps of agglutinated cells, suspending fluid opalescent
+2 Small size clumps of agglutinated cells, suspending fluid opalescent
+3 Medium size clumps of agglutinated cells, suspending fluid opalescent
+4 Large clumps of agglutinated cells, suspending fluid transparent.

Table 4. Results of agglutination tests on isolates examined in Part II

			Antiserum																
			M. avium serotype																
Antigen	No. Isolates Tested	Test[a]	3	5	6	7	9	10	11	12	13	14	15	16	17	18	19	20	*M. paratuberculosis*
M. paratuberculosis	13	T	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	13[b]
		M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	13
M. avium serotype																			
3	3	T	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		M	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	2	T	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		M	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	2	T	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		M	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	3	T	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0
		M	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0
9	2	T	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0
		M	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0
10	6	T	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0
		M	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0
11	3	T	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0
		M	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0
12	3	T	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0
		M	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0

13	2	T	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0
		M	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0
14	3	T	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0
		M	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0
15	2	T	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0
		M	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0
16	3	T	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0
		M	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0
17	2	T	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0
		M	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0
18	3	T	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0
		M	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0
19	2	T	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0
		M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0
20	2	T	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0
		M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0

[a]T = Tube procedure; M = Microagglutination procedure.

[b]Number of isolates showing reaction.

Table 5. Comparison of reactions using the tube and microagglutination procedures on isolates of M. paratuberculosis studied in Part II

		Procedure			
		Microagglutination		Tube	
Antiserum	Maximum[a] Degree of Reaction	No. Showing Reaction	% of Total	No. Showing Reaction	% of Total
F862	0	0	0	0	0
2728	0	0	0	0	0
F862	+1	0	0	0	0
2728	+1	0	0	0	0
F862	+2	2	15.3	0	0
2728	+2	0	0	0	0
F862	+3	5	38.4	1	16.7
2728	+3	3	23.0	1	16.7
F862	+4	6	46.1	12	83.3
2728	+4	10	76.9	12	83.3

[a]0 No reaction
+1 Very small clumps of agglutinated cells, suspending fluid opalescent
+2 Small clumps of agglutinated cells, suspending fluid opalescent
+3 Medium size clumps of agglutinated cells, suspending fluid slightly opalescent
+4 Large clumps of agglutinated cells, suspending fluid transparent.

No cross-agglutination with M. avium isolates or antisera was observed with M. paratuberculosis cultures utilized in Part II.

An analysis of the results of all isolates studied cumulatively (Table 6) demonstrates that M. paratuberculosis antiserum 2728 does show stronger reactions by both methods. In addition most isolates demonstrated +4 reactions in the tube test but the results were divided between +3 (34.8%) and +4 (41.8%) in the microagglutination test depending on the antiserum used.

Reactions of +2 are not uncommon, particularly using the microagglutination procedure. Of the 99 M. avium isolates examined in this study, 52.5% demonstrated a maximum reading of +2 with one or both antisera while 26 of 99 demonstrated a +4 reaction.

Using the tube procedure, 37.3% (37/99) exhibited a +2 reading while 59.5% demonstrated the maximum +4 reaction.

C. Additional Observations

No major differences were found between isolates of M. paratuberculosis originating from different geographical areas of the United States (Table 7). However, isolates originating in the eastern United States tended to have weaker agglutination reactions than those originating from other regions. Isolates originating from the western United States demonstrated stronger reactions particularly using the microagglutination procedure and antiserum 2728.

Table 6. Cumulative tabulation of reactions between *M. paratuberculosis* antisera used in Parts I and II

Antiserum	Maximum Degree of Reaction[a]	Procedure: Microagglutination — No. Showing Reaction	% of Total	Tube — No. Showing Reaction	% of Total
F862	0	1	2.3	1	2.3
2728	0	1	2.3	1	2.3
F862	+1	2	4.6	1	2.3
2728	+1	0	0	0	0
F862	+2	15	34.8	13	30.2
2728	+2	11	25.5	10	23.2
F862	+3	15	34.8	10	23.2
2728	+3	13	30.2	13	30.2
F862	+4	10	23.2	18	41.8
2728	+4	18	41.8	19	44.1

[a]0 No reaction
+1 Very small clumps of agglutinated cells, suspending fluid opalescent
+2 Small clumps of agglutinated cells, suspending fluid opalescent
+3 Medium size clumps of agglutinated cells, suspending fluid slightly opalescent
+4 Large clumps of agglutinated cells, suspending fluid transparent.

Table 7. Serologic reactions of 43 isolates of *M. paratuberculosis* tabulated by geographic origin

Region of United States	*M. paratuberculosis* Antiserum	Test[b]	Reaction[a] 0	+1	+2	+3	+4	Total
East	862	M	1	0	11	6	3	21
		T	1	0	9	2	9	21
	2728	M	1	0	8	8	4	21
		T	1	0	6	5	9	21
North Central	862	M	0	1	2	8	1	12
		T	0	1	0	6	5	12
	2728	M	0	0	2	4	6	12
		T	0	0	1	5	6	12
South	862	M	0	1	0	1	1	3
		T	0	0	2	0	1	3
	2728	M	0	0	1	0	2	3
		T	0	0	1	1	1	3
West	862	M	0	0	1	1	5	7
		T	0	0	2	2	3	7
	2728	M	0	0	0	1	6	7
		T	0	0	2	2	3	7

[a]No. of Isolates showing reaction.

[b]M = Microagglutination Test; T = Tube Test.

Table 8. Serologic reactions of 43 isolates of <u>M. paratuberculosis</u> tabulated by host species

Animal	No. Tested	M. paratuberc. Antiserum	Test[b]	Reaction[a] 0	+1	+2	+3	+4
Aoudad	1	862	T	0	0	1	0	0
			M	0	0	1	0	0
	1	2728	T	0	0	0	1	0
			M	0	0	1	0	0
Bison	1	862	T	0	0	0	0	1
			M	0	1	0	0	0
	1	2728	T	0	0	0	0	1
			M	0	1	0	0	0
Bovine	34	862	T	1	1	11	7	14
			M	1	2	11	11	9
	34	2728	T	1	0	11	9	13
			M	1	0	9	9	15
Big Horn Sheep	1	862	T	0	0	0	0	1
			M	0	0	0	1	0
	1	2728	T	0	0	0	0	1
			M	0	0	0	0	1
Camel	1	862	T	0	0	0	0	1
			M	0	0	1	0	0
	1	2728	T	0	0	0	0	1
			M	0	0	1	0	0
Deer	3	862	T	0	0	0	2	1
			M	0	0	0	3	0
	3	2728	T	0	0	0	1	2
			M	0	0	0	2	1
Porcine	1	862	T	0	0	0	1	0
			M	0	0	1	0	0
	1	2728	T	0	0	0	1	0
			M	0	0	0	1	0
Sheep	1	862	T	0	0	1	0	0
			M	0	0	1	0	0
	1	2728	T	0	0	0	1	0
			M	0	0	1	0	0

[a]Number of isolates demonstrating agglutination.

[b]T = Tube Test; M = Microagglutination Test.

As can be seen from the results tabulated in Table 8, isolates originating from various animal hosts demonstrated agglutination reactions in different degrees to both M. paratuberculosis antisera. Agglutination reactions among bovine isolates of M. paratuberculosis were generally +2 or greater. Thirty-seven percent of all reactions with the tube test using both antisera exhibited the maximum +4 reaction. Maximum +4 reactions using the microagglutination procedure ranged from 25.7% with antiserum 862 to 42.8% with antiserum 2728.

Table 9. Serologic reactions of 43 isolates of M. paratuberculosis tabulated by specimen source

Specimen Source	M. paratubc. Antiserum	Test[b]	Reaction[a] 0	+1	+2	+3	+4	Total Isolates Tested
Fecal Sample	862	T	0	1	10	7	9	27
		M	0	2	8	10	7	27
	2728	T	0	0	8	7	12	27
		M	0	0	7	8	12	27
Lymphatic Tissue	862	T	1	0	2	3	5	11
		M	1	0	4	3	3	11
	2728	T	1	0	0	4	6	11
		M	1	0	2	3	5	11
Intestinal Tissue	862	T	0	0	2	2	1	5
		M	0	0	2	2	1	5
	2728	T	0	0	2	2	1	5
		M	0	0	2	2	1	5

[a]Number showing reaction.

[b]T = Tube procedure; M = Microagglutination procedure.

Although minor differences in agglutination readings were observed, the source of the specimen and the degree of reaction were not closely related (Table 9).

The degree of agglutination reaction between the tube and microagglutination methods as demonstrated by both _M. paratuberculosis_ antisera was compared for statistical validity. In order to determine any differences between antisera 2728 and 862 or between the two procedures used, the results were subjected to the chi square method (14) of statistical analysis. Computed values for both antisera indicated that the p values were less than .005.

V. DISCUSSION

The results reported herein will reveal that *M. paratuberculosis* can be differentiated from *M. avium* by serologic procedures.

Antigenically, *M. paratuberculosis* has been thought to more closely resemble *M. avium* than other species of mycobacteria such as *M. tuberculosis* or *M. bovis*. Evidence for this close-relationship may be found in allergy testing of cattle using avian tuberculin and johnin. Pearson and McClellan (42) found that a high percentage of cattle from which isolations of *M. paratuberculosis* were made demonstrated positive skin reactions to both tuberculin types. Before the use of the complement-fixation test, avian tuberculin was recommended as means of diagnosis (47). Morphologically, the short rods and small, moist, white colonies of *M. paratuberculosis* also resemble *M. avium*.

Although *M. avium* and *M. paratuberculosis* may appear to be related antigenically and morphologically, the lack of cross-agglutination between these two organisms as observed in this study indicate they can be differentiated by serologic methods.

Further evidence that *M. avium* and *M. paratuberculosis* are serologically distinct was observed in immuno-fluorescence studies of these organisms (12). It was also observed

in these same studies that the component responsible for specificity is present in the cell wall as none was observed in protoplasmic fractions of M. avium and M. paratuberculosis.

It is also notable that no significant differences were found among any of the M. paratuberculosis isolates regardless of their geographic, animal host or specimen origins. This synonymity of strains appears to coincide with the cultural and clinical observations of Gilmour (11), whose studies of sheep and cattle isolates of M. paratuberculosis found them to be indistinguishable. Taylor (56) felt that paratuberculosis in sheep was caused by classical M. paratuberculosis but also felt that sub-types could be demonstrated culturally depending on geographic origin.

The observation that both M. paratuberculosis antisera demonstrated no qualitative differences in agglutination regardless of isolate source also serves to add credence to the homogenous nature of the isolates studied.

The previous inability of other investigators to observe differences among M. paratuberculosis strains may have been due to the lack of biochemical and serologic tests of adequate sensitivity and the fastidious growth of the Johne's bacillus. Further research using new and advanced techniques might reveal differences between M. paratuberculosis strains.

Of the 43 isolates of M. paratuberculosis studied, only one failed to be agglutinated by both M. paratuberculosis antisera. The reason for this failure may be (1) the isolate is a separate "serotype" of M. paratuberculosis not possessing agglutinin receptor sites for the antibody used; (2) the isolate was sensitive to the heat treatment used in antigen preparation and was serologically inactivated; (3) laboratory error in standardization of the antigen or in test preparation; or (4) the culture was erroneously identified as M. paratuberculosis.

The isolate most probably represents a different serotype as (1) other isolates of M. paratuberculosis were heat-killed at the same time without adverse effect; (2) the isolate demonstrated mycobactin dependency and typical colony characteristics of M. paratuberculosis; and (3) all cultures were prepared as nearly as possible for serological examination according to the experimental design.

The observation that isolates studied in Part II had a higher proportion of +4 reactions compared to those in Part I was most likely due to the degree of dispersion of the bacterial suspensions. Although all bacterial suspensions were standardized to an optical density of 0.45, some isolates tended to be more granular than others. The granularity did not affect the overall agglutination but may have affected the degree of the reaction. Isolates that

demonstrated minor granularity were observed to show less marked agglutination reactions. Isolates in Part I had more granular characteristics than those in Part II. The less marked agglutination reactions noted in Part I isolates is perhaps due to this observation. Although some isolates demonstrated minor granulation characteristics, in no instance did any isolate autoagglutinate as indicated by complete absence of agglutination in the control wells or tubes.

The greater proportion of +4 reactions observed with antiserum 2728 may be due to an increased agglutinin content in this serum. Both antisera 862 and 2728 had titers of 1:160 but the degree of reaction of 862 at this titer was less than the reaction recorded for 2728.

One can also speculate that the different agglutination reactions between the two M. paratuberculosis antisera may be due to increased avidity of antiserum 2728 to the antigens in the cell walls of the Johne's bacillus. This would result in more efficient binding of the bacterial cells by the agglutinin molecules.

The potential use for serologic examination of isolates of M. paratuberculosis may be two-fold. First, the test will provide an additional method for identifying an isolate as M. paratuberculosis. The current method for differentiating the Johne's bacillus from other acid-fast organisms is based upon a single characteristic, that of mycobactin

dependency. Specific agglutination will provide an additional method for identifying M. paratuberculosis. Secondly, the test would provide the potential for epidemiologic studies on M. paratuberculosis using a serologic method. Although the isolates of M. paratuberculosis observed in the current study appeared for the most part to be serologically identical, the examination of additional M. paratuberculosis isolates and antisera may reveal serologic differences valuable in tracing infections between animal herds. The pioneering work of Schaefer (49) in epidemiologic studies of M. avium only serve to point out the value of serologic testing.

Statistical analysis indicates that either the tube or microagglutination method could be used with confidence in obtaining valid results. The microagglutination test may lend itself to screening large numbers of M. paratuberculosis isolates. In addition, as M. paratuberculosis often grows sparsely on primary culture, the microagglutination test could be applied earlier for quick identification as it requires only a minimal amount of cell suspension as antigen.

VI. SUMMARY

In summary, 43 isolates of *M. paratuberculosis* were tested for agglutination characteristics with two antisera prepared in rabbits hyper-immunized to *M. paratuberculosis*. They were in addition, examined for cross-agglutination with 99 isolates of *M. avium* representing 20 serotypes.

The major conclusions that were gained from the study were (1) isolates of *M. paratuberculosis* lend themselves to serological testing by use of either a microagglutination method or by the agglutination procedure of Schaefer; (2) *M. paratuberculosis* appears to be serologically distinct from all 20 known serotypes of *M. avium* as evidenced by lack of cross-agglutination; (3) the isolates of *M. paratuberculosis* with the exception of varying agglutinability, appear to be serologically homogenous and (4) isolates of *M. paratuberculosis* did not appear to be significantly different serologically regardless of geographical origin, animal host or specimen origin.

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IX. APPENDIX

Table 10. *M. paratuberculosis* isolates used in agglutination studies

Accession #	TB#	Type[a]	Herd	State	Specimen Source[b]	Animal[c]
7500149	34	M. ptb	Shoemaker	Ind.	L.N.	Bov.
7500364	55	M. ptb	Teats	W. Va.	Int.	Bov.
751047	147	M. ptb	Unk.[d]	Wash.	F.	Bov.
7500596	157	M. ptb	Larkin	Conn.	F.	Bov.
751168	162	M. ptb	Johnson	N. Dak.	F.	Bov.
750807	201	M. ptb	Clark	N. Dak.	F.	Bov.
740672	213	M. ptb	Goeres	Ore.	F.	Bov.
740673	215	M. ptb	Burnett	Ore.	F.	Bov.
752029	287	M. ptb	Amer. Breeders	Colo.	F.	Bov.
741088	351	M. ptb	Unk.	Wash.	F.	Bov.
7502134	577	M. ptb	Hinton	Vt.	F.	Bov.
742828	781	M. ptb	Woods	Pa.	L.N./Int.	Bov.
703114	862[e]	M. ptb	Utt	Pa.	F.	Bov.
743316	868	M. ptb	Woods	Pa.	L.N.	Bov.
754134	928	M. ptb	Pocono	Pa.	L.N.	Aoudad
754538	1026	M. ptb	Eastman	Ut.	F.	Bov.
744400	1059	M. ptb	Chester	Mich.	F.	Bov.
745123	1249	M. ptb	Larkin	Conn.	F.	Bov.
724214	1371	M. ptb	Amick	Pa.	F.	Bov.
756860	1530	M. ptb	Wells	Wash.	F.	Bov.
746457	1574	M. ptb	Wells	Ala.	F.	Bov.
757196	1586	M. ptb	Wells	Del.	F.	Bov.
757169	1587	M. ptb	Wells	Del.	F.	Bov.
746990	1690	M. ptb	Rose Zoo	N. Y.	F.	Camel
747339	1781	M. ptb	Cooper	Pa.	F.	Bov.
747494	1800	M. ptb	Adams	Miss.	F.	Bov.
747417	1860A	M. ptb	Sea World	Ohio	F.	Deer
758961	1993	M. ptb	Frahm	Mich.	F.	Bov.
759117	2039	M. ptb	Sea World	Ohio	F.	Deer

749996	2105	M. ptb	Peters	Ind.	F.	Bov.
7412250	2436	M. ptb	Lister	Kans.	Int.	Bov.
7510578	2472	M. ptb	K.C. Zoo	Mo.	F.	Big Horn Sheep
7310810	2726	M. ptb	Gage	Colo.	L.N.	Bov.
35123	2728[e]	M. ptb	Craig	Wis.	Int.	Bov.
7310868	2812	M. ptb	Unk.	Ind.	L.N.	Porc.
7415132	2943	M. ptb	Sea World	Ohio	L.N.	Deer
7311012	2988	M. ptb	Stoner	Pa.	F.	Bov.
7516276	3819	M. ptb	Clark	N. Dak.	F.	Bov.
751289	4210	M. ptb	Catskill	N. Y.	L.N.	Ovine
7311298	4219	M. ptb	Giondia	Fla.	L.N.	Bov.
7311310	4230	M. ptb	Nicolind	Calif.	L.N.	Bov.
734765	4502	M. ptb	Woods	Pa.	L.N.	Bov.
74998	4777	M. ptb	Catskill	N. Y.	Unk.	Bison

[a]M. ptb = M. paratuberculosis.

[b]L.N. = Lymphnode; Int. = Intestine; F. = Fecal.

[c]Bov. = Bovine; Porc. = Porcine.

[d]Unk. = Unknown.

[e]Cultures injected into rabbits for use in producing antiserum.

Table 11. *M. avium* isolates used in agglutination studies

Accession #	TB#	Sero-type	Herd	State	Animal[a]	Source[b]
757497	1634	1	Clark	Calif.	Porc.	VSL
757894	1752	1	Nat'l Zoo Park	Dist. Col.	Finch	VSL
758201	1796	1	Unk.[c]	Pa.	Porc.	VSL
758216	1811	1	Miller	Ga.	Porc.	VSL
758217	1812	1	Frosty Meats	N. C.	Porc.	VSL
758294	1818	1	Taake	Ia.	Porc.	VSL
758516	1877	1	Conn Pkg.	Ct.	Porc.	VSL
748420	1887	1	Unk.	Tex.	Porc.	VSL
758704	1920	1	Nat'l Zoo Park	Dist. Col.	Quail	VSL
758807	1957	1	Honbarrier	Mass.	Porc.	VSL
769804	1989	1	Brimmer	Ia.	Porc.	VSL
749604	2038	1	Unk.	N. Dak.	Porc.	VSL
749607	2041	1	Unk.	Nebr.	Porc.	VSL
759228	2075	1	Shuyler	Nebr.	Porc.	VSL
749809	2081	1	Tayntor	N. Y.	Porc.	VSL
757267	1594	2	Hamilton	Ia.	Porc.	VSL
757503	1639	2	Van Kaalbert	Ia.	Porc.	VSL
757545	1655	2	Hampton	Wis.	Porc.	VSL
758191	1786	2	Unk.	Nebr.	Porc.	VSL
758202	1797	2	Unk.	Wash.	Porc.	VSL
758209	1804	2	Unk.	Mo.	Porc	VSL
757322	1846	2	NADL	Ia.	Bov.	VSL
758567	1886	2	Fortner	Ga.	Porc.	VSL
748421	1888	2	Unk.	Ia.	Porc.	VSL
758905	1969	2	Ga. Farm Bureau	Ga.	Porc.	VSL
758911	1974	2	Patterson	N. Y.	Porc.	VSL
759280	2082	2	Vehlow	Wis.	Porc.	VSL

759288	2088	2	Bloomfield	Ohio	Bov.	VSL
7410806.	2199	2	Unk.	Ia.	Porc.	VSL
7410918	2212	2	St. Joseph Styd.	Mo.	Porc.	VSL
-	577	3	Unk.	Colo.	Unk.	NJH
7511816	2773	3	Hunter	Conn.	Porc.	VSL
-	6197	3	Unk.	Colo.	Unk.	NJH
19649	107	4	Auburn Univ.	Ala.	Porc.	VSL
751643	428	4	Waterloo	N. Y.	Porc.	VSL
755687	1302	4	Umstead	N. C.	Sawdust	VSL
755687	1305	4	Umstead	Ill.	Porc.	VSL
746080	1486	4	Decker	Ill.	Porc.	VSL
746732	1636	4	Sioux City Styds.	Ia.	Porc.	VSL
746979	1834	4	Unk.	Ia.	Porc.	VSL
748701	1953	4	Buffalo Styds.	N. Y.	Porc.	VSL
7411771	2324	4	Weeks	Ala.	Porc.	VSL
7411774	2327	4	Weeks	Ala.	Porc.	VSL
7414048	2755	4	Unk.	Md.	Porc.	VSL
7414728	2886	4	Cal. Primate Ctr.	Calif.	Monkey	VSL
7416450	3120	4	Van Deusen	Ida.	Porc.	VSL
7411766	2319	5	Weeks	Ala.	Porc.	VSL
634443	1237	5	Unk.	La.	Bov.	VSL
12315	67	6	Unk.	Colo.	Unk.	NJH
7400565	193	6	Bovina	Tex.	Bov.	VSL
7600111	11	7	Unk.	Md.	Porc.	VSL

[a]Bov. = Bovine; Porc. = Porcine.

[b]NJH = National Jewish Hospital; VSL = Veterinary Services Laboratory; CDC = Center for Disease Control; NADL = Nat'l Animal Disease Laboratory.

[c]Unk. = Unknown.

Table 11. (Continued)

Accession #	TB#	Sero-type	Herd	State	Animal[a]	Source[b]
756196	1412B	7	Sumner	Ga.	Bov.	VSL
27688	1960	7	Gila Fdyds.	Ariz.	Bov.	VSL
29722	138	8	Camden Export	Ala.	Porc.	VSL
741158	358	8	Schaaf	Mont.	Porc.	VSL
755687	1301	8	Umstead	N. C.	Sawdust	VSL
755814	1318	8	Unk.	N. H.	Bov.	VSL
756203	1419	8	Unk.	Wash.	Porc.	VSL
756204	1420	8	Unk.	Ill.	Porc.	VSL
756994	1550	8	Unk.	Wis.	Porc.	VSL
756995	1556	8	Unk.	Mont.	Porc.	VSL
757590	1662	8	Unk.	Ore.	Porc.	VSL
748006	1856	8	Buck	Ia.	Bov.	VSL
749358	2001	8	Unk.	Md.	Porc.	VSL
759230	2077	8	Unk.	Wash.	Porc.	VSL
7416244	3062	8	Adams	Miss.	Porc.	VSL
33228	1713	9	Kubelsky	Ariz.	Bov.	VSL
34143	2232	9	North Hospital	Pa.	Porc.	VSL
7603753	498	10	Adams	Miss.	Porc.	VSL
7604833	749	10	Umstead	N. C.	Porc.	VSL
766893	1277	10	Dodge NFD	Ga.	Porc.	VSL
676910	1299	10	Umstead	N. C.	Porc.	VSL
28023	2158	10	Copeland	Ind.	Porc.	VSL
34084	2199	10	Morgan Pkg.	Ala.	Porc.	VSL
14186	1424	11	Gower	Tenn.	Bov.	VSL
14604	1610	11	Jenkins	Utah	Bov.	VSL
7512809	2987	11	Umstead	N. C.	Porc.	VSL
30869	650	12	Couteau Platte	La.	Bov.	VSL
756778	1519	12	Mize	Ill.	Human	VSL
33805	2017	12	Coz	Tex.	Bov.	VSL
-	Adcock	13	-	Colo.	Human	NJH

-	Lynn	13	-	Colo.	Human	NJH
751437	382	14	Overturf	Ida.	Porc.	VSL
30566	517	14	Welbourn	Nebr.	Bov.	VSL
7513427	3112	14	Agri. Res. Serv.	Md.	Porc.	VSL
-	2030	15	Unk.	Colo.	Unk.	NJH
-	18587	15	Unk.	Colo.	Unk.	NJH
-	Gamoh	16	-	Colo.	Human	NJH
755166	1184	16	Davis	Ga.	Bov.	VSL
7314682	4424	16	Unk.	Ala.	Porc.	VSL
755142	1170	17	Davis	Ga.	Bov.	VSL
7311124	4024	17	Unk.	Nebr.	Porc.	VSL
7500519	141	18	Ore. Primate Ctr.	Ore.	Monkey	VSL
-	2302	18	Unk.	Ga.	Unk.	NJH
7311275	4194	18	Karnes	Tex.	Porc.	VSL
-	2290	19	Unk.	Unk.	Unk.	CDC
7402359	631	19	Unk.	Unk.	Porc.	VSL
-	Findley	20	-	Colo.	Human	NJH
-	Newberry	20	-	Colo.	Human	NJH

Table 12. Biochemical reactions of M. avium isolates

Accession #	TB#	Sero-type	INH[a] 10ug/ml	TCH[b] 15ug/ml	Neotet[c] 20ug/ml	SM.[d] 2ug/ml	Rifamp[e] .025ug/ml	Tween Hydrol.	Growth[f] Rate
757497	1634	1	+	+	+	+	+	-	S
757894	1752	1	+	+	+	+	+	-	S
75829	1796	1	+	+	+	+	+	+	S
758216	1811	1	+	+	+	+	+	-	S
758217	1812	1	+	+	+	+	+	-	S
758294	1818	1	+	+	+	+	+	-	S
758516	1877	1	+	+	+	+	+	-	S
748420	1887	1	+	+	+	+	+	-	S
758704	1920	1	+	+	+	+	+	-	S
758807	1957	1	+	+	+	+	+	-	S
769804	1989	1	+	+	+	+	+	-	S
749604	2038	1	+	+	+	+	+	+	S
749607	2041	1	+	+	+	+	+	+	S
759228	2075	1	+	+	+	+	+	-	S
749809	2081	1	+	+	+	+	+	-	S
757267	1594	2	+	+	+	+	+	-	S
-	1639	2	+	+	+	+	+	-	S
757545	1655	2	+	+	+	+	+	-	S
758191	1786	2	+	+	+	+	+	+	S
758202	1797	2	+	+	+	+	+	+	S
758209	1804	2	+	+	+	+	+	+	S
757322	1846	2	+	+	+	+	+	-	S
758567	1886	2	+	+	+	+	+	-	S
748421	1888	2	+	+	+	+	+	-	S
758905	1969	2	+	+	+	+	+	-	S
758911	1974	2	+	+	+	+	+	-	S
759280	2082	2	+	+	+	+	+	-	S

759288	2088	2	+	+	+	+	+	-	S
7410806	2199	2	+	+	+	+	+	-	S
7410918	2212	2	+	+	+	+	+	-	S
-	577	3	ND[g]	ND	ND	ND	ND	ND	ND
7511816	2773	3	+	+	+	+	+	-	S
-	6197	3	ND	ND	ND	ND	ND	ND	ND
29649	107	4	+	+	+	+	+	-	S
751643	428	4	+	+	+	+	+		S
755687	1302	4	+	+	+	+	+	-	S
755687	1305	4	+	+	+	+	+	-	S
746080	1486	4	+	+	+	+	+	-	S
746732	1636	4	+	+	+	+	+	-	S
746979	1834	4	+	+	+	+	+	-	S
748701	1953	4	+	+	+	+	+	-	S
7411771	2324	4	+	+	+	+	+	-	S
7411774	2327	4	+	+	+	+	+	-	S
7414048	2755	4	+	+	+	+	+	-	S
7414728	2886	4	+	+	+	+	+	-	S
7416450	3120	4	+	+	+	+	+	-	S
7411766	2319	5	+	+	+	+	+	-	S
634443	1237	5	+	+	+	+	+	ND	S
12315	67	6	+	+	+	+	+	-	S
7400565	193	6	+	+	+	+	+	+	S
7600111	11	7	+	+	+	+	+	+	S

[a]INH = Isonicotinic acid Hydrozide.

[b]TCH = Thiophene 2-carboxylic and hydrozide.

[c]Neotet = Neotetrozolium chloride.

[d]SM = Streptomycin.

[e]RIFAMP = Rifampin.

[f]SM. = Slow grower.

[g]ND = No Data, Received from another laboratory.

Table 12. (Continued)

Accession #	TB#	Sero-type	INH[a] 10ug/ml	TCH[b] 15ug/ml	Neotet[c] 20ug/ml	SM.[d] 2ug/ml	Rifamp[e] .025ug/ml	Tween Hydrol.	Growth[f] Rate
756196	1412B	7	+	+	+	+	+	-	S
27688	1960	7	+	+	+	+	+	-	S
29722	138	8	+	+	+	+	+	-	S
741158	358	8	+	+	+	+	+	-	S
755687	1301	8	+	+	+	+	+	-	S
755814	1318	8	+	+	+	+	+	-	S
756203	1419	8	+	+	+	+	+	-	S
756204	1420	8	+	+	+	+	+	-	S
756994	1555	8	+	+	+	+	+	-	S
756995	1556	8	+	+	+	+	+	+	S
757590	1662	8	+	+	+	+	+	+	S
748006	1856	8	+	+	+	+	+	-	S
749358	2001	8	+	+	+	+	+	-	S
759230	2077	8	+	+	+	+	+	+	S
7416244	3062	8	+	+	+	+	+	+	S
33228	1713	9	+	+	+	+	+	+	S
34143	2232	9	+	+	+	+	+	+	S
763753	498	10	+	+	+	+	+	+	S
764833	749	10	+	+	+	+	+	+	S
766893	1277	10	+	+	+	+	+	+	S
766910	1299	10	+	+	+	+	+	+	S
28023	2158	10	+	+	+	+	+	+	S
34084	2199	10	+	+	+	+	+	+	S
14604	1610	11	+	+	+	+	+	+	S
14186	1424	11	+	+	+	+	+	+	S
7512809	2987	11	+	+	+	+	+	-	S
30869	650	12	+	+	+	+	+	-	S
756778	1519	12	+	+	+	-	+	-	S
33805	2017	12	+	+	+	+	+	-	S

-	Adcock	13	ND	ND	ND	ND	ND	ND	ND
-	Lynn	13	ND	ND	ND	ND	ND	ND	ND
751437	382	14	+	+	+	+	+	-	S
30566	517	14	+	+	+	+	+	-	S
7513427	3112	14	+	+	+	+	+	-	S
-	2030	15	ND	ND	ND	ND	ND	ND	ND
-	18587	15	ND	ND	ND	ND	ND	ND	ND
-	Gamoh	16	ND	ND	ND	ND	ND	ND	ND
755166	1184	16	+	+	+	+	+	-	S
7314681	4424	16	+	+	+	+	+	-	S
755141	1170	17	+	+	+	-	+	+	S
7311124	4024	17	+	+	+	+	+	-	S
7500519	141	18	+	+	+	+	+	-	S
-	2302	18	+	+	+	+	+	-	S
7311275	4194	18	+	+	+	+	+	-	S
-	2290	19	ND	ND	ND	ND	ND	ND	ND
7402359	631	19	+	+	+	+	+	-	S
-	Findley	20	ND	ND	ND	ND	ND	ND	ND
-	Newberry	20	ND	ND	ND	ND	ND	ND	ND

Table 13. Dilution table for antisera used in agglutination tests

Anti-serum #	Sero-type	Dilution Used	Anti-serum	Sero-type	Dilution Used
2116	1	1:160	1610	11	1:160
2864	1	1:160	650	12	1:160
2568	2	1:160	2017	12	1:40
2870	2	1:160	Adcock	13	1:160
577	3	1:320	Lynn	13	1:80
6197	3	1:320	517	14	1:80
22	4	1:160	3151	14	1:160
1342	4	1:80	710	15	1:80
758	5	1:80	653	15	1:160
1658	5	1:80	Gamot	16	1:80
67	6	1:160	1453	16	1:40
407	6	1:160	P-54	17	1:160
311	7	1:160	875	17	1:160
P-49	7	1:80	1107	18	1:80
138	8	1:320	1416	18	1:40
2264	8	1:80	1366	19	1:160
1713	9	1:80	2041	19	1:160
2232	9	1:160	Findley	20	1:80
2158	10	1:160	Newberry	20	1:40
2199	10	1:160	J2728	M.ptb[a]	1:160
1424	11	1:160	F862	M.ptb	1:160

[a]M.ptb = *M. paratuberculosis*.

Table 14. Results of duplicate agglutination tests on all mycobacterial isolates examined in Part I

Isolate TB#	Serotype	Test[a]	M.paratb. F862	M.paratb. 2728	Antiserum Serotype Number 1: 2116	1: 2864	2: 2568	2: 2870	4: 22	4: 1342	8: 138	8: 2264
1634	1	T	0,0	0,0	3,4[b,c]	3,4	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	2,2	2,2	0,0	0,0	0,0	0,0	0,0	0,0
1752	1	T	0,0	0,0	2,4	2,4	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	2,3	2,3	0,0	0,0	0,0	0,0	0,0	0,0
1796	1	T	0,0	0,0	1,4	1,4	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	2,2	2,2	0,0	0,0	0,0	0,0	0,0	0,0
1811	1	T	0,0	0,0	4,4	4,4	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	3,4	3,4	0,0	0,0	0,0	0,0	0,0	0,0
1812	1	T	0,0	0,0	4,4	4,4	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	4,4	4,4	0,0	0,0	0,0	0,0	0,0	0,0
1818	1	T	0,0	0,0	3,4	3,4	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	3,4	3,4	0,0	0,0	0,0	0,0	0,0	0,0
1877	1	T	0,0	0,0	4,4	4,4	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	4,4	4,4	0,0	0,0	0,0	0,0	0,0	0,0
1887	1	T	0,0	0,0	1,2	3,4	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	1,2	1,2	0,0	0,0	0,0	0,0	0,0	0,0
1920	1	T	0,0	0,0	3,4	3,4	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	2,3	2,3	0,0	0,0	0,0	0,0	0,0	0,0
1957	1	T	0,0	0,0	1,4	1,4	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	2,2	2,2	0,0	0,0	0,0	0,0	0,0	0,0
2038	1	T	0,0	0,0	1,2	3,4	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	3,4	3,4	0,0	0,0	0,0	0,0	0,0	0,0
2041	1	T	0,0	0,0	1,2	3,4	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	3,4	3,4	0,0	0,0	0,0	0,0	0,0	0,0

2075	1	T	0,0	0,0	1,4	1,4	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	1,2	1,2	0,0	0,0	0,0	0,0	0,0	0,0
2081	1	T	0,0	0,0	1,2	3,4	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	2,3	2,3	0,0	0,0	0,0	0,0	0,0	0,0
1989	1	T	0,0	0,0	1,2	1,2	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	2,2	2,2	0,0	0,0	0,0	0,0	0,0	0,0
1594	2	T	0,0	0,0	0,0	0,0	1,2	1,2	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	2,2	2,2	0,0	0,0	0,0	0,0
1639	2	T	0,0	0,0	0,0	0,0	1,4	1,3	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	2,2	2,2	0,0	0,0	0,0	0,0
1655	2	T	0,0	0,0	0,0	0,0	1,3	1,2	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	2,2	2,2	0,0	0,0	0,0	0,0
1786	2	T	0,0	0,0	0,0	0,0	1,4	1,3	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	1,2	1,2	0,0	0,0	0,0	0,0
1797	2	T	0,0	0,0	0,0	0,0	3,4	1,3	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	2,2	2,2	0,0	0,0	0,0	0,0
1804	2	T	0,0	0,0	0,0	0,0	1,3	1,3	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	2,2	2,2	0,0	0,0	0,0	0,0
1846	2	T	0,0	0,0	0,0	0,0	1,1	3,4	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	2,3	1,1	0,0	0,0	0,0	0,0
1886	2	T	0,0	0,0	0,0	0,0	3,4	3,4	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	3,4	3,4	0,0	0,0	0,0	0,0
1888	2	T	0,0	0,0	0,0	0,0	1,2	3,4	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	2,3	1,1	0,0	0,0	0,0	0,0

[a]T = Tube Test; M = Microagglutination Test.

[b]First Digit = First Reading 6 h(T) 3h(M) Second Digit = Second Reading 18h(T) 6h(M).

[c]0 = No agglutination; +1 = very small clumps of agglutinated cells suspending fluid opalescent; +2 = small clumps of agglutinated cells suspending fluid opalescent; +3 = medium size clumps of agglutinated cells suspending fluid slightly opalescent; +4 = large clumps of agglutinated cells suspending fluid transparent.

Table 14. (Continued)

Isolate TB#	Sero- type	Test[a]	M. paratb.		Antiserum Serotype Number							
					1	1	2	2	4	4	8	8
			F862	2728	2116	2864	2568	2870	22	1342	138	2264
1969	2	T	0,0	0,0	0,0	0,0	3,4	3,4	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	3,4	3,4	0,0	0,0	0,0	0,0
1974	2	T	0,0	0,0	0,0	0,0	3,4	3,4	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	3,4	3,4	0,0	0,0	0,0	0,0
2082	2	T	0,0	0,0	0,0	0,0	3,4	3,4	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	3,4	3,4	0,0	0,0	0,0	0,0
2088	2	T	0,0	0,0	0,0	0,0	3,3	3,3	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	3,3	3,3	0,0	0,0	0,0	0,0
2199	2	T	0,0	0,0	0,0	0,0	1,2	3,4	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	2,3	1,2	0,0	0,0	0,0	0,0
2212	2	T	0,0	0,0	0,0	0,0	1,2	3,4	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	2,3	1,2	0,0	0,0	0,0	0,0
107	4	T	0,0	0,0	0,0	0,0	0,0	0,0	1,2	3,4	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	1,2	1,2	0,0	0,0
428	4	T	0,0	0,0	0,0	0,0	0,0	0,0	2,2	3,3	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	2,2	3,3	0,0	0,0
1302	4	T	0,0	0,0	0,0	0,0	0,0	0,0	2,4	1,1	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	2,3	1,2	0,0	0,0
1305	4	T	0,0	0,0	0,0	0,0	0,0	0,0	1,3	1,1	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	2,2	1,2	0,0	0,0
1486	4	T	0,0	0,0	0,0	0,0	0,0	0,0	1,1	1,4	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	3,4	3,4	0,0	0,0
1636	4	T	0,0	0,0	0,0	0,0	0,0	0,0	1,1	2,3	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	3,4	3,4	0,0	0,0

1834	4	T	0,0	0,0	0,0	0,0	0,0	0,0	1,2	3,4	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	2,3	2,3	0,0	0,0
1953	4	T	0,0	0,0	0,0	0,0	0,0	0,0	2,3	3,4	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	-	3,4	0,0	0,0
2324	4	T	0,0	0,0	0,0	0,0	0,0	0,0	2,2	3,3	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	2,3	3,3	0,0	0,0
2327	4	T	0,0	0,0	0,0	0,0	0,0	0,0	2,3	3,4	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	2,3	3,4	0,0	0,0
2755	4	T	0,0	0,0	0,0	0,0	0,0	0,0	2,4	2,4	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	2,4	2,2	0,0	0,0
2886	4	T	0,0	0,0	0,0	0,0	0,0	0,0	3,3	3,3	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	3,3	3,3	0,0	0,0
3120	4	T	0,0	0,0	0,0	0,0	0,0	0,0	2,2	1,1	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	2,2	1,1	0,0	0,0
138	8	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	1,2	3,4
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	3,4	3,4
358	8	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,3	3,4
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	3,4	3,4
1301	8	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,1	2,2
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,1	2,2
1318	8	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	3,3	3,3
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	3,3	3,3
1419	8	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	1,4	1,4
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,2	2,2
1420	8	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,4	2,4
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,2	2,2
1550.	8	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,4	3,4
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,2	2,2
1556	8	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,4	3,4
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,2	2,2
1662	8	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,4	2,4
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,2	2,2
1856	8	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,3	3,4
		M	0.0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	3,4	3,4

Table 14. (Continued)

Isolate TB#	Sero-type	Test[a]	M. paratb. F862	M. paratb. 2728	1 2116	1 2864	2 2568	2 2870	4 22	4 1342	8 138	8 2264
2001	8	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	1,2	3,4
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	3,4	3,4
2077	8	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	1,4	1,4
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,2	2,2
3062	8	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	3,4	3,4
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	3,3	3,3
201	M.ptb	T	1,3	2,3	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	1,3	2,3	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
213	M.ptb	T	2,3	2,3	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	3,4	3,4	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
215	M.ptb	T	1,2	1,2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	1,2	3,4	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
351	M.ptb	T	1,2	1,2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	3,4	3,4	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
781	M.ptb	T	1,2	1,2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	1,2	1,2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
862	M.ptb	T	1,3	2,3	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	1,3	1,3	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
868	M.ptb	T	2,4	2,4	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	2,4	2,4	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
1026	M.ptb	T	1,2	1,3	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	1,2	1,3	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0

(Antiserum Serotype Number: columns 1 2116 through 8 2264)

1059	M.ptb	T	1,1	1,2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	1,1	1,2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
1249	M.ptb	T	1,2	1,2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	1,2	1,2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
1371	M.ptb	T	1,2	1,2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	2,3	2,3	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
1530	M.ptb	T	1,3	2,3	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	1,3	2,3	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
1574	M.ptb	T	1,2	2,3	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	1,2	1,2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
1586	M.ptb	T	1,2	1,2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	1,2	1,2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
1690	M.ptb	T	3,4	3,4	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	1,2	1,2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
1781	M.ptb	T	1,2	1,2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	1,2	1,2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
1800	M.ptb	T	1,2	1,2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	2,3	3,4	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
1860A	M.ptb	T	2,3	3,4	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	2,3	2,3	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
2105	M.ptb	T	1,3	1,3	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	1,2	2,2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
2436	M.ptb	T	1,3	1,3	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	1,3	1,3	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
2472	M.ptb	T	2,4	3,4	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	2,3	3,4	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
2726	M.ptb	T	1,3	1,4	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	1,3	1,4	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
2812	M.ptb	T	2,3	2,3	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	1,3	1,4	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
2943	M.ptb	T	2,3	2,3	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	1,3	2,3	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
2988	M.ptb	T	1,2	1,2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	1,2	1,2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0

Table 14. (Continued)

Isolate TB#	Sero-type	Test[a]	M. paratb. F862	M. paratb. 2728	Antiserum Serotype Number 1: 2116	1: 2864	2: 2568	2: 2870	4: 22	4: 1342	8: 138	8: 2264
4210	M.ptb	T	1,2	2,3	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	1,2	1,2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
4219	M.ptb	T	3,4	3,4	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	3,4	3,4	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
4230	M.ptb	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
4502	M.ptb	T	1,2	2,3	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	1,2	1,2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
4777	M.ptb	T	3,4	3,4	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	1,2	2,3	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0

Table 15. Results of duplicate agglutination tests on all mycobacterial isolates examined in Part II (serotypes 1-10)

			Antiserum										
			Serotype Number										
			M.ptb.		1	1	2	2	3	3	4	4	5
Isolate TB#	Serotype	Test	F862	2728	2116	2864	2568	2870	577	6197	22	1342	758
577	3	T[a]	0,0[b]	0,0	0,0	0,0	0,0	0,0	3,4[c]	3,4	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	3,4	3,4	0,0	0,0	0,0
2773	3	T	0,0	0,0	0,0	0,0	0,0	0,0	2,2	2,4	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	2,3	2,3	0,0	0,0	0,0
6197	3	T	0,0	0,0	0,0	0,0	0,0	0,0	3,4	3,4	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	3,4	3,4	0,0	0,0	0,0
2319	5	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	3,4
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,2
1237	5	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	3,4
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	3,4
67	6	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
193	6	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
11	7	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
1412B	7	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
1960	7	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0

[a]T = Tube Test; M = Microagglutination Test.

[b]First Digit = First Reading 6h(T) 3h(M) Second Digit = Second Reading 18h(T) 6h(M).

[c]0 = No agglutination; +1 = Very small clumps of agglutinated cells suspending fluid opalescent; +2 = small clumps of agglutinated cells suspending fluid opalescent; +3 = medium size clumps of agglutinated cells suspending fluid slightly opalescent; +4 = large clumps of agglutinated cells suspending fluid transparent.

5	6	6	7	7	8	8	9	9	10	10
1658	67	407	311	P49	138	2264	1713	2232	2158	2199
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
3,4	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
3,3	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
2,3	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
1,2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	2,2	2,2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	2,2	2,2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	3,3	3,3	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	2,3	2,3	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	2,2	2,3	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	2,3	2,3	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	3,4	3,4	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	3,3	3,3	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	2,3	2,3	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	2,2	2,2	0,0	0,0	0,0	0,0	0,0	0,0

Table 15. (Continued)

			Antiserum										
			Serotype Number										
			M. ptb.		1	1	2	2	3	3	4	4	5
Isolate TB#	Serotype	Test	P862	2728	2116	2864	2568	2870	577	6197	22	1342	758
1713	9	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
2232	9	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
498	10	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
749	10	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
1277	10	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
1299	10	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
2158	10	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
2199	10	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
1424	11	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
1610	11	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
2987	11	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
650	12	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
1519	12	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
2017	12	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Adcock	13	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Lynn	13	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
382	14	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
517	14	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
3112	14	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0

5	6	6	7	7	8	8	9	9	10	10
1658	67	407	311	P49	138	2264	1713	2232	2158	2199
0,0	0,0	0,0	0,0	0,0	0,0	0,0	3,4	3,4	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	3,4	3,4	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	1,2	1,2	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,2	2,2	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	3,4	3,4
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,4	2,4
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,3	2,3
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	3,3	3,3
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,3	2,3
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,3	2,3
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,3	2,3
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,3	2,3
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	3,4	3,4
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,3	2,3
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,4	2,4
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,4	2,4
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0

Table 15. (Continued)

Isolate TB#	Serotype	Test	Antiserum Serotype Number: M. ptb. F862	M. ptb. 2728	1 2116	1 2864	2 2568	2 2870	3 577	3 6197	4 22	4 1342	5 758
2030	15	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
18587	15	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Gamoh	16	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
1184	16	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
4424	16	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
1170	17	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
4024	17	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
141	18	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
2302	18	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
4194	18	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
2290	19	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
631	19	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Findley	20	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Newberry	20	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
34	M.ptb	T	2,4	3,4	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	3,3	4,4	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
55	M.ptb	T	2,4	3,4	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	2,4	3,4	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
147	M.ptb	T	3,4	3,4	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	3,4	3,4	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
157	M.ptb	T	2,4	3,4	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	2,4	4,4	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
162	M.ptb	T	2,4	3,4	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	2,3	3,4	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0

5	6	6	7	7	8	8	9	9	10	10
1658	67	407	311	P49	138	2264	1713	2232	2158	2199
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0

Table 15. (Continued)

Isolate TB#	Serotype	Test	M. ptb F862	M. ptb 2728	1 2116	1 2864	2 2568	2 2870	3 577	3 6197	4 22	4 1342	5 758
287	M.ptb	T	3,4	3,4	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	4,4	4,4	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
577	M.ptb	T	2,4	2,4	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	2,2	3,3	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
928	M.ptb	T	3,4	3,4	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	3,4	4,4	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
1587	M.ptb	T	1,4	3,4	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	2,2	3,3	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
1993	M.ptb	T	3,4	3,4	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	4,4	4,4	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
2039	M.ptb	T	2,4	3,4	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	2,3	4,4	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
2728	M.ptb	T	1,3	1,3	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	1,3	1,3	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
3819	M.ptb	T	2,4	3,4	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	2,3	3,4	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0

Antiserum columns grouped by Serotype Number (M. ptb, 1, 2, 3, 4, 5).

5	6	6	7	7	8	8	9	9	10	10
1658	67	407	311	P49	138	2264	1713	2232	2158	2199
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0

Table 16. Results of duplicate agglutination tests on all mycobacterial isolates examined in Part II (serotypes 11-20)

			Antiserum							
			Serotype Number							
			11	11	12	12	13	13	14	14
Culture	Serotype	Test	1424	1610	650	2017	Adcock	Lynn	517	3151
571	3	T[a]	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
2773	3	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
6197	3	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
2319	5	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
1237	5	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
67	6	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
193	6	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
11	7	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
1412B	7	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
1960	7	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
1713	9	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
2232	9	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
749	10	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0.0	0,0	0.0	0,0	0.0	0,0
1277	10	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
1299	10	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
2158	10	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
2199	10	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0.0	0.0	0.0	0.0	0.0	0.0	0,0	0,0

[a]T = Tube test; M = Microagglutination Test.

15	15	16	16	17	17	18	18	19	19	20	20
710	653	Gamoh	1453	P54	875	1107	1416	1366	2041	Find-ley	New-berry
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0

Table 16. (Continued)

			Antiserum							
			Serotype Number							
			11	11	12	12	13	13	14	14
Culture	Serotype	Test	1424	1610	650	2017	Adcock	Lynn	517	3151
1424	11	T	2,2[b]	2,3[c]	0,0	0,0	0,0	0,0	0,0	0,0
		M	2,2	2,3	0,0	0,0	0,0	0,0	0,0	0,0
1610	11	T	1,2	2,3	0,0	0,0	0,0	0,0	0,0	0,0
		M	1,2	2,3	0,0	0,0	0,0	0,0	0,0	0,0
2987	11	T	2,3	3,4	0,0	0,0	0,0	0,0	0,0	0,0
		M	2,3	2,3	0,0	0,0	0,0	0,0	0,0	0,0
650	12	T	0,0	0,0	2,3	3,4	0,0	0,0	0,0	0,0
		M	0,0	0,0	2,2	2,2	0,0	0,0	0,0	0,0
1519	12	T	0,0	0,0	2,2	3,4	0,0	0,0	0,0	0,0
		M	0,0	0,0	2,2	3,3	0,0	0,0	0,0	0,0
2017	12	T	0,0	0,0	1,1	1,1	0,0	0,0	0,0	0,0
		M	0,0	0,0	1,2	1,2	0,0	0,0	0,0	0,0
Adcock	13	T	0,0	0,0	0,0	0,0	1,2	1,2	0,0	0,0
		M	0,	0,0	0,0	0,0	1,2	1,2	0,0	0,0
Lynn	13	T	0,0	0,0	0,0	0,0	1,2	1,2	0,0	0,0
		M	0,0	0,0	0,0	0,0	1,2	1,2	0,0	0,0
382	14	T	0,0	0,0	0,0	0,0	0,0	0,0	3,4	3,4
		M	0,0	0,0	0,0	0,0	0,0	0,0	2,2	3,3
517	14	T	0,0	0,0	0,0	0,0	0,0	0,0	2,2	2,2
		M	0,0	0,0	0,0	0,0	0,0	0,0	2,2	1,2
3112	14	T	0,0	0,0	0,0	0,0	0,0	0,0	2,3	2,3
		M	0,0	0,0	0,0	0,0	0,0	0,0	2,2	2,2
2030	15	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
18587	15	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Gamoh	16	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0

[b]First Digit = First Reading 6h(T) 3h(M) Second Digit = Second Reading 18h(T) 6h(M).

[c]0 = No agglutination; +1 = very small clumps of agglutinated cells suspending fluid opalescent; +2 = small clumps of agglutinated cells suspending fluid opalescent; +3 = medium size clumps of agglutinated cells suspending fluid slightly opalescent; +4 = large clumps of agglutinated cells suspending fluid transparent.

15	15	16	16	17	17	18	18	19	19	20	20
710	653	Gamoh	1453	P54	875	1107	1416	1366	2041	Findley	Newberry
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
2,2	-	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
1,2	1,2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
2,2	2,3	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
1,2	1,2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	2,2	2,2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0

Table 16. (Continued)

			Antiserum							
			Serotype Number							
			11	11	12	12	13	13	14	14
Culture	Serotype	Test	1424	1610	650	2017	Adcock	Lynn	517	3151
1184	16	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
4424	16	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
1170	17	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
4024	17	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
141	18	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
2302	18	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
4194	18	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
2290	19	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
631	19	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Findley	20	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Newberry	20	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
34	M.ptb	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
55	M.ptb	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
147	M.ptb	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
157	M.ptb	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
162	M.ptb	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
287	M.ptb	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
577	M.ptb	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
928	M.ptb	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0

15	15	16	16	17	17	18	18	19	19	20	20
710	653	Gamoh	1453	P54	875	1107	1416	1366	2041	Find-ley	New-berry
0,0	0,0	1,2	2,2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	2,2	3,3	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	2,2	2,2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	1,2	1,2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	1,2	2,2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	2,3	2,3	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	1,2	1,2	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	1,2	1,1	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	1,2	1,2	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	2,4	3,4	0,0	0.0	0.0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	2,2	2,2	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	2,2	2,2	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	2,3	2,2	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	1,2	2,3	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	1,3	2,3	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,2	2,2	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,3	2,4	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	1,2	2,3	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	1,2	1,2
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	1,2	1,2
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	1,2	1,2
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	1.2	2,2
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0

Table 16. (Continued)

Culture	Serotype	Test	Antiserum Serotype Number 11: 1424	11: 1610	12: 650	12: 2017	13: Adcock	13: Lynn	14: 517	14: 3151
1587	M.ptb	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
1993	M.ptb	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
2039	M.ptb	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
2728	M.ptb	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
3819	M.ptb	T	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		M	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0

15	15	16	16	17	17	18	18	19	19	20	20
710	653	Gamoh	1453	P54	875	1107	1416	1366	2041	Find-ley	New-berry
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0