Comparison of CF and HI Tests on Psittacosis-LGV Serums

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WITHIN recent years, public health laboratories have received an unprecedented number of requests for the laboratory diagnosis of specimens from patients with suspect infections incriminating members of the psittacosis-lymphogranuloma venereum (LGV) group of viruses. Since most of the laboratories are not equipped to prepare their own psittacosis antigens for the performance of complement fixation (CF) tests, it has been necessary to rely upon commercially available sources for their supply. It has also been the practice, and at times the necessity, to substitute LGV for psittacosis CF antigens in tests on serums from cases of psittacosis on the basis that one is dealing with a group-specific antigen-antibody reaction. Meyer (*1*) stated that half of his serums positive by psittacosis CF test exhibited no reaction with lygranum antigen dosages indicated on the label. Volkert and Christensen (*2*), however, concluded from their data that both antigens in two unit dosages may be utilized for routine testing. The present study represents a comparison of three tests using psittacosis and LGV antigens on individual human serums.

Methods

The psittacosis antigen (*3*) consisted of heavily infected allantoic fluids which were phenolized and subsequently lyophilized after concentration to one-fifth the original volume. Control antigen was prepared in the identical manner using normal embryonated eggs of the same age and batch.

Lygranum CF antigen was purchased from commercial sources, and the control antigen was the one accompanying the viral antigen.

The CF test for this study was that described by Sigel and co-workers (*4*). Both psittacosis and lygranum CF antigens were titrated by the block technique with ornithosis positive serum and two units used in the respective tests. Complement was added to the antigen-antibody system in a dosage of 1.8 to 2.0 units. All test serums were preliminarily absorbed with packed sheep erythrocytes and then inactivated at 56° C. for a period of 30 minutes before testing.

The psittacosis hemagglutinating antigen was also prepared from infected embryonated eggs. The infected allantoic fluid was centrifuged at 5,000 × gravity for 1 hour and the supernatant fluid, which served as the hemagglutinin, was stored at temperatures below −30° C. The hemagglutination-inhibition (HI) test was performed according to the method of Hilleman and co-workers (*5*, *6*). All serums for this test were first absorbed with murine erythrocytes and then inactivated at 56° C. for 30 minutes.

Results

One hundred and six "negative" serums were selected to ascertain the baseline reaction of the HI test. Selections were based on a series of preliminary tests performed on serums received in the laboratory showing negative CF reactions in dilutions of 1:2 when tested with psittacosis

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Table 1. Nonspecific inhibition of serums by psittacosis HI test

Serum dilution	Number	Percent
<1:16	30	28.3
1:16	64	60.3
1:32	12	11.5
1:64	0	0

and lygranum CF antigens. These serums were also found to be negative by CF tests for certain members of the neurotropic group of viruses routinely tested in the diagnostic laboratory.

It may be seen from table 1 that nonspecific inhibition may occur in this test in serum dilutions through 1:32. Most of the hemagglutinin inhibitions appeared in serum dilutions of 1:16. A serum dilution of 1:64 must, therefore, be used for the baseline reactivity to avoid nonspecificity.

A total of 103 positive serums were tested for the comparative test series. Most of these serums (87 percent) were taken from patients presenting a clinical history of, or exposure to, members of the psittacosis-LGV group of viruses (ornithosis and lymphogranuloma venereum). The results of the three serologic tests performed on the same serums are shown in tables 2, 3, and 4.

It is apparent from table 2 that psittacosis CF antigen was able to detect a larger number of positive serums than lygranum CF antigen. Ten of the serums (9.7 percent) showed some positive titer with psittacosis CF antigen when lygranum CF antigen was unable to react at the lowest dilution of serum tested (1:2). Thirty-six (34.9 percent) of the serums elicited the same titer when tested with both CF antigens. Higher titers with psittacosis CF antigen were obtained with 61.2 percent of the serums as compared with lygranum CF antigen tested on the same serums, whereas only 3.9 percent of the serums gave higher titers with lygranum antigen.

Table 2. Number of serums reacting to psittacosis and lygranum CF tests

Lygranum CF Titers

Psittacosis CF Titers: Serum dilution	<1:2	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	Total
1:2	4	9	1								14
1:4	3	12	8	2							25
1:8		3	10	6							19
1:16	1			10	8						19
1:32	1	1		2	7	4					15
1:64	1				2	6					9
1:128								1			1
1:256										1	1
Total	10	25	19	20	17	10	0	1	0	1	103

Table 3. Number of serums reacting to psittacosis CF and psittacosis HI tests

Psittacosis HI Titers

Psittacosis CF Titers: Serum dilution	<1:16	1:16	1:32	1:64	1:128	1:256	1:512	1:1,024	Total
1:2	1	10	3						14
1:4	1	11	5	6	2				25
1:8		5	4	5	3	2			19
1:16			2	3	10	4			19
1:32			1	5	6	1	2		15
1:64				1	2	4	1	1	9
1:128								1	1
1:256							1		1
Total	2	26	15	20	23	11	4	2	103

Table 4. Number of serums reacting to psittacosis HI and lygranum CF tests

Lygranum CF Titers	Psittacosis HI Titers								
Serum dilution	<1:16	1:16	1:32	1:64	1:128	1:256	1:512	1:1,024	Total
<1:2		3	2	1	2	1	1		10
1:2	2	14	6	2		1			25
1:4		7	4	4	3	1			19
1:8		2	1	7	8	2			20
1:16			2	5	6	3	1		17
1:32				1	4	3	1	1	10
1:64									0
1:128								1	1
1:256									0
1:512							1		1
Total	2	26	15	20	23	11	4	2	103

From tables 3 and 4 one observes that an equal number of serums (60) showed positive HI titers (considering the baseline titer of the HI test to be 1:64 or higher) from both the psittacosis CF and lygranum CF groups. Five of the serums (4.9 percent) exhibiting no reaction with lygranum CF antigen in dilutions of 1:2 or higher elicited HI titers of 1:64 through 1:512. More serums (33 percent) tested with lygranum CF antigen in low dilutions (up through 1:8 dilution of serum) gave positive III titers than serums tested with psittacosis CF antigen (18 percent) in the same dilution range. Of the 93 serums which were positive by both psittacosis CF and lygranum CF tests, only 55 (or 59.1 percent) were positive to the psittacosis HI test.

Discussion

The results presented above indicate that a nonspecific reaction may be obtained in the psittacosis HI test in serum dilutions through 1:32. At a serum dilution of 1:32, 11.5 percent of the 106 serums tested showed this type of reaction. Hilleman and co-workers (*5*) have indicated from a smaller group study that human serum titers of less than 1:40 are not considered significant when tested with meningopneumonitis hemagglutinating antigen. Studies conducted by Ephrati-Elizur and Bernkopf (*7*) show that 6 percent of their apparently normal serums reacted nonspecifically in dilutions of 1:40 and 2 percent in dilutions of 1:80. They, therefore, considered HI titers above 1:80 as falling outside of the normal range of nonspecificity. It should, however, be pointed out that this unfavorable property of the HI test may not necessarily invalidate the application of this test to diagnostic procedures. The serologic criterion of an infection is generally taken as a fourfold or higher increase in titer of the convalescent phase serum over the acute phase specimen. Although individual serums elicit considerable variations in the nonspecific property, the demonstration of a rise in HI antibody with paired serums may provide significant diagnostic information.

Meyer and Eddie (*8*) state that LGV antigens from any source are unsatisfactory for the serodiagnosis of psittacosis infections and that only psittacosis antigens should be utilized in the CF test. A large number of our reactive serums were derived from patients with clinical histories indicating infections of the psittacosis-LGV group or presenting a history of exposure to birds. Our results verify the fact that the psittacosis antigen generally exhibits a higher sensitivity than the lygranum antigen in serums with positive psittacosis CF tests.

Summary and Conclusion

Three tests were compared for the detection of antibodies to the psittacosis-LGV group of viruses.

The psittacosis HI test exhibits nonspecific reactions in serum dilutions through 1:32. This places a limitation on its practical application except where a significant antibody rise in paired serums can be demonstrated.

Only 59 percent of the serums positive by both psittacosis CF and lygranum CF tests were also positive by the psittacosis HI test.

With the current techniques employed, the psittacosis CF antigen reacts with a larger number of serums and in general gives higher titers than the lygranum CF antigen.

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Local Health Departments and Rehabilitation

Local health departments with trained and experienced staff would be immensely helpful in all areas of service to the handicapped, according to the conclusions of a 1956–57 study of rehabilitation services for the deaf and hard of hearing in Metropolitan Boston. The study was conducted by the Rehabilitation Council, United Community Services of Metropolitan Boston.

Lack of adequate local public health services is basic to many of the problems of availability and use of rehabilitation services, the council concluded. They mentioned these problems in particular: hearing testing in parochial schools, followup of screening test failures, development of ancillary services in suburban and rural areas, and adequate speech reading and auditory training facilities in public schools.

Many of the findings of their study, the council pointed out, are applicable to all the handicapped.