BENTONITE, LATEX, AND CHOLESTEROL FLOCCULATION TESTS FOR THE DIAGNOSIS OF TRICHINOSIS

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AT THE Communicable Disease Center of the Public Health Service the bentonite flocculation test (BFT) is used routinely for the diagnosis of trichinosis. This test has been under continual evaluation for sensitivity and specificity since 1952. As part of this evaluation and to keep abreast of new developments, we have attempted, whenever possible, to compare the BFT with new tests and reagents as they have become commercially available. The trichina-latex antigen sold by Hyland Laboratories has been tested intensively. The antigen for the Suessenguth and Kline flocculation test marketed by the LaMotte Company and the reagents for a bentonite flocculation test and a latex test prepared by the Difco Laboratories have received limited evaluations. The accumulated results of our studies may be of help to local laboratories in interpreting their tests. They do not constitute an endorsement or rejection of the products examined.

Several particle agglutination tests have been used for the diagnosis of trichinosis. Suessenguth and Kline (*1*) adapted the Kline test for syphilis to a test for trichinosis and used cholesterol particles coated with various trichina antigens. They reported high sensitivity for both human and animal trichinosis (*2–4*). Campbell (*5*) and Coudert and Coly (*6*) used coated collodion particles, and Leikina and Poliakova (*7*), carmin particles in their tests. Vogel and co-workers (*8*) employed cholesterol-lecithin particles. Bozicevich and associates (*9*) introduced the use of bentonite particles. A series of papers from the CDC laboratory has reported evaluations of the bentonite test for detection of infection in pigs (*10*) and in humans (*11*). The efficacy of Melcher's antigen (*13*) and of metabolic antigen (*12*) has been reported as well as that of lyophilized reagents (*14*). Innella and Redner (*15*) were the first to describe the use of latex particles coated with trichina antigen. Their test was essentially a modification of the latex test for rheumatoid arthritis developed by Singer and Plotz (*16*). Muraschi and co-workers have been using the latex test since 1957 and recently published their evaluation of a slide latex-particle agglutination test for trichinosis (*17*).

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Material and Methods

Antigens. As reference antigen, Melcher's acid soluble portion of trichina larvae (*18*) was used in the CDC bentonite flocculation test. Two lots of Hyland trichina-latex antigen numbered 366R2 with expiration dates of October 10, 1961 (lot 1), and January 21, 1962 (lot 2), were used. Lot 1 was tested during July and August 1961, and lot 2 from August 1961 through April 1962. One lot of Difco-sensitized latex particles and the reagents for the Difco bentonite flocculation test, which consist of dried bentonite particles and dehydrated antigen, were evaluated.

Tests. The bentonite flocculation test used as the standard test for comparison in this evaluation was performed as follows: Serums were diluted 1:5, 1:10, and 1:20 with saline, and all positive serums were diluted serially to titer. One drop of antigen was added to 0.1 ml. of diluted serum, and the mixture rotated by machine for 15 minutes. Details for performing the test are reviewed by Kagan (*19*).

The Hyland latex test was performed as described in the brochure accompanying the

reagent. One drop of inactivated human serum (undiluted) was placed on a clean glass slide. One drop of well-suspended latex antigen was added, mixed with a stick, and spread to cover an area about 2 cm. in diameter. The slide was then rotated by hand for 2 minutes. Reactions were recorded as positive when flocculation was seen during the first minute of rotation and weakly positive when flocculation appeared between 1 and 2 minutes. Both these reactions are defined as positive in the latex test protocol; they were differentiated in this study only for comparison with results of other tests. A granular appearance which, under 30× magnification, appeared to be caused by fine floccules was recorded as a ± (negative) reaction.

The tube test was performed with the Difco latex antigen according to the procedure described by Innella and Redner (*15*). One-tenth milliliter of the patient's serum was placed in a test tube (8.5 mm. x 75 mm.), and 0.1 ml. of the sensitized latex suspension was added. The tube was shaken gently and incubated at 37° C. for 30 minutes. It was then centrifuged at 2,000 rpm for 3 minutes, and the results were read by gently shaking the tube to distribute the sedimented latex in the menstrum. Agglutination of the sensitized latex was observed by holding the tube almost horizontally. The supernates in the 4+, 3+, and 2+ tubes were sparklingly clear, whereas the supernates in the 1+ and ± tubes were slightly cloudy. No agglutination of the latex was reported as a negative test.

The Difco latex antigen also was used in a slide test by adding one drop to a drop of serum on a slide. The drops were thoroughly mixed with an applicator stick or toothpick and spread out over an area of approximately 1–2 cm.[2] The slide was rotated gently for 3 minutes. Clumping appeared with positive serums during this time.

The Difco bentonite test was performed in the same manner as the CDC test. In addition to using the Difco reagents we tested the Difco antigen with CDC bentonite particles.

The Suessenguth-Kline flocculation slide test was preformed as described in the brochure. A small amount (0.05 ml.) of undiluted serum was placed in the well of a paraffin-ringed slide. One drop of the antigen emulsion was added from a 25-gauge needle, and the mixture rotated for 4 minutes at a speed of 120 rpm on a mechanical rotator. Results were evaluated with a microscope and recorded as 4+, 3+, 2+, or + on the basis of the degree of flocculation. Only 4+ and 3+ reactions were considered positive in this study. About half of the serums were also titrated by serial dilution.

Serums. In the first evaluation of the Hyland latex antigen (lot 1), the following serums were tested: (*a*) 66 serums from patients with "proved" trichinosis infection, defined as patients with a positive muscle biopsy or with clinical symptoms in a proved outbreak or with a rising titer on serial serum examination; (*b*) 18 serums positive by the bentonite flocculation reaction (clinical histories of these patients were not available, and these cases were classified as "unproved" trichinosis; serums with low titers were deliberately chosen for study in this group and two samples lost titer on storage); (*c*) 260 serums from persons infected with other parasitic or fungal diseases; and (*d*) serums from 20 healthy males. All these serums had been stored frozen for various periods of time.

In the evaluation of lot 2 of the Hyland antigen, 294 serums sent to the laboratory for diagnosis of possible trichinosis were tested over a period of 6 months as they came into the laboratory. In this group 37 serums were from patients with "proved" infection, as defined above. Also with this antigen, serum from 14 patients in a trichinosis outbreak were retested 6 (or 7) months and 14 months after the initial examination.

The Difco latex and bentonite antigens were

Table 1. Comparison of the Hyland latex test (HLT) with the bentonite flocculation test (BFT) on specific and nonspecific serums for the diagnosis of trichinosis

Serums	Number tested	Number positive by BFT	Number positive by HLT
"Proved" trichinosis	66	62	54
"Unproved" trichinosis	18	16	12
Other diseases	260	1	1
Healthy males	20	0	0

Table 2. Comparison of the Hyland latex test (HLT) with the bentonite flocculation test (BFT) on serums submitted for routine diagnosis of trichinosis

BFT reaction	HLT reaction		Total
	Positive	Negative	
Positive	49	9	58
Negative	14	222	236
Total	63	231	294

used to test 10 serums from healthy males, 10 serums from persons with diseases other than trichinosis, and 44 serums positive by the CDC bentonite flocculation test.

In the evaluation of the Suessenguth-Kline reagent, 117 serums from Greenland, including 21 from American Army personnel and 96 from Eskimos, were tested in 1959; 211 serums from Greenland, including 25 from American Army personnel, 167 from Eskimos, and 19 from dogs, in 1960; and 35 serums submitted for routine diagnosis from persons in the United States in 1961.

Results

The titers obtained with lot 1 of the Hyland latex antigen are shown in table 1. Of the 66 serums from patients with "proved" infection, the latex test was positive on 54, and the bentonite test on 62. Three of the four negative reactions with the bentonite antigen were obtained on initial serum samples drawn from two patients with positive results on biopsy examination and from one patient with clinical symptoms in the same outbreak. The fourth negative serum was a second sample drawn 4 days after the initial sample from the patient with symptoms. All three patients had positive bentonite flocculation tests approximately 18 days after ingestion of infected pork. The latex antigen test was negative with all serums negative by the bentonite test. It was also negative on eight serums positive in low titer by the BFT from patients with clinical symptoms.

On the serums from patients with "unproved" trichinosis, the latex test gave fewer positive reactions than the bentonite test. Disagreement between the results of the two tests occurred only on serums with BFT titers between 1:5 and 1:20. Identical results were obtained with the two tests on serums from patients with other diseases and on those from healthy males.

Results of the tests made with lot 2 of the Hyland antigen are outlined in table 2. All serums were submitted for routine diagnosis and were fresh when tested. Of 294 serums, 58 were positive by the bentonite flocculation test, and 63 by the latex test. A high level of accordance between the two tests was observed, with differences on only 23 of 294 serums tested.

Table 3 shows a comparison of the titers obtained by the bentonite flocculation and the Hyland latex tests. Counting the 2+ bentonite reactions and the ± latex reactions, 497 serums were negative by both tests. Fourteen serums were negative by the bentonite tests and positive by the latex test, and 23 serums were positive by the bentonite test and negative by the latex. One hundred and twenty-eight serums were positive by both tests. (Included in this group are 14 serums shown in table 4 but not in tables 1 and 2.) There was a suggested correlation between the low titers by flocculation test and weak positive reactions with the latex antigen; the differences between the two

Table 3. Comparison of bentonite flocculation titers and Hyland latex reactions obtained on 662 serums tested for trichinosis

BFT titer	Hyland latex reaction			
	Negative —	Negative ±	Weakly positive	Positive
Negative:				
—	482	5	7	3
2+	8	2	3	1
Positive:				
5	7		6	9
10	9	2	7	11
20	3	2	7	23
40			1	11
80			1	8
160			1	15
320				10
640				7
1,280				9
2,560				1
5,120				1
Total	509	11	33	109

Table 4. Comparison of bentonite flocculation titers and Hyland latex reactions on serums from 14 patients in a trichinosis outbreak

Patient No.	February 1961, BFT titer	August 1961		April 1962	
		BFT titer	HLT reaction	BFT titer	HLT reaction
1	1:2, 560	1:640	+	1:320	+
2	1:1, 280	1:320	+	1:320	+
3	1:1, 280	1:40	+	1:20	+
4	1:20	[1] 1:10	+	1:5	−
5	1:640	1:160	+	1:160	+
6	1:320	1:40	+	1:20	+
7	1:320	1:160	+	1:40	+
8	1:40	1:10	+	1:5	−
9	1:20	1:5	+	−	−
10	1:640	1:80	+	1:20	+
11	1:640	[1] 1:320	+	1:80	+
12	1:320	1:20	+	1:20	−
13	1:10	[1] 1:5	+	−	−
14	1:80	[1] 1:20	+	−	−

[1] September 1961.

tests, however, are not entirely quantitative since 19 serums were positive by bentonite and completely negative by latex.

To determine whether reactions positive by one test and negative by the other represented false positive or false negative reactions, histories and records of specimens were checked whenever possible. Fourteen serums were negative by BFT and positive with the latex antigen. Specimens submitted at later dates from three of these patients were negative by both tests. No data were available on the remaining 11. Of the 23 serums positive by BFT and negative by the latex test, 4 were early specimens collected during a trichinosis outbreak and 7 more can be assumed to be from persons with infection on the basis of history and later tests. It also should be noted that two serums from two patients with positive biopsies and acute disease were negative by both tests. Later serums were positive by both tests.

The results obtained with serums collected over a period of 14 months from 14 patients in a single outbreak are tabulated in table 4. The initial specimens were tested in February 1961 by BFT only. Latex tests were performed on the serums collected 6 (or 7) months and 14 months later. The bentonite test showed loss of titer on the second and third specimens, and three persons became negative in 14 months. The latex test became negative on the specimens from these persons and also on specimens from three other persons. There is, however, good agreement between the two tests.

We tested Difco bentonite particles and trichina antigen and Difco sensitized latex particles in a very limited evaluation during the fall of 1960. Results of the tests are shown in table 5. Both Difco and CDC bentonite test reagents gave only negative reactions on serums from healthy persons and persons with other diseases. Of the 20 serums positive with the CDC reagent, 18 were positive with the Difco reagent. The titers with the Difco reagent were in general approximately one dilution lower, although Difco trichina antigen gave the same results on CDC bentonite particles as did CDC antigen. With the slide latex and tube latex tests, the serums from healthy persons and persons with other diseases also were negative. Of the 20 serums positive by the CDC–BFT, 14 were positive with the slide test, and 17 were

Table 5. Comparison of the Communicable Disease Center bentonite flocculation test (CDC–BFT) with a Difco bentonite flocculation test (Difco–BFT) and two Difco latex tests for the diagnosis of trichinosis

Serums	Number	Number positive by—			
		CDC-BFT	Difco-BFT	Difco latex slide test	Difco latex tube test
"Proved" trichinosis	20	20	18	14	17
"Unproved" trichinosis	24	24	(1)	18	(1)
Other diseases	10	0	0	0	0
Healthy males	10	0	0	0	0

[1] Not tested.

Table 6. Comparison of the Suessenguth-Kline (S–K) test with the bentonite flocculation test (BFT) for trichinosis

Serums	Number tested	Number positive by BFT	Number positive by S–K
Greenland survey, 1959	117	5	35
Eskimos	96	5	25
U.S. Army males	21	0	10
Greenland survey, 1960	211	13	34
Eskimos	167	12	29
Dogs	19	0	4
U.S. Army males	25	1	1
U.S. diagnostic	35	4	8

positive with the tube test. The serums with low flocculation titers among the serums positive by the CDC–BFT were the specimens which were negative with the latex test.

Another group of 24 serums positive by the bentonite flocculation test were later tested with Difco latex and Hyland latex antigens as well as with our bentonite flocculation test. Of these serums, 18 were positive with the Difco latex, and 19 were positive with the Hyland latex. Again the low titer serums by the flocculation test were the serums which were negative by both latex tests. While results of the two latex tests were similar, the tests using the Hyland latex antigen were much easier to read.

In our experience, the commercial Suessenguth-Kline antigen gives many more positive reactions not associated with clinical disease than the bentonite reagent. Of 117 serums from Greenland tested in 1959, 5 were positive by the BFT and 35 were positive by the S–K test (table 6). Twenty-one of the 35 were positive only when the undiluted serums were tested. Of 211 serums, including 19 dog serums, from a second Greenland survey in 1960, 13 serums were positive by the BFT and 30 by the S–K. All dog serums were negative by the BFT, whereas 4 were positive by the S–K. Of 35 serums submitted for routine diagnosis in 1961, 4 were found positive by the BFT, whereas 8 were positive by the S–K. All serums positive by the BFT were positive by the S–K test. Recently, serums collected during a large trichinosis outbreak were tested in another laboratory with the S–K reagent and in our laboratory with the bentonite antigen. The results of this study, which will be published elsewhere, confirm our previous conclusions that the S–K test gives many more positive reactions than the bentonite test.

Discussion

Since the bentonite flocculation test was used as the reference procedure in these studies, its reliability warrants discussion. This test has been used routinely for 8 years in our laboratory. On the basis of published (*10–14*) and unpublished data collected during this time we have concluded that it is positive on serums from persons with acute infection but does not detect the presence of encysted larvae. Positive reactions appear from 2 to 3 weeks after ingestion of infected food, with titer rising to a peak or plateau in 4 to 8 weeks followed by a decrease at unknown rate for an undetermined length of time which may depend on the severity of the initial infection. The titers range from 1:5 to 1:5,120, with the majority from 1:20 to 1:80. The test has a very high degree of reproducibility, and it does not cross-react with serums from other parasitic infections (unpublished evaluation). We believe the flocculation test does not detect very light subclinical infections.

Several problems in the serologic diagnosis of trichinosis need further study. These include false positive reactions in cases of periarteritis nodosa, mononucleosis, typhoid fever, tuberculosis, or diseases producing reversed albumin-globulin ratios (*19*); the question of how long persons retain a serologic titer after infection; and the interpretation of BFT titers of 1:5 or 1:10 which do not rise and may disappear fairly rapidly.

We are unable to explain positively why a serum is positive with one test and negative with another. Since the antigens used are crude and the particles have different physiochemical properties, it may be that different antigen fractions are adsorbed selectively on the different particles. It is also reasonable to assume that the sensitized particles in the various flocculation tests may be activated by different antibodies or may respond quantitatively to different amounts of antibody in the serum. The later point could account for the fact that most discrepancies between bentonite and latex are in the 1:5 to 1:20 titer range.

In our hands the latex test was satisfactory. The test is easy to perform, the antigen is very stable, the sensitivity, while not quite as high as the bentonite test, is nevertheless excellent. The specificity of the test is about equal to that of the bentonite test. Because it is easy to perform and requires little or no training, yet offers a high degree of accuracy, the latex test may be the test of choice for the laboratory receiving only occasional serums for the diagnosis of trichinosis.

The cholesterol reagent tested in our laboratory has a higher level of sensitivity than the BFT and therefore gave many more positive reactions. The test is too sensitive for use in diagnosing trichinosis in humans. Further evaluation for sensitivity and specificity with animal serums is indicated.

Summary

The bentonite flocculation test used for the diagnosis of trichinosis at the Communicable Disease Center, Public Health Service, was compared with latex tests employing two commercially available antigens and a flocculation test with a cholesterol-lecithin antigen, also commercially available.

All the tests were easy to perform and to read.

Results obtained on serums from patients with known diseases and on serums submitted for diagnosis of trichinosis showed that the latex test with either antigen was about equal in specificity to the bentonite test and only a little less sensitive. The cholesterol flocculation test gave more positive reactions than the BFT.

These evaluations indicate that a latex-trichina test can be used satisfactorily in laboratories receiving only occasional serums for diagnosis.

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