Serological Reagents Company

320 NORTH MERIDIAN STREET
INDIANAPOLIS 4, INDIANA, U. S. A.
CABLE ADDRESS: SEROLOGIC INDIANAPOLIS INDIANA

Dear Doctor:

THE NEW MAZZINI CARDIOLIPIN MICROFLOCCULATION TEST ANTIGEN is now available commercially. Also available are the Mazzini serum test slides, spinal fluid test slides and antigen emulsion dispenser.

Under an exclusive arrangement with Mazzini Serodiagnostic Laboratory, which formulates and standardizes each lot of antigen, we are able to offer and guarantee a product of superior and uniform reactivity and specificity at all times.

The advantages of the new Mazzini Cardiolipin Microflocculation Test for Syphilis (*The Journal of Immunology,* Vol. 66, No. 2, Feb., 1951) over the Mazzini Lipoidal Test are:

1. No ripening period of the emulsion is necessary. The antigen emulsion can be used immediately after its preparation.
2. The cardiolipin test requires 40% less serum for its performance.
3. The sensitivity of the cardiolipin test is greater by at least 4%. Moreover, the number of reactions obtained in the 3^+ and 4^+ range as compared to those obtained in the 1^+ and 2^+ range is much greater, thus facilitating reading and interpretation.
4. In a study of 282 cases of individuals suspected of being false positive reactors, the lipoidal test reacted positively in 98.22%, whereas, the cardiolipin test was positive in only 54.6%.
5. The reliability of the cardiolipin spinal fluid test is greatly increased since this test does not require the alteration of either the spinal fluid or the antigen emulsion, whereas, in the lipoidal test it is necessary to alter the spinal fluid before the test can be performed.
6. Unlike the unavoidable variations in the reactivity of different lots of Mazzini Lipoidal Antigen of at least 25%, it is possible to maintain a uniform level of variation of negligible proportions in the Mazzini Cardiolipin Antigen.

In addition, the Mazzini Cardiolipin Test has the following advantages over any other Flocculation Test:

1. Automatic elimination of zonal reactions, thus preventing grave errors in high titer infectious syphilis.
2. Requires 40% less patient's serum.
3. The sensitivity and specificity are as great, and usually greater.
4. *It is the only spinal fluid flocculation procedure in which neither the stability of the antigen emulsion nor the spinal fluid is altered.*
5. The sensitivity and specificity of the spinal fluid test are equal or greater than any good complement fixation or flocculation test.

A complimentary sample of the antigen for your evaluation will be forwarded to you upon request.

SEROLOGICAL REAGENTS COMPANY

C. M. Hudgens

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Sales Manager

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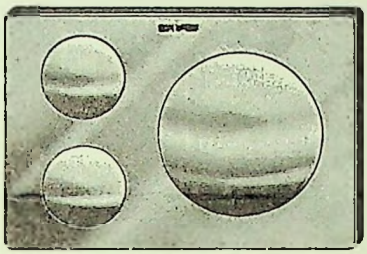


MAZZINI MICROFLOCCULATION TEST SLIDES

For the Test with Serum:

Size: 3" × 2"

Concavities: 10, each 16 mm. in diameter.

Design: 3 concavities in the upper row, 4 concavities in the middle row, and 3 concavities in the lower row.

Unlike the usual design of serological test slides, the arrangement of the concavities is such that in the event any of the four corners of the slide is chipped or broken, the entire 10 concavities will still be usable. This design also prevents the unavoidable contact of the fingers of the technician with the serum of the upper right and lower right concavities during microscopic examination.

Thickness: 6 mm. thick. Twice the thickness of the conventional slide. This feature enables the technician to handle the slide with ease during examination of the reactions. An added feature is its resistance to shock.

Depth: 1.75 mm. deep. The increased depth eliminates the spillage of serum from one concavity into another.

The surface of the slide is frosted for marking.

For the Test with Spinal Fluid:

This slide is 3" × 2" × 6 mm. thick.

2 concavities located on the left side of the slide measure 20 mm. in diameter × 1.75 mm. deep. 1 concavity on the right side of the slide measures 38 mm. in diameter × 1.75 mm. deep.

The surface of the slide is frosted for marking.

This slide supersedes the one described in *The Journal of Immunology*, Vol. 66, No. 2, February, 1951.

2. Add 1 drop of antigen emulsion (the same emulsion as used for the serum test) to each chamber containing spinal fluid.
3. Rotate the slide holder on a mechanical shaker for 10 minutes at 100 to 120 rpm.
4. Immediately after this rotation, add 2 drops of 0.9% saline solution from a medicine dropper to the chamber containing 0.05 ml of spinal fluid, none to the others.
5. Rerotate the slide holder for an additional 20 minutes at 80 to 100 rpm.
6. Read and record the reactions in the same manner as described for the serum test. Report the result of the strongest reaction obtained regardless of which of the 3 quantities it might be.

Example:

0.05 ml	0.2 ml	0.5 ml	
1+	2+	4+	Positive
N	1+	3+	Positive
4+	2+	2+	Positive
2+	4+	3+	Positive
N	1+	2+	Weakly Pos.
N	N	1+	Weakly Pos.

Supplementary Quantitative Test with Spinal Fluid

A strongly positive spinal fluid should be further quantitated in the following manner:

1. Prepare serial dilutions of spinal fluid (1:2 to 1:16, and higher if necessary).
2. Pipette 0.5 ml of each dilution into the respective 30 ml chamber of the glass slides.
3. Add 1 drop of antigen emulsion (the same emulsion as used for the serum test) to each chamber containing spinal fluid.
4. Rotate the slides on a mechanical shaker for 10 minutes at 100 to 120 rpm.
5. Rerotate for an additional 20 minutes at 80 to 100 rpm.
6. Read and record the reactions of each spinal fluid dilution.
7. Report in terms of the highest dilution giving a positive result in the same manner as described for the quantitative test with serum.

SEROLOGICAL REAGENTS COMPANY
320 North Meridian Street
Indianapolis 4, Indiana, U. S. A.

MAZZINI

CARDIOLIPIN ANTIGEN

For the microflocculation test for syphilis.

Compounded and standardized by
MAZZINI SERODIAGNOSTIC LABORATORY

Preparation of Serum

The patient's serum is separated from the clot by centrifuging and heated for 30 minutes in a water-bath at 56° C. Inspection of sera for precipitated substances following the heating period should be done as a matter of routine, if present, they should be removed by centrifugalization. Sera should be reheated for 10 minutes if re-examined more than 4 hours after the original heating period.

The Test Slides

The glass slide for the serum test is a specially designed slide 3" x 2", having 10 chambers, 16 mm in diameter. This slide has been found to produce the most satisfactory results with this test. The glass slide for the spinal fluid test contains 7 chambers, 16 mm in diameter and 2 chambers 38 mm in diameter.

The Antigen Emulsion Dispenser

The antigen emulsion is dropped from a small syringe, or from an "observation tube" No. 420 LST, fitted at one end with a 25-gauge needle and on the other with a rubber nipple. The observation tube dispenser is inexpensive, more convenient and more efficient than the syringe.

Preparation of the Emulsion

The reagents for the test consist of two solutions: (1) cholesterolized antigen solution and (2) buffered saline solution. The antigen emulsion is prepared daily, is kept at room temperature and can be used throughout the day.

1. Pipette 0.4 ml of buffered solution to the bottom of a 30 ml round bottle.
2. With a 1 ml pipette, measure 0.4 ml of cholesterolized antigen (measurement is made from tip of pipette). Hold the bottle in the left hand and

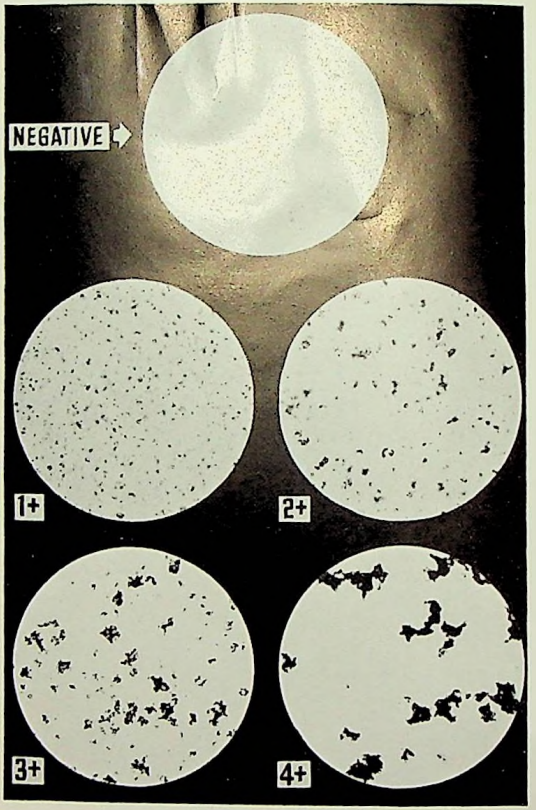
imparting a rapid and constant rotating motion to the bottle add the antigen directly and at once, blowing out whatever antigen is left in the pipette. Draw the emulsion into and out of the pipette exactly six times, returning all the emulsion left in the pipette on the last mixture.

3. Add 2.6 ml of buffered solution rapidly. Cork the bottle with a paraffin coated cork or one covered with tin foil and shake from bottom to cork and back 50 times in 15 seconds. The emulsion is then ready for immediate use and continues usable for the entire day. The emulsion should be mixed gently each time it is used.

Qualitative Test with Serum

As a check on the antigen emulsion and the 0.9% sodium chloride solution, known positive serum, weakly positive serum and the saline solution should be tested prior to performing the tests.

1. With a 1 ml pipette, place 0.03 ml of each patient's serum in the corresponding chamber of a glass slide which has been made grease and lint free.
2. Discharge 1 drop of antigen emulsion from an observation tube fitted with a 25-gauge needle, held at a 45° angle, to each serum.
3. Rotate the slide holder by mechanical shaker for 4 minutes at 160 to 180 rpm, or by hand at 120 rpm.
4. Immediately after this rotation, add 1 drop of 0.9% salt solution from a medicine dropper, having an opening to deliver approximately 0.05 ml (the quantity need not be exact) to each chamber containing serum and rerotate the slides for an additional 4 minutes at 100 to 120 rpm, either by hand or by mechanical rotator.
5. Examine the results under the low power (16 mm) objective of the microscope with bright light. Record the results as follows: No clumping, negative (reactions which give the antigen particles a coarse appearance, but no distinguishable aggregation, are also read as negative); very small but definite clumps, 1+; small clumps, 2+; medium size clumps, 3+; large clumps, 4+. An alternative method of reading and reporting may be used as follows: No clumping or coarse reactions, negative; 1+ and 2+, weakly positive; 3+ and 4+, positive.



Positive and weakly positive reactions must be checked before reporting the results in order to prevent errors in pipetting, accidental spilling of sera and incorrect recording. If zonal reactions should be suspected or are evident after carrying out the test procedure, the serum should be diluted serially from 1:2 to 1:64 or higher, if necessary, and retested according to the quantitative procedure.

Quantitative Test with Serum

1. Prepare serum dilutions of 1:2 to 1:64, and higher if necessary, in the following manner:
   a. Pipette 0.5 ml of buffered solution, or unbuffered 0.9% saline if the former is not available for this purpose, into each of 6 (or more) test tubes.
   b. Add 0.5 ml of heated serum to the first tube and mix thoroughly, but avoid foaming.
   c. Transfer 0.5 ml of the diluted serum from the first to the second tube and mix thoroughly.
   d. Continue transferring and mixing from one tube to the next until all dilutions have been made. Allow the mixing pipette to remain in the last tube in the event that higher dilutions may be necessary.
2. Pipette 0.05 ml of each dilution into the corresponding chamber of a glass slide.
3. Add 1 drop of antigen emulsion (the same as is used for the qualitative test) to each serum on the slide.
4. Rotate the slide holder by mechanical shaker for 4 minutes at 160 to 180 rpm, or by hand at 120 rpm.
5. Read and record the results in the same manner as described for the qualitative test.
6. Report the results in terms of dilution units according to the highest dilution producing a positive reaction (3+ or 4+). Thus, if a 3+ reaction is obtained in a dilution of 1:16, the report should read: 16 dilution units.

Test with Spinal Fluid

Centrifuge spinal fluids at 2,000 rpm for 5 minutes. Decant the clear supernatant fluids into clean test tubes.

Spinal fluids are tested *without* heating.

Bloody spinal fluids are unsatisfactory for examination.

Performance of the Test

1. Pipette 0.05 ml and 0.2 ml of spinal fluid into two separate 16 mm chambers and 0.5 ml into the 30 mm chamber of a glass slide. Known positive and negative spinal fluids should be included as controls for the spinal fluid series.