SIMPLIFIED RAPID DIFFERENTIATION OF TYPHUS FEVER RICKETTSIA AGGLUTINATION ON THE SLIDE-GLASS

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Sero-immunological studies of rickettsia have long been rather tardy in progress owing to its cytotropic nature and the fact that it grows not on the ordinary culture media, in other words, for being difficult to obtain it pure and in abundance.

This difficulty has been at last overcome and a remarkable advance in its serological and immunological directions has been made since 1938 when Barkine¹⁾ and Cox²⁾ have published their new method of culturing rickettsia in the yolk-sac of the developing chick embryo and especially after the vaccine has been prepared by ether extract according to Craigie's method since 1941. And now the both rickettsia suspensions, *Rickettsia prowazekii* and *R. typhi* are made as the antigens for the complement fixation test to differentiate the epidemic typhus from the murine (Topping,⁴⁾ Wilcocks,⁵⁾ Bengtson,⁶⁾ Tamiya⁷⁾ and Kitaoka⁸⁾). These suspensions may be employed for the *Rickettsia* agglutination test though they are more concentrated as an antigen, not only in differentiation of the typhus fever but also capable of analysis on antigenic structures of the agents by means of the absorption test (Fitzpatrick,⁹⁾ Wilcocks⁷⁾ and Kitaoka¹⁰⁾).

Simplified rapid differentiation of epidemic typhus from the murine on the slide-glass is owing to the success of preparing the pure rickettsia suspension and the specific rickettsia agglutination is observed on the slide-glass with the specific antiserum against the rickettsia suspension by the technic similar to the field agglutination kit of Weil-Felix reaction (Kitaoka¹¹⁾). It is to be noted that the reaction is, when the same care as in practice of Weil-Felix reaction on the slide-glass is paid, so specific that man can differentiate rapidly the both epidemic and murine typhus each other at the bed-side.

MATERIAL FOR THE TEST AND METHOD

The Antigen (rickettsia suspension): As Merrill¹²⁾ has mentioned in his report, it is essential to use an antigen of high concentrated suspension of rickettsia, so minute as to 0.3 to 0.5 μ in diameter, to make agglutination to be visible by naked eyes. Number of rickettsia in each cc of the fresh vaccine prepared by us according to Cox-Craigie's method is calculated approximately 500 to 900 millions. If the vaccine contains a large particle it should be centrifugalized first at the low rotation, 2,000 or 3,000 rpm for five minutes to sedimentate the large particle and then the supernatant

is centrifugized at 5,000 or 6,000 rpm for an hour. The supernatant is discarded and the sedimentation resuspended in saline. The same procedure is repeated three or four times and the sedimentation is resuspended approximately in one-fortieth (1/40) of the original volume with the saline added with formalin at 0.5% and mixed with normal rabbit serum at 0.2% to avoid a spontaneous agglutination during its preservation and with a drop of 5% methyl-blue aqueous solution to read the result of the agglutination easily. The fresh antigen should be titrated with four units of the homologous standard antiserum and eight or sixteen units of antigen are to be used for the test unless they show any cross reaction or are too thin to read by naked eyes. If the antigen were used with a cross reaction the result would be read not correctly and sometimes reversely. The antigen is useful for several months if preserved in the refrigerator.

Positive and negative control sera; It is very important to note that both positive and negative sera are used as control to make sure the result of rickettsia agglutination with a test serum. The positive serum is obtained in the convalescent stage of epidemic and murine typhus cases, or, from the rabbit immunized with *Rickettsia prowazekii* or *R. typhi* respectively. The immune sera are properly diluted for use to avoid the cross reaction. If the highly immune serum were used as a positive control serum the test sample would be misjudged reversely because of a cross reaction. The same caution should be paid for the human convalescent serum. It is preferable to use the antiserum of which the titration for rickettsia agglutination is four or eight units without showing any cross reaction. The negative control serum is obtained from the normal human or rabbit.

Equipment and glass ware (see illustration); All equipments necessary to carry out the field agglutination kit for Weil-Felix reaction are also needed here.

- (1) Standard droppers. Two dropper-bottles for eye-lotion are so regulated with the tip as to measure 1.0 cc by 25 drops, or one drop to measure 0.04 cc, of the antigens *R. prowazekii* and *typhi* respectively.
- (2) Standard loops. According to Kudicke's¹³ report a loop of two mm diameter is made to load some 0.003 cc of serum or blood to be tested.
- (3) Slide-glasses. Two well cleaned slide-glasses, bearing 3 circles of some 1.5 cm diameter drawn by dermatographic pencil, are used for each serum.

Procedure; (1) One loopful of the serum to be tested is spread round, some 1.5 cm diameter over the center circle each of two slide-glasses mentioned above. In case with blood two loopfuls are used for a blood contains the serum in the amount of about one half.

The materials should be spread wide to mix well both antigen and antibody, a false reaction may result otherwise. Kudicke recommends to dry in the air in case with blood. Such dried specimen of blood on slide-glasses may well preserve for several days for the test. Blood is usually drawn from ear-lap, finger-tip or the median vein, however, the clearer result can be observed with the serum and not with the blood.

(2) Positive and negative control sera; Two slide glasses so bearing the serum or blood to be tested over the center circles as described above are now to furnish with a loopful of positive control serum of *R. prowazekii* in one glass, and of *R. typhi* in the other, spread round some 1.5 cm in diameter, over the circles on the left side, and with a loopful of negative control serum in each right side.

(3) One drop of the rickettsia suspension (antigen) of *R. prowazekii* and of *R. typhi* is added to all three circles drawn on each slide-glass respectively to cover the serum or blood to be tested as well as the control serum. The compounds are thoroughly mixed by shaking the slides gently.

(4) Reading; While the simple slide-glass method of Weil-Felix reaction is dull in agglutination of *Proteus* OX19 at the temperature below 20°C., rickettsial suspensions

are found to be agglutinated in their respective positive sera even lower than 11°C ranging from one to 5 minutes. The test serum (or blood) exhibiting so positive agglutination against *Rickettsia prowazekii* as seen in the positive control serum and being negative against typhi, may undoubtedly be understood of epidemic typhus and vice versa. If some serum shows positive reaction against either [rickettsiae, the serum is to be further diluted to some extent to avoid the cross reaction by adding a few drops of the rickettsia suspension. One drop of such suspension is, as a rule, good enough for a loopful of the serum to be tested, i.e. the serum is diluted to approximately 1:14. If two drops of the suspension are mixed with a loopful of serum the serum is diluted 1:28.

When the rickettsial suspension is dropped to two loopfuls of the blood which have been spread and dried over the slide-glass, the hemolysed serum is mixed well with an antigen, however, if fibrines or blood cells are liberated the reading of the result is not easy.

Remarks; (1) The antigen should contain abundant rickettsia enough for agglutination to be visible by naked eyes and the titer of the antigen should not be so high that the cross reaction occurs.

(2) Dilution of the serum to be tested should always be minded if it is adequate or not.

(3) Room temperature at which the test is to be done should not be too low. Regulation of the room temperature may be necessary because the low temperature apt to suppress generally an agglutination.

(4) Both positive and negative control sera should be tested at the same time to confirm positivity and negativity of the test sample.

Specificity of the reaction mentioned above is re-approved by the results in the complement fixation test and rickettsia agglutination in the test tubes. How soon after the onset of the disease does the reaction began to prove positive? In two cases trials on the 6th and 8th days of the disease were negative while in 15 cases from the 9th day of the disease to 6 months after onset the positivity has been proved.

DISCUSSION

Simplified slide-glass method for Widal's reaction, agglutination test on the slide glass of *Brucella bacillus*, field agglutination kit of Weil-Felix reaction and others have already been reported respectively. And now rickettsia agglutination on the slide-glass is described. That the agglutination of rickettsia so minute as to be 0.3 to 0.5 μ is visible by naked eyes is approved logically by Merrill.¹³⁾ Serological differentiation of epidemic typhus from murine, which had long been thought to be specially difficult has become able through the rickettsia agglutination and the complement fixation test with the specific antigen prepared from rickettsia suspension made according to the Cox-Craigie's method.

Rickettsia antigen for the agglutination test on the slide-glass is prepared as mentioned above. It should be pointed out that the titration of antigen is to be

estimated to make an adequately concentrated rickettsia suspension to avoid a cross reaction.

A typical epidemic typhus is generally different to some extent in its clinical findings, causative agent, animal experiment, vector and epidemiological aspects from a murine typhus. The prophylactic measurement against epidemic typhus is distinct from the other, however, often confused each other in the mild case on the clinical findings.

Recent progress in serological studies in differentiation of one from the other has made it possible to differentiate each other by means of the complement fixation test, rickettsia agglutination test and neutralization test. If these tests would be done completely the training is necessary and they are expensive. Our slide-glass method for the rickettsia agglutination test is so simple and performed so easily at the bedside as to that for Weil-Felix reaction. Furthermore it is specific for respective rickettsia and the differentiation each other is possible within a few minute under the following condition where the adequately concentrated rickettsia suspension and adequately diluted test serum are used and the temperature at which the test is done is not too cold for agglutination to occur. The results is made sure by those in both positive and negative sera. Anyway we must keep it in mind that the rickettsia agglutination on the slide-glass is an antigen-antibody reaction though it is simple.

It may be noteworthy there was a convalescent serum positive in dilution 1 : 1280 in Weil-Felix reaction which proved positive to either antigen of *Rickettsia prowazekii* or *R. typhi*. Such cross reaction was, however, overcome with further dilution of the test serum by adding drops of the antigen. In other words the serum was diluted to the extent differentiating each other. Furthermore it is to be emphasized that the rickettsia agglutination on the slide-glass is still able to differentiate the typhus fever from the other febrile disease even if the differentiation of epidemic typhus from the murine is impossible.

SUMMARY

Simplified slide-glass method for rickettsia agglutination was described in detail. The antigen should be titrated with the standard antiserum. If the adequately concentrated rickettsia suspension is used as antigen and the test sample is adequately diluted and the room temperature is not too cold for agglutination to occur, the rickettsia agglutination on the slide-glass is able to differentiate epidemic typhus from murine. It is simple and easily done at the bedside and suitable for mass examination. It proves positive in 15 cases ranging from the 9th day of the disease to 6 months after onset. If the antigen may miss the specific partial antigen for respective rickettsia owing to be poorly prepared or wrong preserved it is no more able to differentiate the typhus fever each other. But it is suitable to differentiate the typhus fever from the other febrile diseases. In other words it serves as a screening test for the typhus fever from the other febrile diseases like the complement fixation test with the soluble antigen.

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