29. Detection of Rheumatoid Factor from Dried Bloodstains

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Stapp and Berks (1949)¹⁾ and other researchers²⁾⁻¹²⁾ established the methods to detect neutralizing antibodies in dried bloods on filter paper. This method is convenient for pediatric practice. In the field of forensic medicine, King *et al.* (1974) proved the presence in bloodstains of antibodies produced by parasitic infections¹³⁾ and pollen allergies^{14),15)} by indirect fluorescent antibody technique and radio-allergosorbent test. They considered that the test of these antibodies can offer valuable informations, such as victims' clinical circumstances and native places.¹⁶⁾

This report concerns to the detection of rheumatoid factor (RF) from bloodstains. Methods for this study is applicable to the practice of forensic medicine because of relatively high incidence of rheumatoid diseases and simplicity of technique.

Materials and methods. 1) *Bloodstain*: Blood from adult patients with rheumatoid arthritis was supplied by the Department of Collagen Diseases, School of Medicine, Juntendo University. Blood samples were drawn by venepuncture and immediately immersed to the sheets of gauze which were previously washed to remove starch and air-dried. Blood from so-called sero-negative rheumatoid arthritis patients were not used. Control bloodstains were prepared by using the bloods obtained from healthy and RF negative adults. To confirm the classification of patient and control groups, small quantities of sera, separated from the donors bloods, were tested to detect RF at the day of sample collection. Test method for these fresh sera was followed to the indications of reagent manufactures. The gauze sheets with bloods were completely dried on clean filter papers at room temperature, and stored in petri dishes placed on a desk in our laboratory, of which circumstance temperatures were about 10 to 25°C. For the experiments of heating and treatment with dithiothreitol or organic solvent, bloodstains of strong RF were used after 1 week from preparation.

2) *RF detecting latex reagents*: Four kinds of reagents were commercially obtained. Remaining one reagent was prepared by mix-

ing 1 part of 0.5% "high stability type" latex particles¹⁷⁾ and 1 part of human IgG (0.5 mg/ml) purified by the method of Kendall,¹⁸⁾ followed by heating for 120 min at 56°C in a water bath.

3) *RF detection test*: To 1 ml of 0.05 M glycine buffer (pH 8.2) in a tube was immersed about 1 cm² of the gauze stained with about 1.3 mg of the dried blood. After heating the tube for 5 min at 50°C in a water-bath, the gauze was 7 to 8 times poked by a clean glass rod, and 1 drop (about 0.03 ml) of extracted fluid was taken by a pipette. The extract and an equal quantity of RF detecting latex reagent was mixed on a hole glass (glass plate with concaved holes), which was continuously rotated for 1 min by hands or slide test rotator (Kayagaki Works, Tokyo). The hole glass was then allowed to stand for 10 min at room temperature and at the conditions to prevent evaporation, e.g. storage in a humidified chamber or coverage with another glass plate. Results were read under weak magnification by a hand lens or concaved mirror. Intensities of reactions were classified to ++(strong positive), +(moderate positive), w(weak positive), tr(trace occurrence of agglutination) and negative. Test results were determined to be positive for RF, when agglutination of the grades, weak or stronger, occurred.

4) *Dithiothreitol treatment of serum and bloodstain extract*: The method of Olson *et al.*¹⁹⁾ was used.

5) *Treatment of bloodstains with methanol or acetone*: The gauze with bloodstain was dipped into the acetone or methanol in a tube for 10 min, and completely dried on a clean filter paper.

Results. Preliminary experiments of this study showed that the RF in bloodstain extracts caused weak agglutination compared with RF detection test for sera by the routinely used latex fixation technique. Accordingly appropriate property of RF detecting latex reagent suitable to this study was considered to show high reactivity especially toward weak RF. This qualification was sufficed by two among five latex reagents. These two reagents, one of which was commercially obtained and the other was prepared by our laboratory, did not cause nonspecific agglutination even after 20 min after mixing with bloodstain extracts. This brought about the increase of sensitivity due to prolonged reacting time.

Table I shows the results obtained by using the reagents prepared by our laboratory. Times for agglutination appearance was gradually prolonged by the lapse of time after staining of bloods. Weak RF lost its activity within 4 weeks, whereas potent RF retained its reactivity for 30 weeks. Table II shows the summarized results of the RF detection from bloodstains. A half of RF positive samples were proved the presence of RF after 5 weeks storage. All the bloodstains, show-

Table I. Agglutination reaction of latex particles coated with denatured human IgG by bloodstain extracts

RF	Times for result reading	Time lapse after staining of bloodstains			
		1 week	4 weeks	10 weeks	30 weeks
Strong positive	1 min	+	+	+	tr
	2 min	++	++	+	tr
	5 min	++	++	++	w
	10 min	++	++	++	w
Weak positive	1 min	—	—	—	—
	2 min	tr	—	—	—
	5 min	+	—	—	—
	10 min	+	w	—	—
Negative	1 min	—	—	—	—
	2 min	—	—	—	—
	5 min	—	—	—	—
	10 min	—	—	—	—

ing positive reactions after 10 weeks, were of the patients having strong RF in their sera. All bloodstains prepared from RF negative bloods caused no agglutination. Reproducibility of this test was determined to be good, since repetitious tests performed by two persons showed closely similar results. The RF in bloodstains did not agglutinate latex particles coated with native human IgG. Extracts from three RF positive bloodstains lost their activities after dithiothreitol treatment, and similar results were obtained by a parallel experiment

Table II. Correlation between time lapse after staining and numbers of positive case

RF	Numbers tested	Time lapse after staining of bloods				
		1 week	4 weeks	10 weeks	30 weeks	50 weeks
Positive	14	11(78.6%)	9(64.3%)	7(50.0%)	4(28.6%)	1(7.1%)
Negative	20	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)

Table III. Influence of heating and immersion to organic solvents on activity of RF in a bloodstain

Treatments	Agglutination of latex particles coated with denatured human IgG	
None		+
Heating at 100°C	10 min	+
	30 min	—
Immersion to acetone	10 min	+
	30 min	+
Immersion to methanol	10 min	—
	30 min	—

using the sera in solution. This result indicated that RF in the bloodstains, as in fresh sera, were in most parts consisted of IgM.

As shown in Table III, the RF in a bloodstain scarcely weakened after 10 min heating at 100°C in a oven, and it completely disappeared after an additional 20 min heating. Immersion of bloodstains into methanol destroyed the RF activity, whereas equal treatment with acetone scarcely decreased the potentialities.

Discussion. Rheumatoid arthritis is the most frequent autoimmune disease of which prevalence rate in general population is about 1 to 3%; and 65 to 75% of the patients are determined to be RF positive by the agglutination test using latex particles coated with denatured human IgG.²⁰⁾ Because of its relatively high incidence, estimation of this disease from bloodstain may have a value for the practice of forensic medicine.

The RF detection method reported here is simple in procedure and good in reproducibility. Some previous reports described that the nonspecific agglutination of latex particles sometimes disturbed the result determination of RF detection test in clinical laboratory.²¹⁾ However, the use of suitable RF detecting reagents for this study prevented the occurrence of such undesirable phenomenon.

According to the results of this study, bloodstains contaminated with oily substances were suggested to be washed with acetone, because this treatment caused no decrease of RF activity. The activity in bloodstain was considered to be rather thermostable, because it was proved after incubation in a 100°C chamber for 10 min.

Previous reports²²⁾⁻²⁴⁾ indicated that the presence of isohemagglutinins in bloodstains were proved for over 7 months by means of using sensitive techniques, and antibodies related to some parasite infections and pollen allergy could be detected after several months storage.^{13), 14)} In our studies prior to this study showed that the sensitivity of RF was much improved by adopting some modified techniques, and RF in bloodstains could be proved in most samples even after 1 year from staining. However, researches along this line were abandoned, because feeble anti-human IgG factors other than RF in non-rheumatoid persons²⁵⁾⁻²⁹⁾ sometimes caused positive reactions unless the stain tested elapsed two weeks or so after preparation.

It has been considered that the resistance of IgM antibodies toward denaturation is weak relative to antibodies belonging to IgG. For example, Cohen *et al.* (1969)³⁰⁾ revealed that IgM antibody to yellow fever rapidly declined regardless storage temperature, whereas IgG antibody with same specificity kept its activity for a long period of time. In the results of this study, however, strong RF in bloodstains resisted to dryness for over 30 weeks, although the factors in blood-

stains were determined to be principally IgM. These results may be the indication of strong potentialities of RF of some cases. It can be conceivable conclusion that the blood is probably originated from rheumatoid patients, if the bloodstain tested is positive for RF. The accuracy of this estimation increased according as time lapse after staining of blood, because only the strong RF, produced in most instances by advanced rheumatism, were detectable for over 10 weeks. When no RF is detected, on the other hand, it can be concluded that the bloodstain is of non-rheumatoid persons, because RF negative rheumatism is not infrequent, and weak RF may lose its activity due to dryness.

Conclusion. A detection methods for rheumatoid factor in dried bloodstain was described. By using appropriate kinds of latex reagents coated with denatured human IgG, presence of the rheumatoid factor was proved from the bloodstain positive for the factor. Strong rheumatoid factor in bloodstains could be detected after 30 weeks from staining.

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