DISCUSSION

1. An increased pH above 7.70 causes a delay in precipitate formation. It is therefore important to use buffered sodium citrate as the anticoagulant. Sodium oxalate produces too alkaline a pH.
2. The presence of heparin, or contamination with a few red cells, does not alter the results of this test.

FIBRIN-SPLIT PRODUCTS

Fibrin- (fibrinogen) split products may be demonstrated in the blood of patients with primary fibrinolysis and during the process of disseminated intravascular coagulation with secondary fibrinolysis.

The Thrombo-Wellcotest procedure described here is a rapid, sensitive test for fibrin- (fibrinogen) split products present in the blood. The normal level of serum fibrin-split products in the adult is 2.1 to 7.7 μg per ml.

Thrombo-Wellcotest

REFERENCE

Wellcome Reagents Limited: *Thrombo-Wellcotest. Rapid Latex Test for Detection of Fibrinogen Degradation Products*, Wellcome Research Laboratories, Beckenham, Kent, England, 1977.

REAGENTS AND EQUIPMENT

1. Sample collection tubes (contain thrombin to cause rapid and complete clotting, and soya bean enzyme inhibitors to prevent the breakdown of fibrin).
2. Glycine buffer.
3. Latex suspension. (The latex particles have been sensitized with an anti-fibrin-split product globulin.)
4. Positive control serum.
5. Negative control serum.
6. Glass test slide.
7. Disposable pipet droppers.
8. Disposable mixing rods.
   (Note: All preceding reagents are available from Wellcome Reagents Division, Burroughs Wellcome Co., Research Triangle Park, N.C. 27709.)
9. Test tubes, 10 × 75 mm.

SPECIMEN

Obtain 3 ml of whole blood. Immediately transfer from the syringe 2 ml to the sample collection tube. These tubes may also be used with a Vacutainer system and will draw 2 ml of blood. As soon as the blood is in the tube, mix well by inverting several times.

PRINCIPLE

Whole blood is added to thrombin (to ensure complete clotting) and soya bean enzyme inhibitors (to prevent any breakdown of fibrin). After incubation, the patient's serum is diluted and mixed with anti-fibrin-split products latex particles. If fibrin- (fibrinogen) split products are present, the latex particles will agglutinate.

PROCEDURE

1. Obtain the blood specimen and place 2 ml into the sample collection tube. Immediately mix the tube by inverting several times.
2. The blood in the sample tube should clot quickly, within one-half minute. As soon as clotting has occurred, ring the clot with an applicator stick to allow good clot retraction.
3. Allow the blood sample to stand at room temperature, or 37°C, for 30 minutes to allow for clot retraction.
4. As soon as the serum has separated from the clot, remove the serum from the sample collection tube with a disposable dropper. (If red cells are present in the serum, centrifuge serum and separate from the cells. The serum may be obtained more quickly by centrifuging the specimen after clotting is complete.)
5. Label two 10 × 75-mm test tubes for each patient sample being tested, 1:5 and 1:20.

6. Place 0.75 ml of glycine buffer into each of the preceding test tubes.
7. Mark two of the rings on the glass slide to correspond to the two tubes: mark one 1:5 and the second 1:20. Label the third ring for the negative control and the fourth ring for the positive control.
8. Add five drops of patient's serum to the tube labeled 1:5, and one drop of serum to the tube marked 1:20. Mix both tubes well.
9. Using a disposable test tube dropper, transfer one drop of the patient's 1:20 serum dilution to the appropriate ring on the glass slide; place one drop of the 1:5 dilution in the 1:5 labeled area on the glass slide. Add one drop each, of the negative and positive control, to the appropriately labeled rings on the slide.
10. Mix the latex suspension of the anti-fibrin-split products globulin vigorously. Immediately add one drop to each of the serum dilutions.
11. Using the disposable mixing rods, stir each serum–latex mixture, beginning with the 1:20 dilution. Spread mixture over the entire area of the ring.
12. Stir the mixtures for 2 minutes, using a back and forward motion. Examine each solution for macroscopic agglutination.
13. Interpretation of results. No agglutination in either of the patient mixtures indicates that no fibrin-split products are present, or that fibrin-split products are present in a concentration less than 10 μg per ml. Agglutination present in the 1:5 dilution but none present in the 1:20 dilution indicates that fibrin-split products are present in a concentration greater than 10 μg per ml but less than 40 μg per ml of serum. Agglutination present in both dilutions of serum is indicative of a concentration of fibrin-split products greater than 40 μg per ml of serum. The negative control should show no agglutination, whereas the positive control will show agglutination.

DISCUSSION

1. If the patient to be tested is receiving heparin, Reptilase-R should be added to the patient's blood in the sample collection tube in order for clotting to occur. (Add 1.0 ml of distilled water to the Reptilase-R. Pipet 0.2 ml of the reconstituted Reptilase-R to 2 ml of the patient's whole blood. Mix.)
2. If agglutination occurs in the 1:20 dilution of serum (in step 12 above) but not in the 1:5 dilution, an error has been made in the procedure and the test should be repeated.
3. If agglutination occurs in both patient dilutions, further dilutions may be made to determine a more accurate concentration.
4. This procedure may also be performed using a urine sample. For the exact procedure, the reader is referred to the Thrombo-Wellcotest instruction booklet.
5. The sample tubes may be kept at room temperature.
6. The centrifuged serum sample may be refrigerated for up to 1 week or stored at −20°C for longer, before performing the test.
7. False positive results may occur in patients with rheumatoid arthritis (patients positive for the rheumatoid factor).