(Petechiae are minute hemorrhages under the skin and appear as small bruises.) A positive tourniquet test (presence of numerous petechiae) will be found in thrombocytopenia, purpura, and von Willebrand's disease.

REFERENCE

Cartwright, G.E.: *Diagnostic Laboratory Hematology*, Grune & Stratton, Inc., New York, 1963.

REAGENTS AND EQUIPMENT

1. Stethoscope.
2. Blood pressure cuff.

PRINCIPLE

An inflated blood pressure cuff on the upper arm is used to apply pressure to the capillaries. At the end of 5 minutes, the arm is examined for petechiae. If a patient has thrombocytopenia, there will not be enough platelets present to maintain capillary integrity, and small bruises will form on the arm.

PROCEDURE

1. Apply a blood pressure cuff on the upper arm, above the elbow, and take a blood pressure reading.
2. Inflate the blood pressure cuff to a point halfway between the systolic and diastolic pressures. (However, never exceed a pressure of 100 mm of mercury.) Maintain this pressure for 5 minutes.
3. Remove the blood pressure cuff.
4. Examine the forearm, hands, and fingers for petechiae. Disregard any petechiae within one-half inch of the blood pressure cuff, since this may be due to pinching of the skin by the cuff.
5. The test results are graded roughly as follows:
   1+ = A few petechiae on the anterior part of the forearm.
   2+ = Many petechiae on the anterior part of the forearm.
   3+ = Multiple petechiae over the whole arm and back of the hand.
   4+ = Confluent petechiae on the arm and back of the hand.

DISCUSSION

1. An alternative procedure uses the inflated blood pressure cuff at a pressure of 80 mm of mercury, regardless of the patient's blood pressure.
2. The test should not be repeated on the same arm within 7 days.
3. At times, the petechiae may not appear until several minutes after the blood pressure cuff has been removed. If this occurs, these petechiae should be included in the grading of the test results.
4. For a more quantitative test, a circle, 5 cm in diameter, may be drawn on the anterior surface of the forearm, about 4 cm below the anterior bend of the elbow. If this is done, the test is performed as just described, and the number of petechiae present in the circle is counted. In normal patients, there should be no more than 10 to 20 petechiae present. The one drawback to this method occurs when many petechiae appear on the arm, but very few are present within the circle.

TEST FOR PLATELET FACTOR-3 AVAILABILITY

Platelets activated during the coagulation process liberate platelet factor-3, a phospholipid, which is essential for normal blood coagulation. Normal values for this method are determined by the correlation of patient and control results. The patient's platelet-rich plasma should give a clotting time similar in length to that of the normal platelet-rich control plasma. Increased clotting times occur in thrombocytopenia and in defects in platelet factor-3 availability, as found in thrombasthenia and some uremic patients.

REFERENCE

Hardisty, R.M., and Ingram, G.I.C.: *Bleeding Disorders, Investigations and Management*, Blackwell Scientific Publications, Oxford, 1965.

REAGENTS AND EQUIPMENT

1. All glassware in this test must be siliconized or plastic because platelets adhere to glass.
2. Water bath, 37°C.
3. Calcium chloride, 0.025 M.
   Anhydrous calcium chloride 1.38 g
   Distilled water 500 ml
4. Light kaolin suspension.
   Kaolin 0.50 g
   Tris buffer, pH 7.35 100 ml
   (For preparation of Tris buffer, refer to section, Thrombin Time, Reagents and Equipment.)
   Store at 4°C.
5. Normal platelet-poor control plasma.
6. Normal platelet-rich control plasma.
7. Reagents and equipment, as used for the platelet count.
8. Test tubes, 13 × 100 mm.
9. Stopwatch.

SPECIMEN

Platelet-rich and platelet-poor patient plasmas and normal control plasmas. Collect blood and mix one part 0.11 M sodium citrate with nine parts whole blood. Collect two tubes of blood from both the patient and normal control.

PRINCIPLE

Equal parts of patient's platelet-rich plasma (PRP) are mixed with normal control platelet-poor plasma (PPP). A second mixture of equal parts of patient's platelet-poor plasma and normal control platelet-rich plasma is made. Kaolin is added to activate the platelets. Calcium chloride is added and the clotting time of the mixtures recorded. Platelet counts are performed on the platelet-rich plasmas of both the patient and normal control.

PROCEDURE

1. For platelet-rich plasma, centrifuge one tube each of the patient's and normal control blood at 1000 RPM for 10 minutes. Remove the plasma from the cells. For platelet-poor plasma, centrifuge both the patient's and normal control blood at 2500 RPM for at least 20 minutes. Remove the plasma from the cells.
2. Label eight 13 × 100-mm test tubes as shown in Table 14 and pipet the indicated plasmas into each tube.
3. Place tube No. 1 in the water bath. Add 0.2 ml of the well-mixed kaolin suspension.
4. Set a clock for 27 minutes. (This mixture is to be incubated for 20 minutes.)
5. At one-minute intervals, repeat step 3 starting with tube No. 2, then tube No. 3, and so forth until kaolin suspension has been added to all eight tubes in the water bath.
6. When the clock has 7 minutes remaining (20 minutes after kaolin was added to tube No. 1), add 0.2 ml of 0.025 M calcium chloride to tube No. 1 and simultaneously start a stopwatch.

TABLE 14. MIXTURES FOR PLATELET FACTOR-3 AVAILABILITY TEST

TUBE NO.	PRP	PPP
1, 8	0.1 ml control	0.1 ml control
2, 7	0.1 ml control	0.1 ml patient
3, 6	0.1 ml patient	0.1 ml control
4, 5	0.1 ml patient	0.1 ml patient

7. Using the tilt-tube method, determine the clotting time. It should be approximately 30 seconds.
8. Continue to add 0.2 ml 0.025 M calcium chloride to each succeeding tube at 1-minute intervals. That is, when the clock has 6 minutes remaining, add calcium chloride to tube No. 2, and so forth, so that calcium chloride is added to each tube exactly 20 minutes after the kaolin was added.
9. Average the duplicate results and record.
10. Perform a platelet count on the patient's platelet-rich plasma, and the normal platelet-rich control plasma.
11. Interpretation of results: the plasma mixtures in tubes No. 2 and No. 7, and in No. 3 and No. 6, differ only in their source of platelets. If the average clotting times of these two groups agree within 2 to 3 seconds of each other, and the platelet counts on the patient's and normal control platelet-rich plasmas are within 100,000 to 300,000 per cu mm, the patient has no significant defect in platelet factor-3 availability. If, however, the clotting time in tubes No. 3 and No. 6 are more prolonged and differ more widely from tubes No. 2 and No. 7, this may be due to decreased platelets (thrombocytopenia) in the patient or to defective platelet factor-3 availability. If the platelet count performed on the patient's platelet-rich plasma is within the range of 100,000 to 300,000 per cu mm, the prolonged clotting time is probably due to defective platelet factor-3 availability. A patient with abnormal platelet function generally has a clotting time about 15 seconds longer than the control.

PLATELET ADHESIVENESS TEST

One of the functions of platelets is their participation in hemostasis, where they adhere to each other and to the walls of damaged blood vessels to form a hemostatic plug. The adhesiveness of blood platelets is measured, in vitro, by their ability to adhere to glass surfaces. The normal values for this test are 26 to 60% platelet adhesiveness. Decreased values, using the procedure to be described, are found in thrombasthenia, where there is a qualitative disorder in platelets, von Willebrand's disease, and in some cases of myeloid metaplasia and thrombocythemia. Increased platelet adhesiveness has been reported in venous thrombosis, pulmonary embolism, coronary disease, following splenectomy, and in diabetes mellitus.

Salzman Method

REFERENCE

Salzman, E.W.: Measurement of platelet adhesiveness, a simple in vitro technique demonstrating an abnormality in von Willebrand's disease, J. Lab. Clin. Med., 62, 724, 1963.

REAGENTS AND EQUIPMENT

1. A double-ended, 20-gauge, Vacutainer needle.
2. Hypodermic needle, 20 gauge.
3. Vacutainer holder.
4. Vacutainer tubes (two) containing EDTA anticoagulant.
5. Siliconized ML-ML adapter, obtainable from Becton-Dickinson Company, Rutherford, N.J.
6. Siliconized 3200 A adapter, obtainable from Becton-Dickinson Company, Rutherford, N.J.
7. Polyvinyl tubing (inner diameter of 0.113 inch).
8. Grease-free, soda-lime-silica glass beads, with an average diameter of 0.0185 inch. (Obtainable from Minnesota Mining Company as "Superbrite," type 070.)
9. Siliconized nylon mesh, with openings of 0.002 inch.