# Miniaturization of Leukocyte Bactericidal Function Tests (37464)

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Tests of polymorphonuclear neutrophil leukocyte (PMN) bactericidal function are now an integral part of the systematic investigation of recurrent pyogenic infections in children. Such tests are generally performed on separated washed leukocytes and require volumes of blood (2–5 mls) which may be impractical to obtain repeatedly from small infants. To overcome this disadvantage, we examined the feasibility of miniaturizing the test by collecting the blood in heparinized capillary tubes, diluting the sample and by assaying the leukocyte bactericidal activity in the heparinized, diluted whole blood.

*Material and Methods.* Siliconized or heparinized glassware was used throughout. White cell counts on all samples were carried out immediately prior to testing using a Coulter counter.

Initial studies were conducted on paired venous and capillary blood samples from adult volunteers and later ones on similar paired samples from healthy infants. Samples were collected by finger or heel prick in heparinized capillary tubes. Following collection, the 0.2 ml of blood in the capillary tube was expelled into a screw-capped test-tube containing 3.8 ml of heparinized 0.85% saline to give a final dilution of 1:20. The bactericidal activity of the leukocytes in this diluted sample of whole blood was compared with that of neutrophils harvested from 2.0 ml of heparinized venous blood from the same individual after overlayering and separation on a methyl cellulose/diatrizoate solution, the standard procedure in our laboratory (1).

The whole blood samples diluted in 0.85% saline and the separated PMN samples suspended in 0.85% saline were treated in the same manner. Each sample was centrifuged at 800*g* for 5 min, the supernate was decanted and the pellet was resuspended in an equal volume of 0.85% heparinized saline. This procedure was repeated and the cells in the pellet were resuspended in an equal volume of Hanks' balanced salt solution (HBSS). An equal volume of *Staphylococcus aureus* (Oxford strain) in HBSS was added to give a 1:1 ratio of white cells/bacteria. Fresh pooled human serum was then added to a final concentration of 5%. This mixture was incubated at 37° on a tilting table mixer for 30 min to allow phagocytosis and then centrifuged at 800*g* for 5 min. The supernate was decanted and replaced by an equal volume of HBSS containing 100 units/ml of penicillin and 100 μg/ml of streptomycin to kill extracellular bacteria. The leukocytes, containing ingested bacteria, were incubated in this medium for 3 hr on the tilting table mixer and aliquots were withdrawn at 0, 1, 2 and 3 hr. Each of these samples was centrifuged at 800*g* for 5 min, the supernate was decanted and replaced by an equal volume of HBSS, the centrifugation was repeated and the cell pellet finally was resuspended in 2.0 ml distilled water to lyse the cells. Cell clumping was discouraged by a 10 sec disaggregation on a vortex mixer. Two tenfold dilutions were then made in distilled water, a 0.02 ml sample was withdrawn from each using a calibrated dropping pipette and this was spotted onto blood agar plates. The drops were evenly spread with glass spreaders and after overnight incubation at 37° the colonies were counted. Results were expressed as the log-reduction in viable count over the 3-hr period.

*Results.* The reduction in viable count using heparinized whole capillary blood at a

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TABLE I. $Log_{10}$ Reduction in No. Viable Bacteria/ml (3 hr).

| Subject | Separated washed PMN | PMN in diluted (1:20) whole blood |
|---|---|---|
| Adults | | |
| 1 | 1.9, 1.85, 1.8 | 1.8, 1.8, 1.75 |
| 2 | 1.7, 1.8, 1.8, 1.8 | 1.8, 1.75, 1.8, 1.75 |
| 3 | 1.75, 1.65, 1.8 | 1.8, 1.7, 1.65 |
| Infants | | |
| 1 | 2.1 | 1.9 |
| 2 | 1.8 | 2.0 |
| 3 | 2.05 | 2.0 |
| 4 | 2.0 | 1.85 |
| 5 | 1.9 | 1.7 |
| 6 | 1.9 | 1.8 |
| 7 | 1.75 | 1.8 |
| 8 | 1.95 | 1.8 |
| 9 | 2.0 | 1.85 |
| 10 | 1.7 | 1.95 |
| 11 | 1.8 | 1.8 |
| 12 | 1.85 | 1.8 |
| 13 | 1.8 | 2.0 |
| 14 | 1.75 | 1.9 |
| 15 | 1.95 | 1.6 |
| 16 | 2.2 | 1.85 |
| 17 | 1.65 | 1.8 |
| 18 | 1.9 | 2.1 |
| 19 | 1.85 | 1.9 |
| 20 | 2.0 | 2.1 |
| CGD | 0.7 | 0.6 |
| Carrier | 1.1 | 1.05 |

dilution of 1:20 compared closely with that obtained using separated, washed PMN and both methods produced results within the normal range, $>$ 1.5 log drop in 3 hr (2). Replicate tests on 3 adults and isolated tests on 20 healthy infants by the miniaturized method gave results which agreed closely with those obtained by the conventional test procedure. Tests on the leukocytes of a case of chronic granulomatous disease (CGD) and on the cells of his carrier mother also gave results in the expected range (Table I).

*Discussion.* The advantage of smaller volumes of blood for leukocyte bactericidal function tests is most apparent in pediatric practice where the 2–5 ml of blood required by the standard methods and generally obtained by venipuncture may be impractical to obtain repeatedly from small children. The miniaturized method described here requiring as little as 0.2 ml of blood, conveniently procured by heel prick, is particularly suitable for studies in this age group.

That any effects of the erythrocytes on the leukocyte bactericidal function test can largely be ignored is confirmed in this study (3).

The miniaturized assay system has other advantages. Sequential and detailed studies of PMN activity can be performed. Time is saved in the laboratory through the omission of a separation procedure. The effect of separation solutions on PMN function, a factor recently suggested to contribute to alterations in PMN activity, is avoided (4). Higher PMN yields are attainable using whole blood as even the most effective separation techniques result in an unavoidable reduction in available leukocytes (5).

There are two obvious limitations to the capillary method. Firstly, because the test is performed on leukocytes in heparinized whole blood it may be necessary to use a resistant test organism if the patient is receiving antibiotics. Secondly, in profound neutropenia there may be insufficient numbers of neutrophils to permit an accurate estimation of leukocyte bactericidal activity.

Although the capillary nitroblue tetrazolium (NBT) dye reduction test is the customary screening test for chronic granulomatous disease of childhood the diagnosis must be substantiated by a test of bactericidal activity. The miniaturized test described is the most convenient type of test for this purpose. In addition there are other conditions such as familial myeloperoxidase deficiency where the NBT test fails to indicate a defect and is accordingly inappropriate. In these the miniaturized version of the leukocyte bactericidal function test is preferred (6).

*Summary.* The neutrophil bactericidal function test can be performed on as little as 0.2 ml of capillary blood if diluted heparinized whole blood is used. This miniaturized version of the test saves time, can be repeated on small infants and avoids the effects of neutrophil separating solutions. Results by this method compare well in accuracy and reproducibility with standard techniques.

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