Improved Method for Cultivation of *Brucella* from the Blood. (20815)

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In the diagnosis of *Brucella* infections, particularly in man, positive blood cultures have been recognized as one of the most infallible clinical criteria. Unfortunately, even the most widely adopted method for such blood cultures, namely Castaneda's double medium technic(1) suffers from the disadvantage that cultivation in liquid media cannot be eliminated due to the relative scarcity of organisms in the blood. Such cultivation *in vitro*, even for brief periods, is undesirable because (a) certain lots of a widely used liquid medium, tryptose broth, have proved to contain antibrucellar substances(2), and (b) cultivation in liquid media, particularly in the presence of antiserum, promotes rapid population changes that may lead to the establishment of variant types differing considerably from the type(s) originally present *in vivo*(3). An additional problem associated with blood cultures, namely the removal of inhibitory antibodies and the release of possibly intracellular brucellae by "washing" of the blood specimen, has been investigated by Pickett and Nelson(4) who claim to have obtained a significant increase in positive recoveries following washing of citrated blood with distilled water. However, the latter method also utilized fairly prolonged cultivation in liquid media and has not found widespread acceptance, probably due in part to the authors' controversial claims that they recovered, even from the blood of clinically normal humans, many atypical *Brucella* variants with this technic.

An approach to a more ideal blood culture technic, permitting the recovery of *Brucella* cells directly onto solid media, became possible with the help of membrane filters ("millipore filters"(5)) which are now commercially available.* This new technic has been carried out as follows: One ml of blood is drawn from the heart of an infected rabbit, or from the vein of a patient, and is transferred immediately into a sterile test tube containing 0.1 ml of a heparin solution (100 USP units). Three ml of tryptose saline are used to wash down the sides of the tube and the mixture of heparinized blood and tryptose saline is centrifuged for 30 minutes at 2500 RPM. After the supernate is drawn off, the cell sediment is suspended in 3 ml of sterile distilled water and permitted to stand for a period of 30 minutes. The laked blood sample is then filtered through a sterile membrane filter, employing an O'Leary-Volpe type holder for the filter. Filtration is carried out under negative pressure and usually takes from 30 to 40 minutes. When the entire sample of laked blood has passed through the filter, the disc is removed with the help of sterile forceps and is placed on the surface of a solid medium in Petri dishes (2-1 agar(3) has been employed in all studies so far). If *Brucella* cells are present on the surface of the membrane filter they will develop into visible colonies within 3 to 4 days. The detection of such colonies can be facilitated by the incorporation of 0.5% of 2,3,5 Triphenyltetrazolium chloride into the agar. Colonies may be picked from the surface of the membrane filter and can be subcultured onto appropriate media for further identification.

In order to determine whether this method would permit the quantitative recovery of a known number of *Brucella* cells, a number of experiments were carried out with sterile blood samples to which a known number of *B. suis* cells had been added. One ml samples from a saline suspension containing approximately 10 cells per ml were processed in 3 different ways: (a) direct counts were made on the cell suspensions by the pour plate method, (b) 5 ml of sterile saline was added to each sample and the suspensions were then filtered through membrane filters which were subsequently placed on the surface of 2-1 agar plates, and (c) samples were added to heparinized blood obtained from normal rabbits

* Obtainable from Lovell Chemical Co., Watertown, Mass.

TABLE I. Recovery of *Brucella suis* Cells from Infected Rabbits by Membrane Filter Method and by Castaneda Method at Successive Days after Infection.

Days after inoculation	A*		B		C		D	
	Membrane filter	Castaneda	Membrane filter	Castaneda	Membrane filter	Castaneda	Membrane filter	Castaneda
1	3	+	12	+	0	—	1	—
3	179	+	100	+	54	+	69	+
5	21	+	34	+	3	+	Not run	+
7	2	+	10	+	2	—	243	+
9	5	+	6	+	0	—	8	+
11	24	+	2	—	1	—	1	—
13	0	—	1	—	0	—	0	—
15	0	—	2	—	0	—	1	—
17	0	—	0	—	0	—	0	—
19†	0	—	1	—	2	+	0	—

* Capital letters represent different animals. Figures are numbers of colonies found on membrane filter discs. Positive recoveries by Castaneda method are recorded as plus, non-recovery as minus.

† No positive recoveries from the 21st to the 27th day.

and processed by the above described technic. In 6 replicate tests for each procedure the average recovery of cells from these one ml samples was (a) 11 cells by pour plate method, (b) 13 cells after filtration of the saline suspensions through membrane filters, and (c) 10 cells after filtration of treated blood suspensions through membrane filters. These results indicate that the new technic permits quantitative recovery of *Brucella* cells, at least when they are present extracellularly.

Numerous tests have been made on supernates obtained following centrifugation of laked blood specimens, but *Brucella* cells have never been recovered from such supernates; therefore, all supernates are now discarded in the regular routine.

Finally, comparative tests have been made on the recovery of *Brucella* cells from the blood of infected rabbits at various times after exposure of the animals to *B. suis* employing both the Castaneda method and the above described membrane filter technic. Six rabbits were inoculated intravenously with approximately 1 x 10^7 organisms. On the following day and every other day thereafter, for a period of 27 days, a 2-ml sample of blood was taken from the heart of each of the infected rabbits. One ml of blood was then processed according to the membrane filter technic and the other one ml of heparinized blood was treated according to the method described by Castaneda(1). The results of these tests are shown in Table I and indicate that the recoveries obtained with the help of the new method are as good, and probably better, than those obtainable by previously utilized methods. In addition to the ability to culture *Brucella* cells directly onto solid medium without any growth period in liquid media, this method also appears to permit a quantitative estimation of the extent of bacteremia. It is probable that the same technic may be applicable to blood cultures of other bacterial pathogens.

Summary. A new technic for the recovery of *Brucella* cells from the blood of infected hosts is described. This technic employs filtration of laked blood specimens through membrane filters and permits the recovery of *Brucella* cells directly on solid media. The new method has proved to be at least as good as previously described blood culture methods and does not possess the disadvantage of cultivation in liquid media which is necessary in other methods. The membrane filter technic also appears to permit a quantitative estimation of the extent of bacteremia.

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