Small-Sample Blood Culture Method for Identification of Bacteria in Central Arterial and Peripheral Blood

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A blood culture technique that utilized small arterial blood samples or peripheral capillary blood was tested in beagle dogs and pig-tailed macaque monkeys. A bolus of $2.0 \times 10^7$ Escherichia coli (ATCC 25922) was injected intravenously into five animals of each species. Blood samples were taken before injection of the organisms and at 5, 12, 25, 30, 60, and 120 min after injection. Arterial blood samples (2.0 and 0.2 ml) and peripheral capillary samples (0.14 ml) were taken at each sampling time. Four plates were prepared from arterial blood for colony counts. All three blood sampling methods were equally effective in detecting sepsis when 10 or more organisms per ml of blood were present. Below this level, the 0.2-ml sample was more effective. Contamination of the peripheral sample with air or skin contaminants was a problem.

In human neonates and in small adult and infant animals, diagnosis of sepsis via standard blood culture methods may require comparatively large and frequent blood samples. The standard 1- to 5-ml blood sample taken for culture from a 0.5- to 1-kg premature human infant, newborn kitten, or puppy may further compromise the patient's failing circulation. Difficulties in finding an accessible vein for blood sampling frequently occur in small animals when sepsis combines with hypovolemia. A method of blood sampling for identification of bacteria from blood cultures that is accurate using small amounts of arterial, venous, or capillary blood would be a useful adjunct for the pediatrician and veterinary clinician.

Preliminary work performed at this facility suggested that a small sample of arterial blood could be used to detect experimental Escherichia coli septicemia in rabbits. The present paper expands this technique to higher species and compares small central arterial versus peripheral capillary blood samples for detection of bacteria from blood culture.

**MATERIALS AND METHODS**

Five adult beagle dogs (two females and three males) weighing 10 to 13 kg and five adult female pig-tailed macaque monkeys (Macaca nemestrina) weighing 4 to 7 kg were utilized in this experiment. Each dog was premedicated with atropine sulfate (0.04 mg/kg), anesthetized for intubation with 2.5% sodium thiamylal (Parke-Davis), and maintained on a gas anesthesia machine via a cuffed endotracheal tube with methoxyfluorane plus oxygen. A PE240 polyethylene catheter was placed in the thoracic aorta via a femoral artery cutdown and connected to a Sanborn 350 recorder for continuous arterial blood-pressure monitoring.

Each monkey was premedicated with 5 mg of ketamine hydrochloride per kg (Parke-Davis) and atropine sulfate (0.04 mg/kg). Sodium thiamyl (2.5%) again was used for intubation, and methoxyfluorane and oxygen were used for maintenance of anesthesia. A PE90 catheter was utilized for arterial pressure and was inserted and connected as described above for the dogs.

During the course of the experimental procedure, each dog received 250 ml of 5% dextrose in water (D5/W) intravenously; the monkeys received 100 ml of D5/W.

For sampling of peripheral blood, the animals' ears were shaved, and a 5-min surgical scrub of the ear was performed with hexachlorophene soap, alcohol, and iodine paint. In the dogs, a site on the inner surface of the pinna of each ear was selected; in monkeys, the edges of the ears were so prepared. A nick was then made in the prepared ear by using a number 11 scalpel blade, and the blood sample was drawn into a sterile micro blood collection tube (micro blood collecting tubes, plain, no. B3095-1; Sherwood Medical Industries, Inc., American Hospital Supply Corp., Evanston, Ill.). The tube was prefilled with sterile liquid heparin (1,000 U/ml) before the sample was collected. A 5-drop (0.14-ml) sample of this heparinized blood was utilized for the culture sample and placed into a Pfizer blood culture bottle containing 50 ml of dextrose phosphate broth with p-aminobenzoic acid and 0.05% sodium polyanethol sulfonate (SPS) (Pfizer Diagnostics Division, Santa Anna, Calif.). The
blood sample was added to the culture bottle by opening the top of the bottle and allowing the 5 drops to enter by force of gravity.

Arterial blood samples (3.5 ml) were withdrawn from the aortic catheter into heparinized 5-ml syringes. The blood was divided into three samples of 1.0, 2.0, and 0.2 ml. The 2.0- and 0.2-ml samples were placed into separate blood culture bottles. Trypticase soy agar (BBL) was melted and then cooled to 48 C prior to adding the 1.0-ml arterial blood sample for a pour plate. All cultures were read at 24 and 48 h. Cultures were assumed to be negative only if they were negative at both readings and no growth occurred on the subculture.

Initial central arterial and peripheral (ear) blood samples were withdrawn. Then, a 2-ml suspension containing 2 × 10⁷ E. coli (ATCC 25922) was injected by a peripheral vein. In the dogs, the suspension was injected via the cephalic vein of the front leg. In the monkeys, the saphenous vein of the rear leg was used for injection. After the injection of bacteria, central arterial and peripheral ear samples were taken at 10, 15, 20, 30, 60, and 120 min. An equal amount of heparinized saline was used to replace the fluid loss after each blood sampling.

**RESULTS**

Figure 1 illustrates the differences in the rate of clearance of E. coli from the arterial blood of dogs and monkeys, as determined by colony counts on pour plates. In the dogs, rapid clearance occurred within the first 30 min after introduction of the bolus of E. coli. The curve for monkeys was more gradual in descent.

In Table 1, the colony counts are compared with the blood culture results in both dogs and monkeys. In both animal species, when 10 or more bacteria per ml of blood were present (as determined by the pour plate method), all three blood culture techniques were equally reliable in detecting bacteremia. With less than 10 organisms per ml of blood, all three methods were less reliable, although the larger volume arterial sample (2.0 ml) was more accurate, especially in the monkeys.

Contamination of the blood culture bottles with gram-positive air or skin flora was a problem only when using the peripheral ear sampling method. In 7 of the 27 0.14-ml peripheral samples taken from the monkeys, contamination of the media with Staphylococcus epidermidis (5 samples) and Bacillus spp. (2 samples) occurred. In the dog experiments, only 2 of the 30 peripheral samples were contaminated with S. epidermidis. One of the 0.2-ml arterial samples in one dog was also contaminated with S. epidermidis.

**DISCUSSION**

This study initially was intended to see whether the 0.2-ml arterial blood sample was as reliable as a 2.0-ml sample in detection of sepsis. A 0.2-ml sample was chosen because, by reduction of the blood volume of a 70-kg man to

**Table 1. Colony count compared with blood culture results**

| Colony count | Arterial blood culture (2 ml) | Arterial blood culture (0.2 ml) | Peripheral blood culture (0.14 ml) |
|--------------|-------------------------------|-------------------------------|-------------------------------|
| Dogs         |                               |                               |                               |
| 300          | 3                             | 3                             | 3                             |
| 200-300      | 2                             | 2                             | 0                             |
| 100-199      | 2                             | 2                             | 2                             |
| 50-99        | 5                             | 5                             | 5                             |
| 25-49        | 2                             | 2                             | 2                             |
| 10-24        | 2                             | 2                             | 1                             |
| 5-9          | 2                             | 2                             | 1                             |
| 0-4          | 4                             | 4                             | 1                             |

| Monkeys      |                               |                               |                               |
| 300          | 15                            | 15                            | 11*                           |
| 200-300      | 2                             | 2                             | 0                             |
| 100-199      | 2                             | 2                             | 2                             |
| 50-99        | 1                             | 1                             | 1                             |
| 25-49        | 2                             | 2                             | 2                             |
| 10-24        | 1                             | 1                             | 1                             |
| 5-9          | 2                             | 1                             | 1                             |
| 0-4          | 4                             | 1                             | 0                             |

*Three samples not performed with colony counts >300/ml.

**Fig. 1. Rate of clearance of E. coli from blood of dogs and monkeys.**
a 3.5-kg newborn child, 0.2 ml of blood from a child is proportional to 4.0 ml in an adult. The use of a peripheral sample was based upon the popularity of the peripheral heel stick as a method of sampling human infants for blood testing, not including blood culture. If sepsis in these animal species could be diagnosed by means of small arterial or peripheral capillary blood samples, then perhaps the information and techniques might be extrapolated to pediatrics and veterinary medicine.

Anesthetized animals are not the same as awake human infants. Anesthesia produces physiological aberrations, including peripheral pooling of blood, arterio-venous shunting, and, in dogs anesthetized with barbiturates, sequestration of a large percentage of the blood volume in the spleen (2). Many of these same physiological problems occur in the septic animal or child, especially when the septicemia is reaching shock proportions.

The use of SPS as an additive for blood culture media has been reported to aid the likelihood of recovery of bacteria in septicemias (3). The beneficial effect of SPS has been attributed to its ability to prevent phagocytosis, its anti-coagulatory effect, and its possible lessening of inhibitory action of nonspecific antibodies (4). This agent has been shown to yield more positive blood cultures when small numbers of organisms are present in the inoculum (3, 5). However, this advantage has been coupled with an increase in the number of contaminants (1). In our study, even though sterile equipment and sterile heparin were used and a complete surgical preparation of the skin was performed for all samples, contamination was significant, especially in the monkeys. In spite of this contamination it was still possible to detect E. coli. Perhaps a closed system between the micro blood collecting tube and the culture bottle would have reduced contamination in our experiment.

These experimental results indicate that, at bacteremia levels of 10 or more organisms per ml of blood, small amounts of arterial blood and even peripheral capillary blood are enough to yield positive blood cultures. This is not to say that if a larger amount of blood is available it should not be collected. What it does indicate is that, if an animal or perhaps even a human neonate is unable to provide 1 to 4 ml of blood for culture, then smaller samples may be adequate. Further work is needed with peripheral sampling methods for culture to reduce the likelihood of contamination.

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