NOTES

Recovery of *Gardnerella vaginalis* from Blood by the Quantitative Direct Plating Method

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**This report describes a case of *Gardnerella vaginalis* sepsis in an obstetric patient whose blood cultures were
negative by the conventional BACTEC system but positive by the quantitative direct plating method, which
involves the direct inoculation of blood samples on chocolate agar and blood agar plates.**

Gardnerella vaginalis, formerly termed *Haemophilus vaginalis*, is frequently isolated from the female genital tract and, in conjunction with anaerobic bacteria, is implicated as a cause of bacterial vaginosis, also known as nonspecific vaginitis (3, 6, 11); it has also been associated with maternal and neonatal septicemia (1, 2, 7, 9, 10, 13). This report describes a case of maternal *G. vaginalis* sepsis in which the blood cultures were negative by the conventional BACTEC radiometric system but positive by a supplementary technique termed the quantitative direct plating (QDP) method, which involves the direct plating of blood samples on chocolate agar and blood agar plates.

Case report. A 34-year-old female, blood type O+, gravida 2 para 0, in premature labor with intact membranes at 26 weeks of gestation, was admitted to the maternity wing of The Children's Hospital of Buffalo on 10 June 1982. The course of the pregnancy was normal until the day of admission, when the patient began to feel mild contractions. The cervix was found to be 2 to 3 cm dilated and 80% effaced. By the time of admission the cervical dilation had progressed to 5 cm and the membranes were bulging, with uterine contractions occurring every 5 min and lasting 40 to 60 s. Measurement of vital signs showed a temperature of 37.6°C, a pulse rate of 91 per min, blood pressure of 114/61, and a respiratory rate of 22 per min. The fetal heart rate was regular at 160 beats per min. An intravenous drip of ritodrine hydrochloride was started immediately, and contractions subsided once the maximum dose of 350 µg/min was reached. The ritodrine infusion was maintained at 350 µg/min for the next 36 h, when the patient began complaining of shortness of breath but denied having chest pains. Her pulse rate was 120 per min and her respiratory rate was 28 per min; bilateral rales were heard at the lung bases. The impression was mild pulmonary overload secondary to ritodrine hydrochloride administration. The shortness of breath was treated with intermittent doses of intravenous furosemide.

At 48 h after admission, a fetal tachycardia of 180 beats per min and late decelerations were noted with contraction. Immediate delivery was recommended because of fetal distress. The leukocyte count of the patient had increased from 10,300 on admission to 18,500 just before delivery. A female infant weighing 900 g, with an Apgar score of 6 at 1 min and 8 at 5 min, was delivered by cesarean section, with 1 g of

intravenous cephalothin given at surgery for prophylaxis. The infant was transferred to the intensive care nursery, where her progress was uneventful.

At 4 h postoperatively, the blood pressure of the mother dropped to 67/44, and her temperature was 39.5°C. She was ashen and experiencing respiratory difficulty. A central venous catheter was inserted and a reading of 13.5 mmHg was obtained. Septic shock and pulmonary edema were diagnosed. She received 2 units of packed erythrocytes and 500 ml of fresh frozen plasma. A dopamine drip of 5 µg/kg per min was started. Two blood cultures were obtained. Initial arterial blood gas test results on 50% O_2 showed 93% saturation, PO_2 −64, pH 7.51, PCO_2 25, base excess −2. Her leukocyte count had dropped to 2,100. In addition to cephalothin, tobramycin at 80 mg every 8 h was administered intravenously. The blood pressure of the patient increased to 125/60 in response to these resuscitative measures. She was intubated and transferred to an intensive care unit. After 3 days of intensive care, the low-output hypotensive state improved dramatically. The final pathology report of her placenta showed extensive chorioamnionitis. The patient was discharged on 16 June 1982.

The two blood cultures were positive for *G. vaginalis* on the basis of the following criteria for identification (14). After 48 h of incubation at 37°C in 5 to 10% CO_2, colonies on chocolate agar appeared as pinpoint and produced no surrounding green discoloration of the agar. Observation of these colonies under the microscope indicated small, pleomorphic, gram-variable organisms. The colonies showed beta-hemolysis on 5% human blood agar and gamma-hemolysis on 5% sheep blood agar. Furthermore, this organism was negative for catalase, oxidase, and utilization of raffinose, but fermented starch and hydrolyzed hippurate. The identification was confirmed by the Division of Laboratories and Research of the New York State Department of Health, Albany, N.Y.

Blood cultures were processed and monitored as described previously (4, 5). In brief, blood cultures consisted of BACTEC aerobic bottles with QDP heparin tubes attached. The blood cultures for the BACTEC and QDP methods were procured at the same time. A total of 3 to 5 ml of blood was inoculated into each BACTEC bottle, and 1.0 ml was injected into each QDP heparin tube. The time elapsed between the collection of the specimens and laboratory arrival was usually 0.5 h or less. The BACTEC bottles were

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TABLE 1. Recovery of *G. vaginalis* from blood by the QDP and BACTEC methods

Technique	Vol of blood (ml) per culture	Time of detection			CFU/ml
		24 h	60 h	5 days	
BACTEC[a]	3–5	−	−	−	
QDP	1.0	−	+[b]		6, 12

[a] Represents BACTEC GI readings, microscopy, and subcultures.

[b] Both blood cultures were positive.

monitored every 4 h for the first 24 h, at 36 h, and on days 2, 3, 4, and 5. When a positive growth index (GI) reading of 30 or more was obtained, subcultures and Gram and methylene blue stains were performed. At 24 h, all BACTEC bottles with a negative GI were subcultured and stained by the methylene blue and Gram methods. On day 5, all negative bottles were discarded. Briefly, for the recently published QDP procedure (4, 5), 0.1 to 0.5 ml each of heparinized blood was directly pipetted onto sheep blood agar and chocolate agar plates and spread with a bacteriological loop. The plates were incubated at 37°C in 5 to 10% CO_2 and inspected for growth every 4 h for 3 days. The number of bacteria per milliliter of blood was calculated.

By the QDP method, *G. vaginalis* was detected in both blood cultures (6 and 12 CFU/ml) within 60 h (Table 1). In contrast, the BACTEC method failed to detect this organism by GI readings; microscopy and subculture results were also negative. Furthermore, the GI readings did not show a significant sequential increase below a reading of 30. It is noteworthy that the BACTEC method gave negative results even though the amounts of blood used per bottle (3 to 5 ml) were larger than those used per QDP tube (1.0 ml of blood assayed per culture). This would indicate that the QDP was positive and the BACTEC was negative not because of a sampling effect favoring the QDP.

G. vaginalis is frequently isolated from the female genital tract and, in conjunction with anaerobes, is implicated as a cause of bacterial vaginosis (3, 6, 11). It has also been implicated primarily in obstetric patients with postpartum endometritis and septicemia with predisposing factors such as septic abortion, premature rupture of membranes, and cesarean section. This organism has also been associated with acute chorioamnionitis and neonatal septicemia (1, 2, 7, 9, 10, 13). It is believed that the source of the bacteremia is closely related to the obstetrical procedures necessary at delivery; the most probable sources are the exposed vascular bed of the operative site or the area of implantation of the placenta.

It is of interest that the QDP method was able to detect *G. vaginalis* bacteremia in this obstetric patient even though smaller amounts of blood were assayed than in the BACTEC system, which failed to detect this agent. In relation to this, La Scolea et al. (4, 5) have reported the difficulty of detecting *Haemophilus influenzae* with the BACTEC sys-

tem. The possibility exists that this may also be the case with other fastidious bacteria which are closely related to *Haemophilus* spp., such as *G. vaginalis*. It has also been observed (8, 12) that human blood agar enhances the isolation and identification of *G. vaginalis* more than sheep blood agar does. Since in the QDP procedure the direct inoculation of human blood on sheep blood agar plates is used, in contrast to the BACTEC system, this could have been a factor in the detection of *G. vaginalis* by the QDP method. On the basis of this preliminary observation, the opportunity exists to perform expanded clinical and experimental studies on several strains of *G. vaginalis* to determine whether a direct plating procedure of human blood samples is superior to a radiometric procedure in recovering the organism. Experimentation is in progress to test this possibility. If this is the case, the QDP procedure could be a valuable supplementary technique in the diagnosis of sepsis in obstetric patients.

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