Effectiveness of Palladium Chloride for the Isolation of Anaerobes

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Because of the increased price of palladium chloride, we investigated the effectiveness of the addition of this ingredient to the culture medium used for the recovery of anaerobes. With our system, the addition of palladium chloride did not consistently improve the recovery rate of bacteria from guinea pig intestinal contents and human fecal material.

The utilization of palladium, either as palladium black or palladium chloride, as a catalyst in bacterial culture media has been known for many years. In 1954 Mylroie and Hungate (6) used palladium black to lower the oxidation-reduction potential of the culture medium for the recovery of methane bacteria in sludge. They found that palladium chloride, which could be added to the media as a suspension, worked as well as palladium black.

Later Aranki et al. (2) reported that palladium black was a necessary ingredient in agar media for the optimal recovery of strict anaerobes when the anaerobic glove box was used. With palladium black overlay on agar plates, they were able to recover a greater number of organisms from both mouse intestine and human gingiva than with medium without palladium black. However, this difference in recovery rate was statistically significant in only one of a number of different experiments. Based on this data, Aranki et al. (2) and others incorporated palladium black in the media they were using to study the normal intestinal flora of mice (4, 7).

In other experiments Aranki and Freter (1) also found that palladium chloride was as effective as palladium black for recovery of anaerobes and that it was much easier to work with in the laboratory. Palladium chloride, unlike palladium black, does not need to be added as a separate layer on the surface of agar plates. Ellner et al. (3) utilized media with palladium chloride to isolate anaerobes from clinical material.

Since the cost of palladium chloride has increased considerably in the past 18 months, we decided to investigate the effectiveness of the addition of palladium chloride to culture medium for recovery of anaerobes from human

fecal material and guinea pig cecal contents. Results show that with our system the addition of palladium chloride did not consistently increase the recovery rate.

Two types of anaerobic glove box chambers were employed. A rigid plexiglass chamber (Germfree Labs, Miami, Fla.) was utilized for the determination of the flora of guinea pig intestine, and a flexible plastic chamber (Coy Manufacturing Co., Ann Arbor, Mich.) was utilized for the study of human fecal samples.

In addition, a GasPak anaerobic jar (BioQuest, Cockeysville, Md.) containing a hydrogen and carbon dioxide generator envelope and a palladium catalyst was used in the study of human fecal flora.

In all studies, organisms were cultured on a medium similar to that described by Aranki and Freter (1), except that *Brucella* agar (Difco, Detroit, Mich.) was substituted for Trypticase soy agar. This modified medium is designated BAF.

Stool specimens were collected in a specimen cup and were placed immediately into a GasPak jar that had been activated previously by the addition of water to the generator envelope. The anaerobic jars containing the specimens were then placed into the anaerobic chamber. Inside the chamber the specimens were removed from the jar and homogenized, and 10-fold serial dilutions of each homogenate were prepared in a prereduced salt solution (5). Hartley strain 13 guinea pigs were sacrificed and immediately taken into the anaerobic chamber. Cecal contents were removed, and 10-fold serial dilutions of the contents were prepared in the prereduced salt solution. A portion (0.1 ml) of each of the human fecal and guinea pig cecal dilutions was spread with a bent glass rod onto the surface of prereduced BAF plates

containing PdCl_2 and plates which did not contain PdCl_2 . Ten replicate plates were prepared from each dilution. Plates were incubated for 6 days before colonies were counted. Total counts were determined by averaging counts obtained from 10 plates containing between 30 and 300 colonies. The Student's *t* test was used to analyze the significance of the difference between plate counts at the 0.05 level.

Fecal homogenates which had been used for inoculation of plates inside the chamber were removed from the chamber, and a new series of 10-fold dilutions was prepared outside the chamber in freshly prepared salts solution. A portion (0.1 ml) of each of these dilutions was inoculated onto BAF plates with and without PdCl_2 which had not been prereduced. Ten replicate plates were made from each dilution. After inoculation, the plates were placed into anaerobic jars. They were incubated at 37 C for 6 days before colonies were counted.

Experiments were designed to compare bacterial counts obtained from human feces and guinea pig cecal contents on medium with and without PdCl_2 in the anaerobic chamber. Results from the human fecal samples are presented in Table 1. Counts from two of the five specimens of human feces were higher on medium without PdCl_2 . In the other three experiments the counts were higher on medium containing PdCl_2 . In evaluating this data statistically, there was a significant difference between counts on the two types of media in only one experiment. In this case there was a greater number of organisms on the medium without PdCl_2 . Similar results were obtained when guinea pig cecal contents were analyzed. Results are presented in Table 2. In two experiments, a greater number of organisms was iso-

lated on medium without PdCl_2 , and in a third experiment the mean count was higher on medium with PdCl_2 . In one case there was a statistically significant difference between the counts on the two media. This was observed in experiment 3, and in this case a higher count was observed on media without PdCl_2 .

Some of the fecal cultures were incubated in anaerobic jars as well as in the anaerobic chamber. Table 1 shows that, under these conditions, three of the specimens produced a greater number of colonies on medium containing PdCl_2 than on medium without PdCl_2 . Significant differences between counts on the two types of media could not be demonstrated in any experiment. As expected, counts obtained from fecal specimens processed in the anaerobic chamber were uniformly higher than counts obtained when anaerobic jars were used.

Recovery of anaerobic bacteria from either human feces or guinea pig cecal contents was not consistently improved by the addition of PdCl_2 to BAF medium. This phenomenon was

TABLE 2. Recovery of bacteria on BAF medium with and without PdCl_2 (guinea pig ceca)

Expt no.	Specimen cultured	Plate counts ^a (anaerobic chamber)	
		PdCl_2	No PdCl_2
1	Guinea pig cecal content	1.00×10^{10}	1.04×10^{10}
2	Guinea pig cecal content	1.10×10^{10}	1.05×10^{10}
3	Guinea pig cecal content	7.32×10^9	9.78×10^9

^a Each of the values represents an average count for ten plates expressed as counts per gram of specimens (wet weight).

TABLE 1. Recovery of bacteria on BAF medium with and without PdCl_2 (human feces)

Expt no.	Specimen cultured	Plate counts ^a			
		Anaerobic chamber		Anaerobic jar	
		BAF medium with PdCl_2	BAF medium without PdCl_2	BAF medium with PdCl_2	BAF medium without PdCl_2
1	Human feces	2.35×10^{11}	1.52×10^{11}	ND ^b	ND
2	Human feces	4.68×10^{11}	4.76×10^{11}	2.48×10^{11}	1.60×10^{11}
3	Human feces	4.93×10^{10}	5.94×10^{10} ^c	ND	ND
4	Human feces	8.57×10^{10}	8.37×10^{10}	4.82×10^{10}	4.77×10^{10}
5	Human feces	2.04×10^{11}	1.84×10^{11}	1.01×10^{11}	9.39×10^{10}
6	Human feces	ND	ND	2.97×10^{10}	3.58×10^{10}

^a Each of the values represents an average count obtained from 10 plates expressed as counts per gram of specimen (dry weight).

^b ND, Not done.

^c Significantly greater count on media without PdCl_2 than on media containing PdCl_2 . Student's *t* test at the 0.05 level.

observed both when the cultures were processed in the anaerobic chamber and when they were incubated in anaerobic jars. In this respect our results differ from those reported earlier by Aranki et al. (2). These investigators demonstrated a uniformly higher recovery of bacteria from human gingival material and mouse cecal contents when the media they used were prepared with a palladium black overlay. However, in only one experiment were counts significantly higher than on medium without the overlay. The discrepancy between their results and ours may be due to the fact that different specimens were examined and a different recovery medium was used in the two laboratories. In some systems addition of PdCl_2 to the medium may be advantageous for recovery of anaerobes; in other systems, such as ours, it may not be. Because of the high cost, it appears prudent to determine the effectiveness of PdCl_2 in a particular anaerobic system before routinely incorporating it into culture medium.

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