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DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service
Centers for Disease Control

Memorandum

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From C. Patton, General Bacteriology Laboratory Branch
Bacterial Diseases Division, CID

Subject Culture Results of Amyl/Butyl Nitrite Samples
Related to Kaposi Sarcoma

To Roger Feldman, M.D.
Director, Bacterial Diseases Division, CID

I. Sample Identity

Four bottles of amyl or butyl nitrite were received from Dr. Martha Rogers, Task Force, Kaposi Sarcoma on August 20, 1981. Twelve additional samples were received on August 28, 1981. The following six samples were selected for testing:

Sender #	CDC #	Source	Date Collected	Amount
A1	C7647	Ambush Bar, San Francisco	7/81	10 ml
A2	C7648	Ambush Bar, San Francisco	7/81	20 ml
C1	C7649	Christopher St. Book Store, New York	7/81	15 ml
C2	C7650	Christopher St. Book Store, New York	7/81	10 ml
-	C7656	Ft. Dicks	-	20 ml
J2	C7659	Jaguar Book Store, San Francisco	8/81	15 ml

II. Testing Procedure

One half of each of the 16 samples was sent to Dr. Liddle, Toxicology Branch, for chemical analysis. The remaining half of the above six samples were processed and cultured for bacteria and fungi on 9/2/81 as indicated below.

A. Untreated samples

1. Microscopy

Each was examined by darkfield microscopy and a smear prepared and stained by Gram's method.

2. Culture

Each was inoculated to one plate/broth each of a) aerobic blood plate (heart infusion agar with 5% rabbit blood - HIA); b) thioglycollate medium; c) heart infusion broth with 5% rabbit blood - HIB); d) anaerobic enriched blood plate (trypticase soy agar with 5% sheep blood, cysteine, yeast extract, hemin and vitamin K) and e) Sabouraud's agar + chloramphenical.

The anaerobic blood plates were incubated and examined by Anaerobe Lab, CDC and the Sabourand's agar slants were incubated and examined by Mycology Lab, CDC. The other media were incubated and read in GBLB for 4 weeks. The HIB was subcultured to HIA on days 2, 7 and 14 after inoculation.

B. Filtration and culture of membrane and eluate

The remaining portion of each sample was passed through a 0.22μ porosity teflon membrane filter. The filter was removed, placed into phosphate buffered saline, and vortexed for 1-2 minutes to elute organisms. The eluate was Gram stained and inoculated to the above five media. The membrane was aseptically cut into 5 pieces and 1 piece was cultured in (or on) the above five media. Media were incubated and subcultured as indicated above.

C. Seeding filtrate with bacteria

Each filtrate was divided into three equal aliquots. One aliquot each was seeded with ∿10^8 cells of 1) 24 hour broth culture of Pseudomonas aeruginosa, 2) 24 hour broth culture of Bacillus cereus and 3) 48 hour broth culture of Mycobacterium fortuitum. The seeded samples were held at 4°C (amyl/butyl nitrite rapidly evaporates and decomposes unless kept cool) and subcultured to the appropriate media on days 1, 2 and 7 after seeding.

III. Results

A summary of results is presented in Table 1.

Table 1. Microscopy and culture results of amyl/butyl nitrite samples

	Microscopy			Culture						Seeded filtrate		
	untreated		eluate	untreated			eluate					
Sample #	Dark-field	Grams	Grams	aerobes	anerobes	fungi	aerobes	anaerobes	fungi	Ps. sp.	Bac. sp.	Myc sp
C7647	Neg[1]	Neg[1]	Neg[1]	NG[2,3]	NG[2]	NG[2]	NG[2,3]	NG[2]	NG[2]	NG[4]	NG[4]	NG
C7648	Neg	Neg	Neg	NG	NG	NG	NG	NG	NG	NG	NG	NG
C7649	Neg	Neg	Neg	NG	NG	NG	NG	NG	NG	NG	NG	NG
C7650	Neg	Neg	Neg	NG	NG	NG	NG	NG	NG	NG	NG	NG
C7656	Neg	Neg	Neg	NG	NG	NG	NG	NG	NG	NG	NG	NG
C7659	Neg	Neg	Neg	NG	NG	NG	NG	NG	NG	NG	NG	NG

1) Neg - i.e. no organisms seen.

2) NG - No growth

3) Thioglycollate medium contaminated (uninoculated control and those inoculated with samples showed fungi ∿14 days after incubation)

4) NG - No growth on subculture

IV. Comments

Based on these studies, the risk of developing a bacterial or fungal infection from sniffing amyl/butyl nitrite seems unlikely since the samples did not appear to be contaminated and relatively resistant bacteria did not survive in the samples.

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C. Patton

cc: Dr. Weaver
Dr. John Feeley