

# **A MICROBE ON THE MOON? SURVEYOR III AND LESSONS LEARNED FOR FUTURE SAMPLE RETURN MISSIONS**

**Formerly (2004)—*Strep.*, *Lies(?)*, and *16mm Film*:  
*Did S. mitis Survive on the Moon? Should Humans be Allowed on Mars?***

**Now—  
*Streptococcus mitis* on Surveyor III - Pheidippides or Rosie Ruiz?**

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# Implications of a False Positive to SR

## Back Contamination: A Candidate Mars Sample Handling Process

Facilities:

- Mobile Retrieval Units (MRU)
- Sample Receiving Facility (SRF)
- Mars Curation Facility (MCF)

- Rapid retrieval and containment

- Preliminary examination/characterization
- Subsampling, documentation
- Preliminary search for extinct/ extant life
- Hazard testing

- Samples declared safe?
- Subsampling
  - Documentation
  - Sample distribution
  - Long-term curation
  - Cold curation

Curation  
(MCF)

Quarantine  
(SRF)

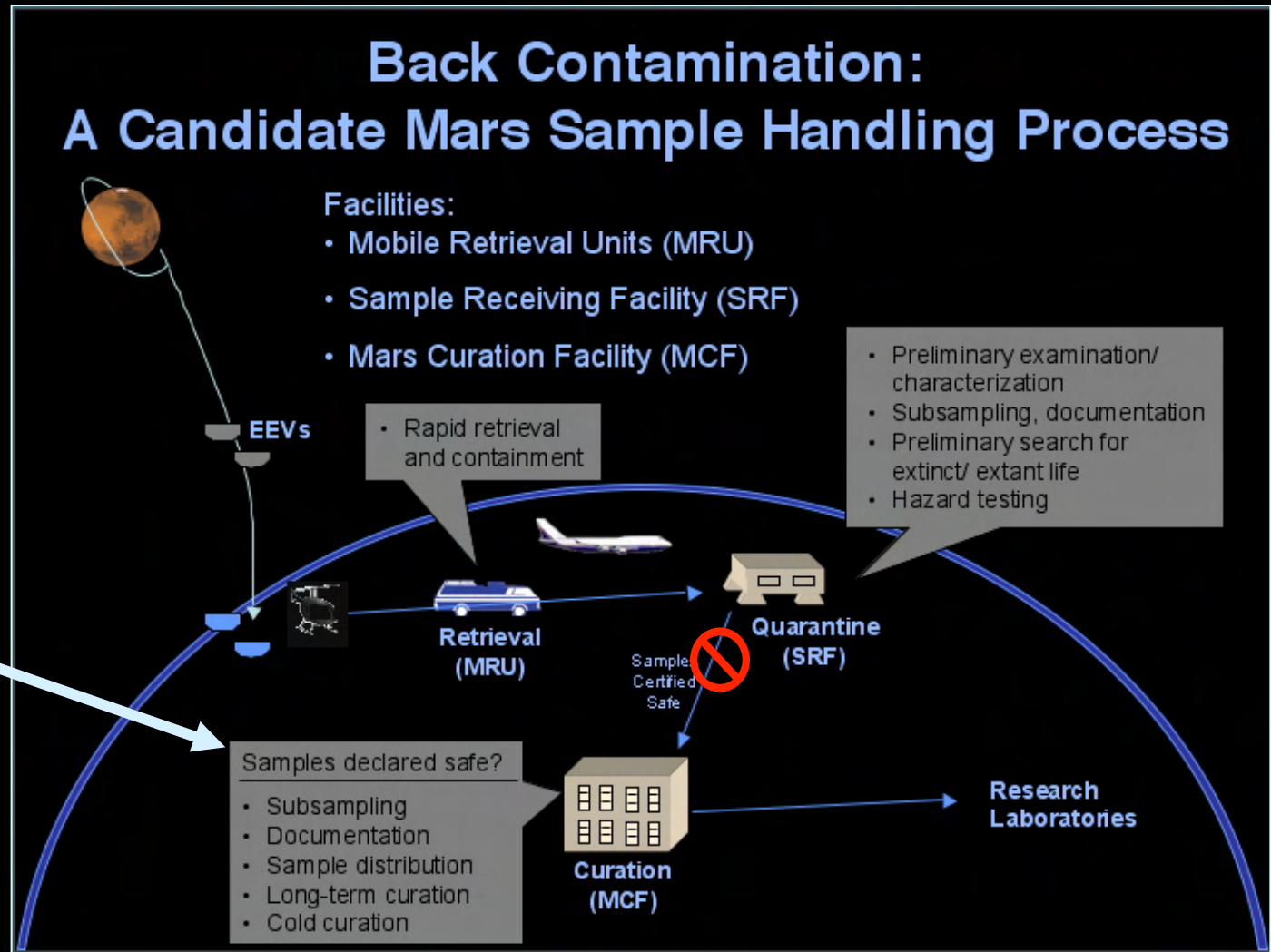
Research  
Laboratories

Sample  
Certified  
Safe

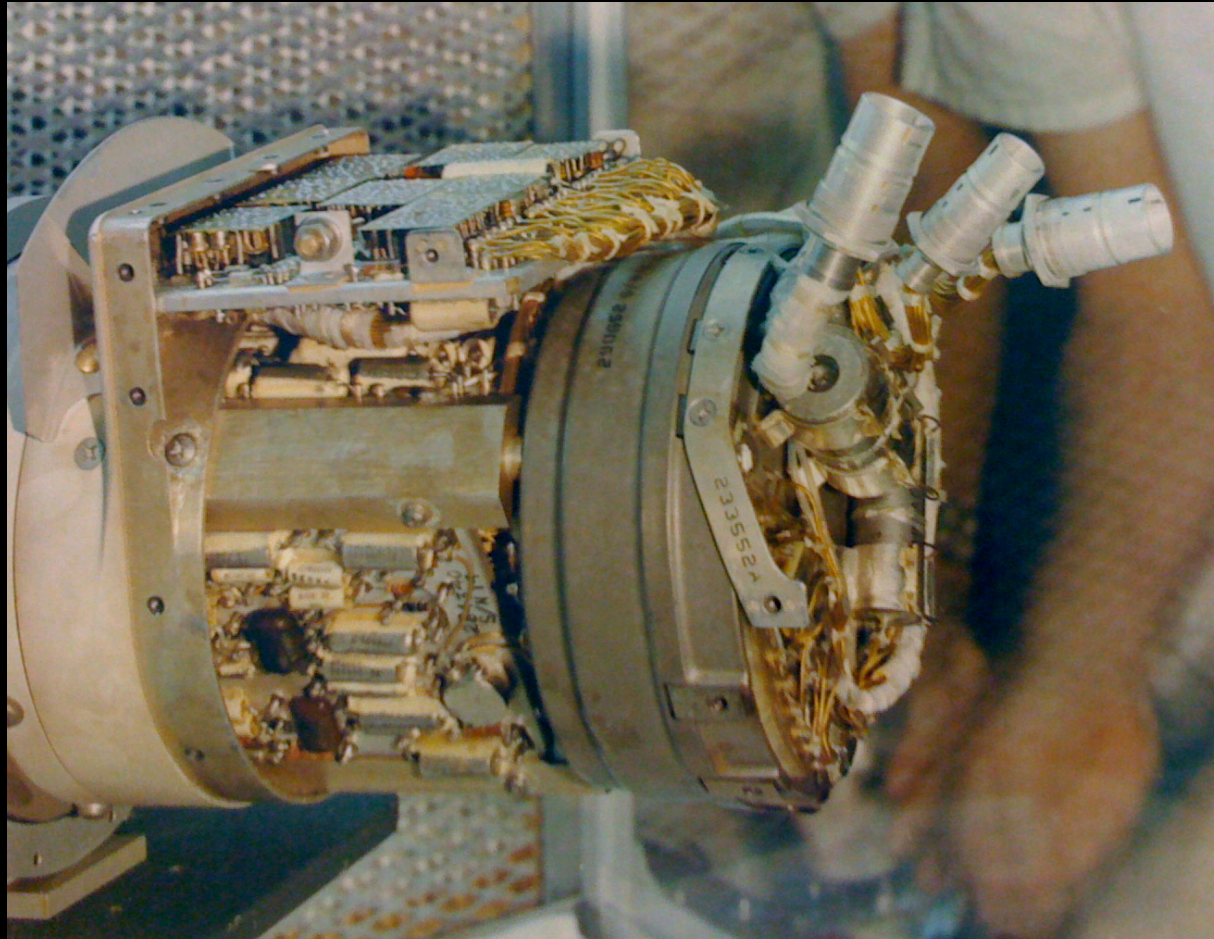
Retrieval  
(MRU)

EEVs

*Never  
Gonna  
Happen...*



# Microbial Noah's Ark?



*Yes, But Which One?*





# Apollo XII – A Mission to the Moon



Crew:

- Charles (Pete) Conrad, Jr.
- Alan Bean
- Richard Gordon, Jr.

Launch: 14 Nov 1969

Landed: 19 Nov 1969

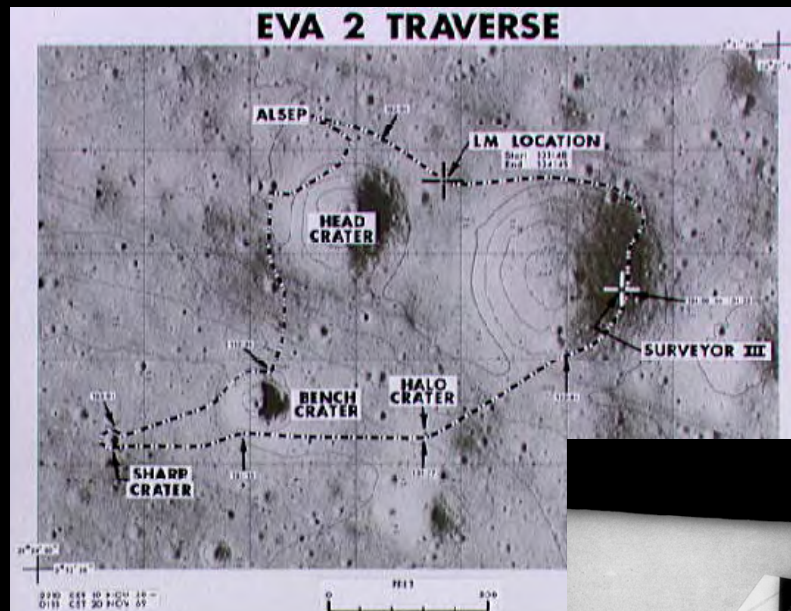
Ocean of Storms

~163 m from Surveyor-III

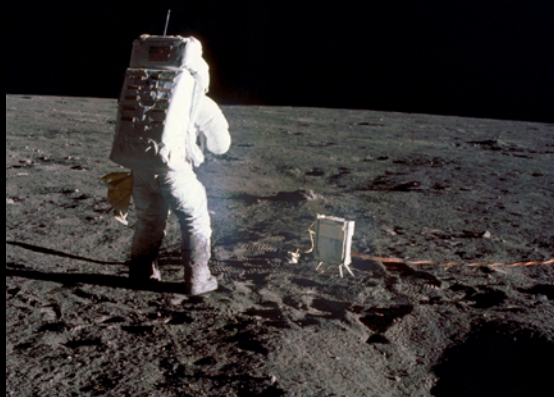




# Apollo XII EVA Operations

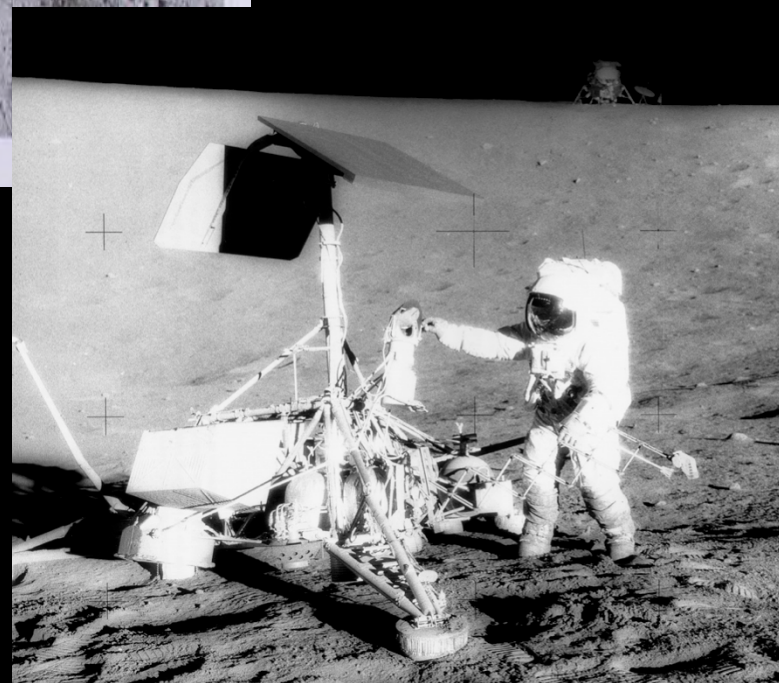


- Crew visited Surveyor III on 2nd EVA, after EVA 1 main science deployments at landing site
- SNAP-27 was deployed for first time



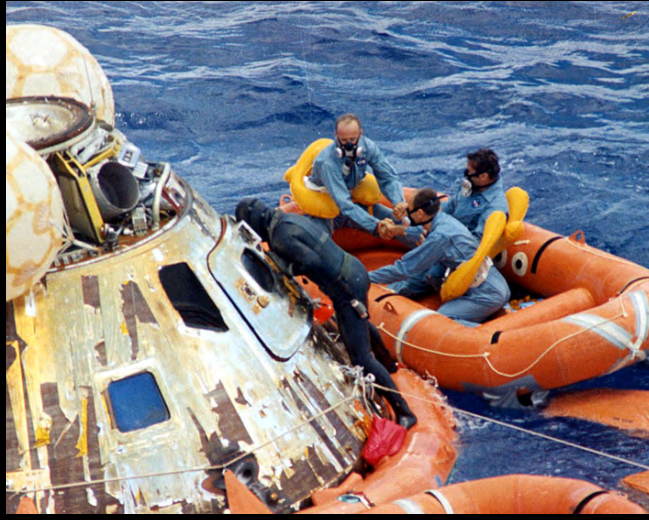
## Surveyor III:

- Launch 17 Apr 1967
- Landed 20 Apr 1967
- Camera retrieved 20 Nov 1969
- Sampled for microbes beginning 8 Jan 1970

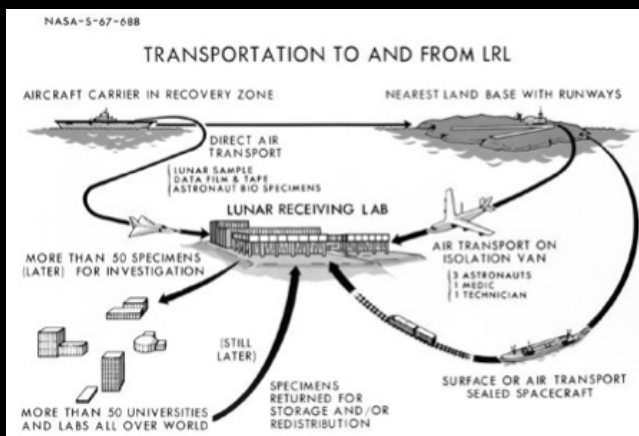
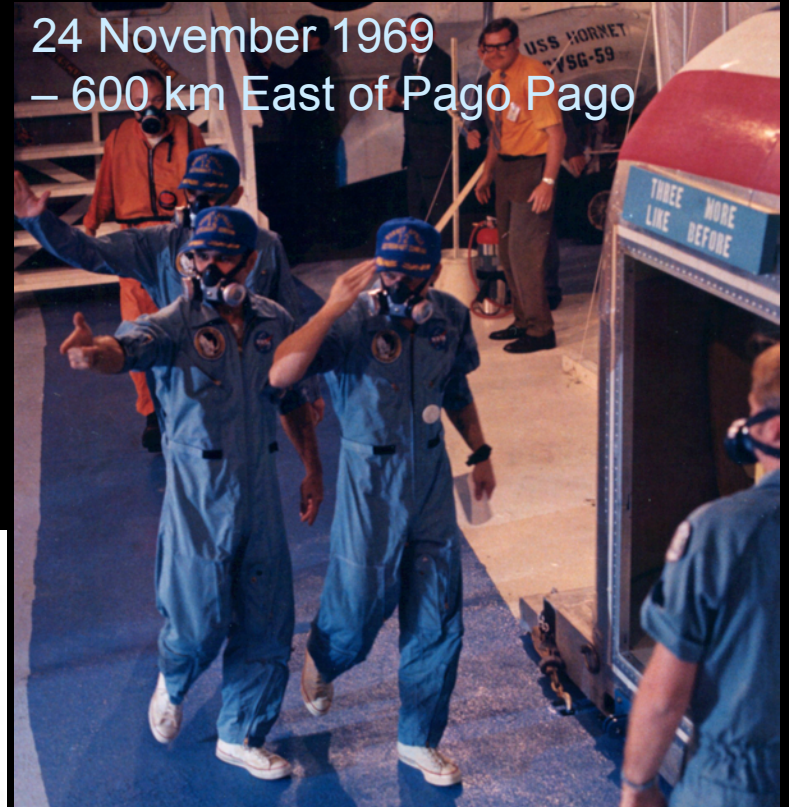




# Apollo XII Returned to the Earth

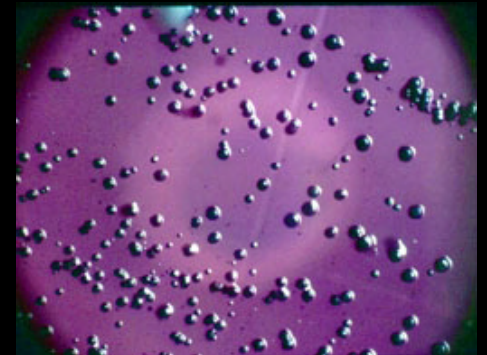
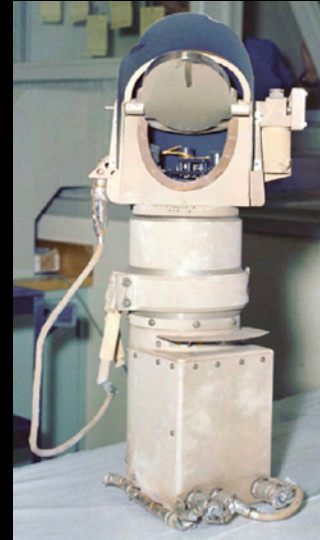


24 November 1969  
– 600 km East of Pago Pago





# ...and so did the Surveyor III Camera



10 December 1969

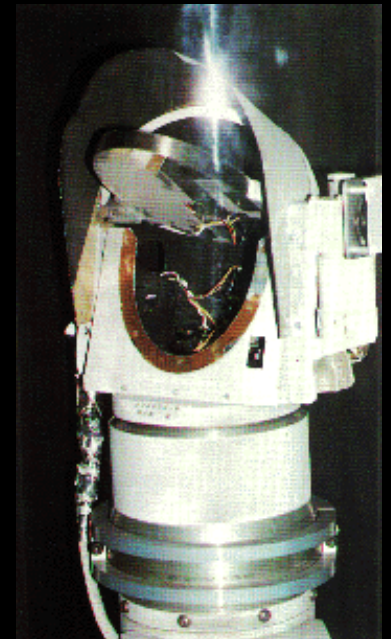


‘They straightened up the cabin, stowing the rock boxes and improvising stowage for the television camera, which Houston wanted to examine. They had carried in considerably more lunar dust than Armstrong and Aldrin had reported; Conrad told Houston they looked like "a couple of bituminous coal miners right at the moment, but we're happy.'”



# Issues wrt *Streptococcus mitis* on the Moon

- 1) Can a microbe survive near-vacuum, and temperature swings from  $\sim -150^{\circ}\text{C}$  to  $\sim 120^{\circ}\text{C}$ ?
- 2) Can a microbe survive the temperatures that were really faced within the Surveyor camera body?
  - Was *S. mitis* ever given the chance?
- 3) Were sufficiently stringent procedures followed:
  - When collecting the camera?
  - When delivering the camera?
  - When conducting the microbial testing?





## Issues wrt *S. mitis* on the Moon (cont.)

1) Can a microbe survive near-vacuum, and temperature swings from  $\sim -150^{\circ}\text{C}$  to  $\sim 120^{\circ}\text{C}$ ?

A spore former, almost certainly, but repeated cycling to  $120^{\circ}\text{C}$  will have a killing effect (D-value  $\sim 1$  day)

Non-spore formers (e.g., *S. mitis*) are much more prone to temperature effects, even under vacuum / lyophilization conditions

Estimates provided by the MSC (now Johnson Space Center) were that the Surveyor III camera reached a maximum temperature of  $\sim 70^{\circ}\text{C}$

- NO VIABLE MICROBES WERE ISOLATED FROM THE SURVEYOR-III CABLES, OR FROM ANY APOLLO SURFACE SAMPLES RETURNED TO EARTH



## Issues wrt *S. mitis* on the Moon (cont.)

2) Can a microbe survive the temperatures that were really faced within the Surveyor camera body?

*NO VIABLE MICROBES WERE ISOLATED FROM 10 OF 11 SAMPLING LOCATIONS (32 OF 33 SAMPLES) WITHIN THE CAMERA BODY—MICROBES WERE RECOVERED FROM THE BACKUP CAMERA (GROUND CONTROL), BUT ONLY IN SMALL NUMBERS (6 LOCATIONS)*

No viable *S. mitis* were ever isolated from the TAT-1 (Type Approval Test Camera) which had remained on Earth

- *Bacillus* sp., *Aspergillus pulvinus*, and *Aureobasidium* sp. were....

*BUT S. mitis WAS ONE OF THE ORGANISMS ISOLATED FROM THE CREW IN ROUTINE MICROBIAL TESTING*



## Issues wrt *S. mitis* on the Moon (cont.)

3) Were sufficiently stringent procedures followed:

- When collecting the camera?

The camera was collected and returned to the LM inside of a sample pack carried by an astronaut.

The camera (in the pack, zipped shut) was stowed in the LM for takeoff from the lunar surface.

*AND S. mitis WAS ONE OF THE ORGANISMS ISOLATED FROM THE CREW IN ROUTINE MICROBIAL TESTING*



## Issues wrt *S. mitis* on the Moon (cont.)

3) Were sufficiently stringent procedures followed:

- When delivering the camera?

The camera was stowed (in the pack, zipped shut) in the Command Module for return to Earth.

The camera was taken to the US (in the pack, zipped shut), and quarantined in the MSC's Lunar Receiving Laboratory.

And there it was placed inside of two Teflon bags, and sealed for storage at room temperature....





# Issues wrt *S. mitis* on the Moon (cont.)



## Issues wrt *S. mitis* on the Moon (cont.)

- 3) Were sufficiently stringent procedures followed:
- When conducting the microbial testing?

“The retrieved TV camera was placed in a laminar-outflow hood...Only those personnel directly responsible for disassembling and sampling the TV camera were permitted in the room. They were clothed in laboratory attire, including surgical caps, face masks, and sterile gloves.” (Mitchell and Ellis, 1971)





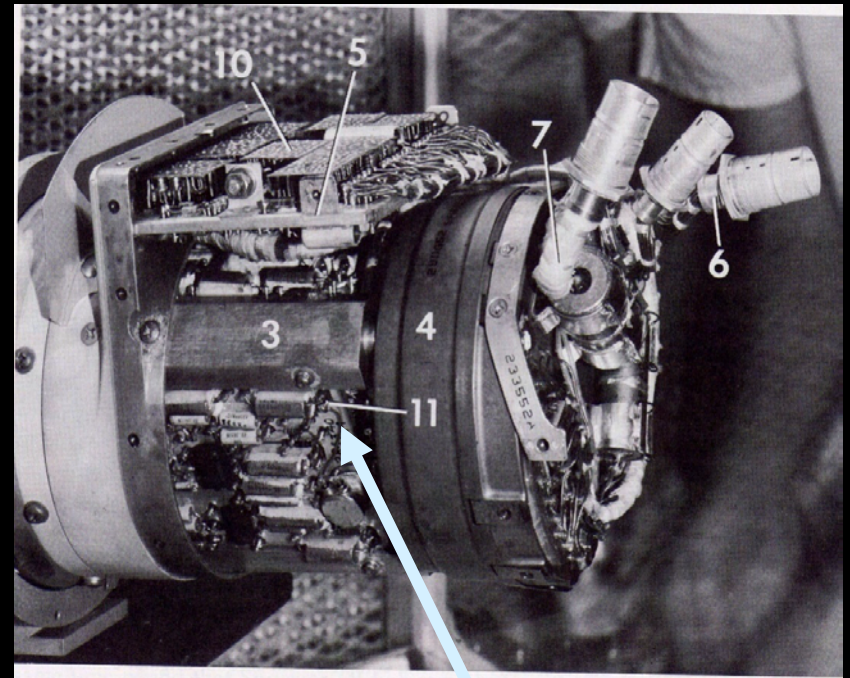
# Issues wrt *S. mitis* on the Moon (cont.)

## 3) Were sufficiently stringent procedures followed:

- When conducting the microbial testing?

“Samples 31, 32, and 33 consisted of bits of polyurethane foam. This foam had been used as insulation between the two aluminum plates of the circuit boards....Only by using long, curved, needle-nosed forceps could one reach through the hole and into the space between the aluminum plates to obtain bits of foam....”

(Mitchell and Ellis, 1971)



## Issues wrt *S. mitis* on the Moon (cont.)

3) Were sufficiently stringent procedures followed:

- When conducting the microbial testing?

No negative control was employed to test sampling procedures (only a positive control, TAT-1).

*S. mitis* was isolated from a colony that grew in the undiluted thioglycollate broth in which a piece of insulating polyurethane foam had been placed.

But wait, what really happened??

Last year, we found the 16mm films, languishing in Maryland. They were viewed and analyzed by all three of us. It wasn't pretty....





# If American Idol Judged Microbiology,



Those Guys Would Have Been Out  
in an Early Round....



Or put more delicately,

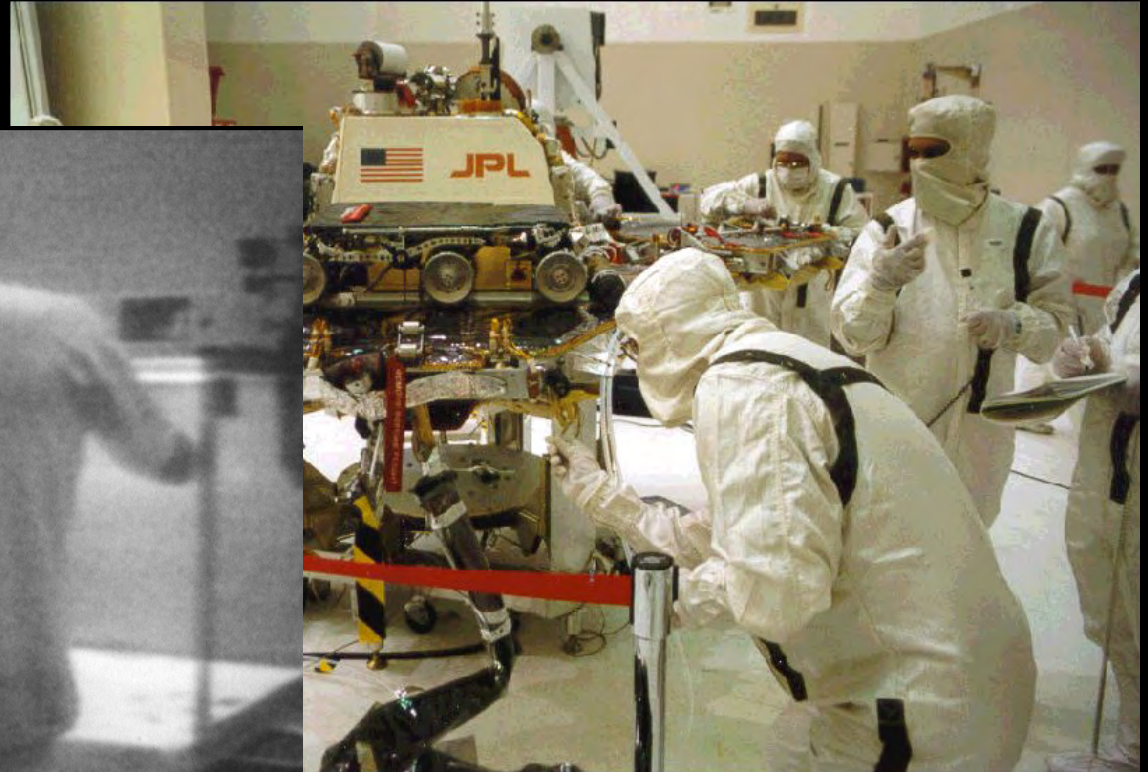
“The general scene does not lend a lot of confidence in the proposition that contamination did not occur.”

– Don Morrison



# Microbiology, and what not to do

- First, the clothes are all wrong....



Final PP Assay, Pathfinder

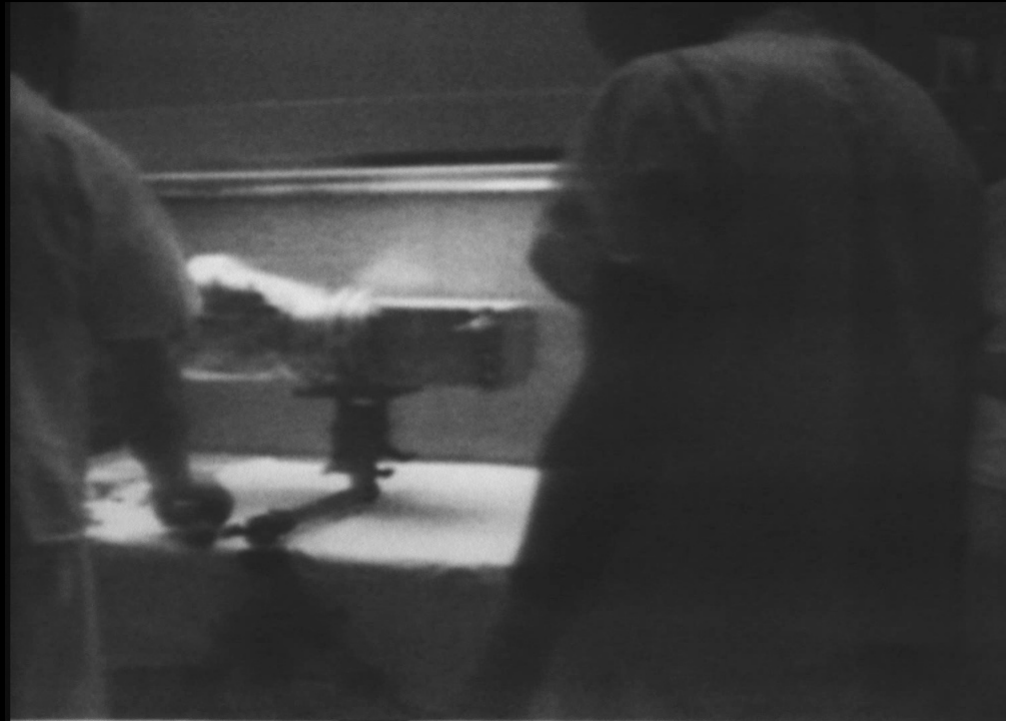
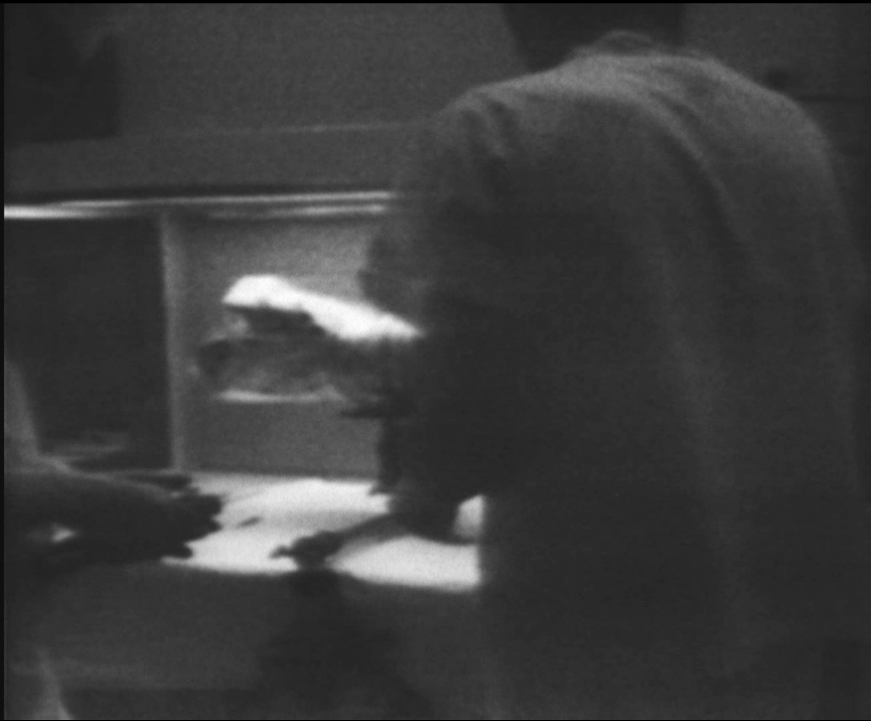


Surveyor III Camera



## Microbiology, and what not to do (cont.)

- And the crew tended to be “less than careful”....



# Microbiology, and what not to do (cont.)

- No gloves, no sleeves, no control....



## Microbiology, and what not to do (cont.)

- A close personal relationship with the subject...



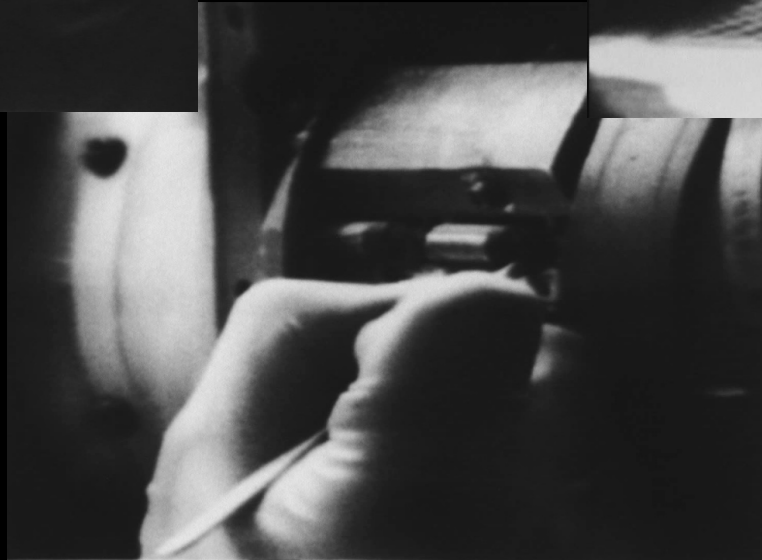
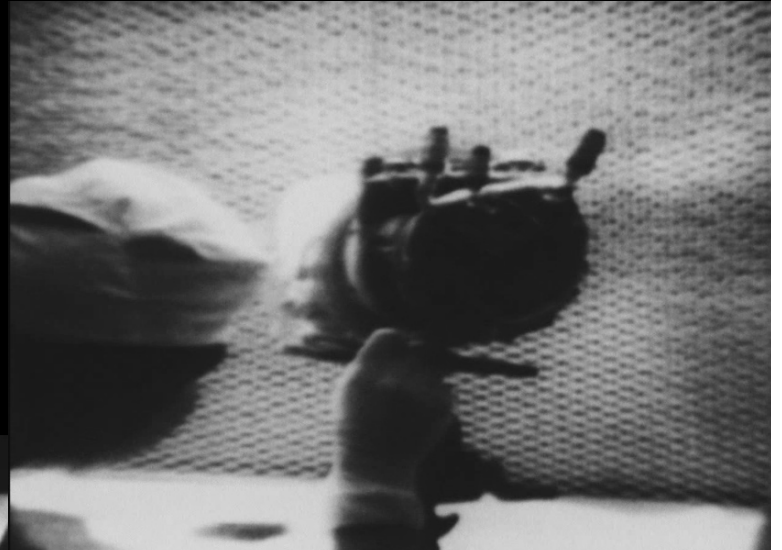
...is not necessarily a good thing, in microbiology.





# Microbiology, and what not to do (fini)

- And after all of that, how can you be sure...



...where your microbes came from?

# Analysis – Don Morrison

## An Anomaly in Foam Sampling

- All of the prior samples were taken with the camera sampling areas on the viewers left. Before taking the foam sample, a worker inserted his upper body into the LFB and visually examines the side of the camera that is toward the filter of the LFB and away from the viewer. He faces the hidden side of the camera's electronics package and examines it, perhaps exposing it to his respiration. After withdrawing from the LFB, the camera is rotated so that the side that was toward the filter of the LFB and examined by the worker is now the visible face and the camera sampling areas are now on the viewer's right. Immediately after the camera rotation, the foam samples are taken (about 29 minutes of disk 22). The collection of the foam sample concludes the sampling exercise. The foam samples were the last taken. This raises a serious question. Because the worker extended his upper body into the LFB and directly faces the area from which the foam sample was taken, it is possible that his exhalations were deposited on the camera, including the foam, causing contamination.



# Analysis – Don Morrison

## An Anomaly in Foam Sampling (cont.)

- If this is the case, then the electronics package surfaces would have been contaminated as well. But, because only the foam samples were taken and were the last sample taken, it is impossible to determine whether or not other areas of the camera were contaminated by the workers respiration. The situation destroys the argument that the foam samples were unique in the sense that the foam locality was better shielded from contamination than the exterior areas of the camera electronics package. The external areas were **not** tested after the potentially contaminating behavior of the worker. Consequently, it cannot be shown that the action of the worker in inserting his upper body into the LFB and facing the hidden side of the electronics package resulted in no contamination from his respiration, even if no contamination occurred. If other adjacent surfaces has been sampled after the foam samples and shown to be free of bacteria, then the action of the worker would be less of an issue. As it is, the possibility that contamination occurred during foam sampling is very



Comments from Judy Allton, March 15, 2010:



# Analysis – Judy Alton

## Comments

- Don has provided a thorough, thoughtful assessment with which I concur.

As to general protocol, I will add that the participants were wearing short sleeve scrubs, thus arms were exposed. Also, that the scrub shirt tails were higher than the flow bench level (and would act as a bellows for particulates from inside the shirt).

We do not see how the tweezers were handled before the sampling.



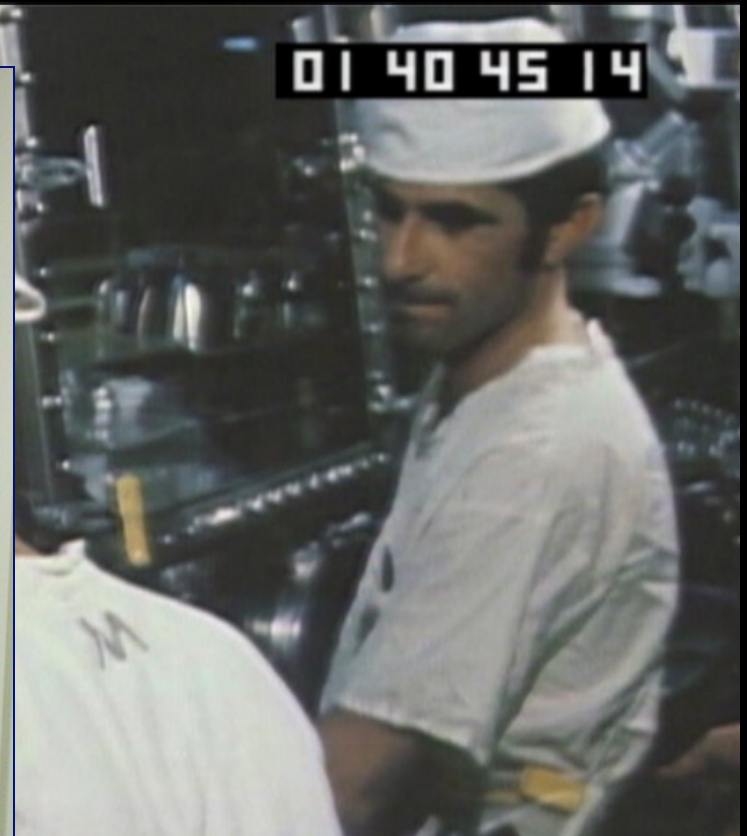
## ***Streptococcus mitis* on the Moon – what to do?**

Just because we don't believe it, doesn't mean it is going to go away...the WWW is forever.

- But we need to think about the microbial aspects of the mission when planning for the return of materials from other planetary bodies
  - Round-trip contamination should be avoided, but anticipated when planning and executing missions
  - Take a microbial inventory before you go
  - Short circuit contamination must be avoided at all costs
  - Robotic sampling can simplify the problem. NASA must engender expertise in this area, and should begin NOW!



We have  
come a long  
way in  
contamination  
control!



Thanks to my co-authors  
– Judy Allton  
– Don Morrison  
who stuck with it, and  
found the record of  
events.





# Major References

## *Streptococcus mitis* on the Moon

- Knittel, M. D., Favero, M. S., Green, R. H. Microbiological sampling of returned Surveyor III electrical cabling. 1971. *Proc. Second Lunar Sci. Conf.* 3:2715-19.
- Mitchell, F. J., Ellis, W. L. 1971. Surveyor III: Bacterium isolated from lunar-retrieved TV camera. *Proc. Second Lunar Sci. Conf.* 3:2721-33.
- Taylor, G. R. 1974. Space Microbiology. *Annual Review of Microbiology* 28:121-137.



# Questions?



