GEMINI BACTERIAL CHALLENGE REPORT

PURPOSE:

The Gemini bacterial challenge test was completed by Analytical Services, Inc., an independent laboratory, to determine the quality of viable bacteria in the Gemini effluent when challenged at the inlet by a high concentration of a specific test organism.

METHOD:

A pure strain of *Pseudomonas aeruginosa* was inoculated into a flask of Trypticase Soy Broth and incubated for 24 hours. Following incubation, enumeration was performed using diluted spread plate technique. The culture was determined to contain 2.0 x 10^9 cultivable bacteria per ml using *Pseudomonas* Isolation Agar (PIA).

The Gemini unit was fed by filtered ultrapure water. Prior to the introduction of the challenge culture a 0.2 *um* absolute sterilizing filter was placed on the Gemini inlet plumbing. Triplicate 1 liter pre-challenged samples were collected from the Gemini inlet and outlet controls. Ten ml of the challenge culture (2 x 10^{10} organisms) were diluted in 500 ml of the feed water and injected downstream of the 0.2 *um* filter into the Gemini while the unit dispensed an equal volume.

Following introduction of the challenge organisms, the Gemini was allowed to recirculate for one minute. After recirculation, three samples were dispensed from the Gemini outlet and collected in 1 liter autoclaved bottles. Viable bacteria in the samples were enumerated using the membrane filter (MFS 0.2 *um*) technique. The membrane filters were aseptically plated on PIA and incubated at 28^0 C. This procedure was repeated for samples collected at 30 minute and 1, 2, 3, 4, 24 and 48 hour post spike injections. No other water was dispensed form the Gemini unit during the test period. Following incubation for 24 hours and five days, bacteria on the plates were enumerated. The Gemini panel resistivity meter reading was noted and the dispensing UV lamp was checked at each sampling.

RESULTS:

During the test period, the Gemini resistivity was equal to or greater than 18.1 meghomn-cm @ 25^0 C. The UV lamp at the dispenser port was operating during each sample event. No viable bacteria were discovered from any of the triplicate 1 liter Gemini outlet samples collected 0 to 48 hours following a *P.Aeruginosa* spike (see table). Viable bacteria numbers as Colony Forming Units (CFU) per Liter are given in the following table:

Replicates	1	2	3
Challenge Spike	2X10^{10}	N/A	N/A
Inlet, pre-spike CFU/L*	0	0	0
Outlet, pre-spike CFU/L	0	0	0
Outlet, 1 minute CFU/L	0	0	0
Outlet,30 minutes CFU/L	0	0	0
Outlet, 1 hour CFU/L	0	0	0
Outlet, 2 hours CFU/L	0	0	0
Outlet, 24 hours CFU/L	0	0	0
Outlet, 48 hours CFU/L	0	0	0

Samples were cultured using Pseudomonas Isolation Agar Colony Forming Units per Liter

SECONDARY TEST:

Samples collected twenty eight days after the spiking of Gemini feed water with 2x10^{10} *Pseudomonas aeruginosa* bacteria cells and analyzed by the Kinetic Turbidimetric Method, was also completed for pyrogens with the following results:

Limulus Amebocyte Lysate (LAL) Results

Sample	Date	Endotoxin
Gemini	3/22/95	0.003/