

Analytical MicroLabs

Industrial Microbiologists

NATA Accredited Laboratory: 14917

Douglas Hocking Research Institute, The Geelong Hospital, Barwon Health, Ryrie St. Geelong, VIC. 3220

Phone: 1300 724 961 Fax: 03- 52603183

ABN 55 167 987 554

Citrofresh 14P Propionibacterium acne testing

Certificate number: AML 17137
Batch number: Citrofresh 3603
Production date: May 2005

Gel or cream used; "Simply scential" pure Aloe Vera gel

Date or period of testing: 10/06/05-28/06/05

Organism tested: 1. Propionibacterium acne ACM 5109

Summary of results: Day 1

	Concentration of Citrofresh and Salicylic acid 0%-0%	Concentration of Citrofresh and Salicylic acid 5%-0%	Concentration of Citrofresh and Salicylic acid 10%-0%	Concentration of Citrofresh and Salicylic acid 5%-2%
Exposure time				
10 minutes	5/5 growth	2/5 growth	0/5 growth	2/5 growth
20 minutes	5/5 growth	1/5 growth	0/5 growth	1/5 growth
24 hours	5/5 growth	0/5 growth	0/5 growth	0/5 growth

0, 1, 2, 3, 4, 5/5 growth represent the positive broth (visible growth) out of 5 Nutrient broth No. 2 in anaerobic condition after 48hrs incubation

Initial bacterial contamination of gel: Mixed Coliform 5.0 x10⁴ orgs/ ml

Initial bacterial load: Propionibacterium acne 7.9 x 10⁸ orgs/ml

Controls:
 1. Recovery Broth contamination in anaerobic condition No growth
 2. Recovery Broth contamination in aerobic condition No growth
 3. Disinfectant contamination in anaerobic condition No growth
 4. Fertility test in anaerobic condition Growth

Summary of results: Day 3

	Concentration of Citrofresh and Salicylic acid 0%-0%	Concentration of Citrofresh and Salicylic acid 5%-0%	Concentration of Citrofresh and Salicylic acid 10%-0%	Concentration of Citrofresh and Salicylic acid 5%-2%
Exposure time				
10 minutes	5/5 growth	2/5 growth	0/5 growth	1/5 growth
20 minutes	5/5 growth	1/5 growth	0/5 growth	1/5 growth
24 hours	5/5 growth	0/5 growth	0/5 growth	0/5 growth

0, 1, 2, 3, 4, 5/5 growth represent the positive broth (visible growth) out of 5 Nutrient broth No. 2 in anaerobic condition after 48hrs incubation

Initial bacterial contamination of gel: Mixed Coliform 5.0 x10⁴ orgs/ ml

Initial bacterial load: Propionibacterium acne 9.5 x 10⁸ orgs/ml

Controls:

1. Recovery Broth contamination in anaerobic condition	No growth
2. Recovery Broth contamination in aerobic condition	No growth
3. Disinfectant contamination in anaerobic condition	No growth
4. Fertility test in anaerobic condition	Growth

Summary of results: Day 5

	Concentration of Citrofresh and Salicylic acid 0%-0%	Concentration of Citrofresh and Salicylic acid 5%-0%	Concentration of Citrofresh and Salicylic acid 10%-0%	Concentration of Citrofresh and Salicylic acid 5%-2%
Exposure time				
10 minutes	5/5 growth	1/5 growth	1/5 growth	0/5 growth
20 minutes	5/5 growth	1/5 growth	0/5 growth	0/5 growth
24 hours	5/5 growth	0/5 growth	0/5 growth	0/5 growth

0, 1, 2, 3, 4, 5/5 growth represent the positive broth (visible growth) out of 5 Nutrient broth No. 2 in anaerobic condition after 48hrs incubation

Initial bacterial contamination of gel: Mixed Coliform 5.0 x10⁴ orgs/ ml

Initial bacterial load: Propionibacterium acne 1.5 x 10⁹ orgs/ml

Controls:

1. Recovery Broth contamination in anaerobic condition	No growth
2. Recovery Broth contamination in aerobic condition	No growth
3. Disinfectant contamination in anaerobic condition	No growth
4. Fertility test in anaerobic condition	Growth

Number of challenge: One (1), repeated 2 consecutive days

Origin of Test Organisms: The University of Queensland, Australia

Enumeration of initial bacterial load: HBA in duplicate-pour-plate technique in anaerobic jar

Incubation temperature and time: 37 degree Celsius, 48 hrs in anaerobic jar

Medium for growth of Test Organisms: Wright- Mundy Dextrose medium (Difco)

Recovery Medium: Nutrient Broth No. 2 (Oxoid)

Diluent for Citrofresh 14P: Sterile Hard Water (Melbourne University)

Flow chart of methode used- Modified Kelsey-Mauer Capacity test:

Preparation of gel / cream, containing Citrofresh (5%,10%) and Citrofresh-Salycilic (5%-2%) acid

↓

3 ml of gel / cream aliquoted and pre-reduced oxygen tension in anaerobic jar for 2 hours

↓

3 ml gel / cream challanged with 1 ml diluted (standard hard water) Propionibacterium acne solution

↓

Exposure time 10 minutes in anaerobic jar

↓

20 ul of gel / cream inoculated into 10 ml, oxygen tension reduced Nutrient broth No. 2

↓

Nutrient broth No. 2 incubated at 35 degree Celsius for 48 hours in anaerobic jar

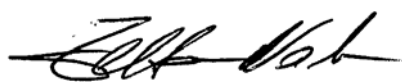
↓

Nutrient broth No.2 subcultured onto HBA (oxygen tension reduced in anaerobic jar for 2 hours)

↓

Incubate HBA at 35 degree Celsius for 48 hours in anaerobic jar to detect growth

Tests repeated at three consecutive days



Zoltan Nack MSc. MBA. MASM
Biologist, Senior Scientist, Analytical MicroLabs