



TECHNICAL SERVICE REPORT NO. 95819

PHARMAID – COSMOCHEM CHEMICALS

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January 2020

**Client:**

PHARMAID (COSMOCHEM CHEMICALS)

**Test Laboratory:**

Technical Service Laboratory  
Thor Specialties  
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## SAMPLES

- 1)ATHENA'S TREASURES Face Mask Avocado MICROCARE PEHG 1%
- 2)ATHENA'S TREASURES Face Mask Avocado 50ml UNPRESERVED
  
- 3)ALOE TREASURES Body Butter Coconut 200ml MICROCARE PEHG 1%
- 4)ALOE TREASURES Body Butter Coconut 200ml UNPRESERVED
  
- 5)ALOE TREASURES Hand Cream Olive Oil 120ml MICROCARE PEHG 1%
- 6)ALOE TREASURES Hand Cream Olive Oil 120ml UNPRESERVED
  
- 7)ATHENA'S TREASURES Hand Cream Aloe & Frangipani MICROCARE PEHG 1%
- 8)ATHENA'S TREASURES Hand Cream Aloe & Frangipani UNPRESERVED
  
- 9)ALOE TREASURES Face Wash Tea Tree 250ml MICROCARE PEHG 1%
- 10)ALOE TREASURES Face Wash Tea Tree 250ml UNPRESERVED

## EXAMINATIONS

Measurement of pH and Redox Potential: **Thor Test Method No 725**  
Screening for Microbial Contamination: **Thor test Method Thor No 201**  
Personal Care Preservative Efficacy Test (Streak plate method) - **Thor Test Method No 206**

## BIOCIDE TESTED

On samples 4-6-8-10 not preserved following addition was tested:

- 1% Microcare® PHDG

## PROCEDURE

### Procedure TM 725 - Measurement of pH and Redox Potential

A pH electrode is calibrated with three standard buffer solutions. After the calibration, the pH value of the sample can be determined.

Reduction potential (also known as redox potential) is a measure of the tendency of a sample to acquire electrons and thereby be reduced; the more positive the potential is, the greater is the sample's affinity for electrons and tendency to be reduced. Redox determination is performed using an appropriate electrode capable of reading the potential in mV.

The redox electrode accuracy is evaluated and checked with a standard solution.

### Procedure TM 201 - Screening of Microbial Contamination

After homogenization, a sterilized loop is used to pick up a small portion of the sample and spread it in petri dishes containing the appropriate cultural media (Nutrient Agar, OGYE Agar, Saboraud Dextrose Agar, SIM medium) to allow the growth of, respectively, bacteria, yeasts, moulds and sulphate reducing bacteria. In this way, the microorganism growth can be separately assessed. The petri dishes and SIM tubes are incubated under the appropriate conditions. For bacteria detection, Nutrient Agar plate, 24-48h at 30 +/- 2°C. For yeasts and moulds detection, OGYE Agar and SDA plates, 48-72h at 26 +/- 2°C. For sulphate reducing bacteria detection, SIM tubes, 48-72h at 30 +/- 2°C. After incubation, the microbial growth is visually evaluated. Appropriate scales are used to determine the extent of the growth in the sample.

### Procedure TM 206 – Personal Care Preservative Efficacy Test (Streak plate method)

The test method comprises the inoculation of a product with blended microbial cultures of known strains (pools) and enumerate the remaining microorganisms at set time points, over a period of 28 days incubation at constant temperature, by streaking onto selective agar plates (TSA or Nutrient agar, for bacteria, and Malt agar for yeasts and moulds). Density growth on the streak is determined by using a subjective numerical rating scale.

It is considered a hard test, created to evaluate the efficacy and the resistance of the preservation system. Depending on the needs in satisfying local Norms (Pharmacopeia or any microbiological test), it is possible, for certain formulation, to pass a challenge test only focused on the speed of a preservation system to reduce the microbial content.

A set of acceptance criteria based on reduction in viability are used to interpret the results.

It is recommended to test three groups of microorganisms:

#### Pool 1 – Bacteria

*Pseudomonas aeruginosa* ATCC 9027  
*Pseudomonas putida* ATCC 31483  
*Burkholderia cepacia* ATCC 25416  
*Klebsiella pneumoniae* ATCC 10031  
*Enterobacter gergoviae* ATCC 33028  
*Escherichia coli* ATCC 8739  
*Staphylococcus aureus* ATCC 6538  
*Staphylococcus epidermidis* ATCC 12228

#### Pool 2 – Yeast

*Candida albicans* ATCC 10231  
*Candida parapsilosis* ATCC 22019

#### Pool 3 - Mould

*Aspergillus brasiliensis* (niger) ATCC 16404  
*Talaromyces pinophilus* (*P. pinophilum*)  
ATCC 36839

The microorganism suspensions are prepared in Physiological solution for bacteria and yeast, in physiological plus Polysorbate 80 for moulds .

**RESULTS**

**Measurement of pH and Redox Potential (TM 725)**

<b>SAMPLE</b>	<b>pH</b>
1)ATHENA'S TREASURES Face Mask Avocado MICROCARE PEHG 1%	5.73
2)ATHENA'S TREASURES Face Mask Avocado 50ml UNPRESERVED	5.74
3)ALOE TREASURES Body Butter Coconut 200ml MICROCARE PEHG 1%	6.33
4)ALOE TREASURES Body Butter Coconut 200ml UNPRESERVED	6.40
5)ALOE TREASURES Hand Cream Olive Oil 120ml MICROCARE PEHG 1%	5.74
6)ALOE TREASURES Hand Cream Olive Oil 120ml UNPRESERVED	5.78
7)ATHENA'S TREASURES Hand Cream Aloe & Frangipani MICROCARE PEHG 1%	5.41
8)ATHENA'S TREASURES Hand Cream Aloe & Frangipani UNPRESERVED	5.63
9)ALOE TREASURES Face Wash Tea Tree 250ml MICROCARE PEHG 1%	5.46
10)ALOE TREASURES Face Wash Tea Tree 250ml UNPRESERVED	5.49

**Screening of Microbial Contamination (TM 201)**

SAMPLE	Growth Rating			
	BACTERIA	MOULDS	YEASTS	SULPHATE REDUCING BACTERIA
	30°C	25°C	25°C	30°C
1)ATHENA'S TREASURES Face Mask Avocado MICROCARE PEHG 1%	0	0	0	-
2)ATHENA'S TREASURES Face Mask Avocado 50ml UNPRESERVED	0	0	0	-
3)ALOE TREASURES Body Butter Coconut 200ml MICROCARE PEHG 1%	0	0	0	-
4)ALOE TREASURES Body Butter Coconut 200ml UNPRESERVED	0	0	0	-
5)ALOE TREASURES Hand Cream Olive Oil 120ml MICROCARE PEHG 1%	0	0	0	-
6)ALOE TREASURES Hand Cream Olive Oil 120ml UNPRESERVED	0	0	0	-
7)ATHENA'S TREASURES Hand Cream Aloe & Frangipani MICROCARE PEHG 1%	0	0	0	-
8)ATHENA'S TREASURES Hand Cream Aloe & Frangipani UNPRESERVED	0	0	0	-
9)ALOE TREASURES Face Wash Tea Tree 250ml MICROCARE PEHG 1%	0	0	0	-
10)ALOE TREASURES Face Wash Tea Tree 250ml UNPRESERVED	0	0	0	-

Growth assessment scale :

**Surface of growth on agar (bacteria and yeast)**

- 0 No growth
- 1 Minimal growth, 1 to 10 visible colonies
- 2 Slight growth, up to 100 colonies
- 3 Significant growth, up to 300 to 400 colonies
- 4 Strong growth, separated colonies remain visible > 400 colonies
- 5 Very strong growth, too many colonies to count but not covering the entire surface
- 6 Total growth, the colonies are virtually inseparable and cover the entire surface

**Surface of growth on agar (moulds)**

- 0 No growth
- X Slight growth
- XX Significant growth
- XXX Strong growth
- XXXX Very strong growth



**Personal Care Preservative Efficacy Test (Streak plate method) (TM 206)**

Results for the bacteria

Bacterial Challenge				
Inoculum (CFU/ml)	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	4 <sup>th</sup>
Viable Count	1.4 x 10 <sup>8</sup>	2.5 x 10 <sup>8</sup>	1.6 x 10 <sup>8</sup>	1.4 x 10 <sup>8</sup>

Test Sample	Evaluation of Bacteria growth:								
	1 <sup>st</sup> inoculation		2 <sup>nd</sup> inoculation		3 <sup>rd</sup> inoculation		4 <sup>th</sup> inoculation		
	6°d	12°d	6°d	12°d	6°d	12°d	6°d	12°d	18°d
1)ATHENA'S TREASURES Face Mask Avocado MICROCARE PEHG 1%	0		0		0		0		
2)ATHENA'S TREASURES Face Mask Avocado 50ml UNPRESERVED	6	6	<i>Contaminated</i>						
3)ALOE TREASURES Body Butter Coconut 200ml MICROCARE PEHG 1%	0		5	6	<i>Contaminated</i>				
4)ALOE TREASURES Body Butter Coconut 200ml UNPRESERVED	6	6	<i>Contaminated</i>						
4 + 1% Microcare® PHDG	0		0		0		0		
5)ALOE TREASURES Hand Cream Olive Oil 120ml MICROCARE PEHG 1%	0		1		1		1	0	
6)ALOE TREASURES Hand Cream Olive Oil 120ml UNPRESERVED	6	6	<i>Contaminated</i>						
6 + 1% Microcare® PHDG	0		0		0		0		



Test Sample	Evaluation of Bacteria growth:								
	1 <sup>st</sup> inoculation		2 <sup>nd</sup> inoculation		3 <sup>rd</sup> inoculation		4 <sup>th</sup> inoculation		
	6°d	12°d	6°d	12°d	6°d	12°d	6°d	12°d	18°d
7)ATHENA'S TREASURES Hand Cream Aloe & Frangipani MICROCARE PEHG 1%	0		3		3		3	0	
8)ATHENA'S TREASURES Hand Cream Aloe & Frangipani UNPRESERVED	4	6	<i>Contaminated</i>						
8 + 1% Microcare® PHDG	0		0		0		0		
9)ALOE TREASURES Face Wash Tea Tree 250ml MICROCARE PEHG 1%	0		5	6	<i>Contaminated</i>				
10)ALOE TREASURES Face Wash Tea Tree 250ml UNPRESERVED	6	6	<i>Contaminated</i>						
10 + 1% Microcare® PHDG	0		0		0		0		

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Results for the yeasts



Yeasts Challenge				
Inoculum (CFU/ml)	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	4 <sup>th</sup>
Viable Count	1.4 x 10 <sup>7</sup>	1.9 x 10 <sup>7</sup>	2.0 x 10 <sup>7</sup>	1.7 x 10 <sup>7</sup>

Test Sample	Evaluation of Yeasts growth:								
	1 <sup>st</sup> inoculation		2 <sup>nd</sup> inoculation		3 <sup>rd</sup> inoculation		4 <sup>th</sup> inoculation		
	6°d	12°d	6°d	12°d	6°d	12°d	6°d	12°d	18°d
1)ATHENA'S TREASURES Face Mask Avocado MICROCARE PEHG 1%	0		0		0		0		
2)ATHENA'S TREASURES Face Mask Avocado 50ml UNPRESERVED	2		6	6	<i>Contaminated</i>				
3)ALOE TREASURES Body Butter Coconut 200ml MICROCARE PEHG 1%	0		1		1		1	0	
4)ALOE TREASURES Body Butter Coconut 200ml UNPRESERVED	6	6	<i>Contaminated</i>						
4 + 1% Microcare® PHDG	0		0		0		0		
5)ALOE TREASURES Hand Cream Olive Oil 120ml MICROCARE PEHG 1%	0		0		0		0		
6)ALOE TREASURES Hand Cream Olive Oil 120ml UNPRESERVED	6	6	<i>Contaminated</i>						
6 + 1% Microcare® PHDG	0		0		0		0		





Test Sample	Evaluation of yeast growth:								
	1 <sup>st</sup> inoculation		2 <sup>nd</sup> inoculation		3 <sup>rd</sup> inoculation		4 <sup>th</sup> inoculation		
	6°d	12°d	6°d	12°d	6°d	12°d	6°d	12°d	18°d
7)ATHENA'S TREASURES Hand Cream Aloe & Frangipani MICROCARE PEHG 1%	0		0		0		0		
8)ATHENA'S TREASURES Hand Cream Aloe & Frangipani UNPRESERVED	0		6	6	<i>Contaminated</i>				
8 + 1% Microcare® PHDG	0		0		0		0		
9)ALOE TREASURES Face Wash Tea Tree 250ml MICROCARE PEHG 1%	0		0		0		0		
10)ALOE TREASURES Face Wash Tea Tree 250ml UNPRESERVED	0		6	6	<i>Contaminated</i>				
10 + 1% Microcare® PHDG	0		0		0		0		

Results for the mould

<b>Fungi Challenge</b>	
<i>Viabile Count</i>	$1.2 \times 10^7$

<b>Sample tested</b>	<b>After 14 days:</b>		<b>After 28 days:</b>	
	<b>Fungal surface development</b>	<b>Control of viable spores (malt development)</b>	<b>Fungal surface development</b>	<b>Control of viable spores (malt development)</b>
1)ATHENA'S TREASURES Face Mask Avocado MICROCARE PEHG 1%	0	0	0	0
2)ATHENA'S TREASURES Face Mask Avocado 50ml UNPRESERVED	0	0	0	0
3)ALOE TREASURES Body Butter Coconut 200ml MICROCARE PEHG 1%	0	0	0	0
4)ALOE TREASURES Body Butter Coconut 200ml UNPRESERVED	0	0	0	0
4 + 1% Microcare® PHDG	0	0	0	0
5)ALOE TREASURES Hand Cream Olive Oil 120ml MICROCARE PEHG 1%	0	0	0	0
6)ALOE TREASURES Hand Cream Olive Oil 120ml UNPRESERVED	0	XX	0	XXX
6 + 1% Microcare® PHDG	0	0	0	0
7)ATHENA'S TREASURES Hand Cream Aloe & Frangipani MICROCARE PEHG	0	0	0	0
8)ATHENA'S TREASURES Hand Cream Aloe & Frangipani UNPRESERVED	0	0	0	0
8 + 1% Microcare® PHDG	0	0	0	0
9)ALOE TREASURES Face Wash Tea Tree 250ml MICROCARE PEHG 1%	0	0	0	0
10)ALOE TREASURES Face Wash Tea Tree 250ml UNPRESERVED	0	0	0	0
10 + 1% Microcare® PHDG	0	0	0	0

Growth assessment scale :

**Surface of growth on agar (bacteria and yeast)**

- 0 No growth
- 1 Minimal growth, 1 to 10 visible colonies
- 2 Slight growth, up to 100 colonies
- 3 Significant growth, up to 300 to 400 colonies
- 4 Strong growth, separated colonies remain visible > 400 colonies
- 5 Very strong growth, too many colonies to count but not covering the entire surface
- 6 Total growth, the colonies are virtually inseparable and cover the entire surface

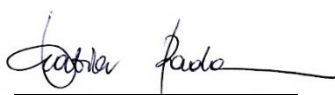
**Surface of growth on agar (moulds)**

- 0 No growth
- X Slight growth
- XX Significant growth
- XXX Strong growth
- XXXX Very strong growth

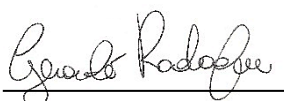
## INTERPRETATION OF RESULTS

To validate a preservative system, it must be capable of withstanding a minimum of 1 successive inoculations and reduce levels of microorganisms to a maximum value of 3 on the rating scale of bacteria and yeasts and must not show signs of fungal development on the surface nor remaining surviving spores on the streaked plates superior to 2X on the rating scale after 28 days of incubation. A sample is considered contaminated if the content of microorganisms is  $\geq 4$  on the rating scale after 14 days incubation for bacteria and yeasts.

In any case, the acceptance criteria are to be discussed with the customer depending on the security level they required.



Technical Director  
*Katia Padoan*



Laboratory Manager  
*Gerardo Radogna*

24.01.2019

Please note that unless otherwise stated, the conclusions and any recommendations, either made or implied, are based on information drawn from examination of the samples identified in this report only. Since these may be influenced by, for example, infection level variations in raw materials, stored component solutions and manufacturing equipment, it is recommended that some appropriate monitoring of microbiological properties be carried out. Use biocides safely. Always read the label and product information before use.