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Consulting Industrial Microbiologists

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Biodet Ref: 09/15190
Client Ref: Dental Powder Analysis

Laser Lifecare Institute
PO Box 106-230
Auckland Mail Centre
AUCKLAND 1143

Attention: Dr Hisham Abdalla

Dear Hisham,

Re: Analysis of Dental Powder

A dental powder previously received at the laboratory for bacterial challenge testing against the organism *Streptococcus mutans* was further analysed over a longer period of time. (*Streptococcus mutans* is commonly found in the mouth, and is regarded as a significant contributor to tooth decay.) The sample was labelled as follows:

Lab. Number	Sample Type	Sample Description	Solution Test Strength
15190	Dental Powder	Ozospa dental powder	1:5

Methodology for Challenge Test:

The challenge method used was based on B.S.3286: 1960 "*Method for Laboratory Evaluation of Disinfectant Activity of Quaternary Ammonium Compounds by Suspension Test Procedure*". This standard describes a test procedure for the laboratory evaluation of the activity of disinfectants, in which a suspension of viable microorganisms is added to solutions containing a pre-determined concentration of the disinfectant and the numbers of organisms surviving after a period of time is determined. A 1:5 solution of dental powder with sterile deionised water was used for the challenge testing.

A neutralising inactivation test had been previously performed to ascertain the ability of the neutralising buffer to stop the action of any antimicrobial agents present in the sample. The results of the analysis showed the product solution was neutralised.

The following organism was used for the challenge testing:

Streptococcus mutans ATCC 25175

The culture was grown on Tryptic Soy Agar and a suspension made to achieve an approximate level of 5.0 x 10^6 colony forming units per ml (cfu/ml) when inoculated into the solution under test. (The identity of the bacterium was revalidated using an APIStrep test.)

0.1ml of this culture was inoculated into 10ml volumes of a blank solution (neutralising buffer) and the product test solution and mixed.

A modification will be made to the method by the addition of 10 ml of the 1:5 Ozospa solution each day, for 4 days. (The blank solution would have 10 ml of Neutralising buffer added.)
The solutions were then sampled at:

T = 0 (Day 0)
T = 1 (At 1 day, prior to adding 10 ml of the 1:5 Ozospa solution)
T = 2 (2 hours after adding 10 ml of the 1:5 Ozospa solution on Day 1)
T = 3 (At 2 days, prior to adding 10 ml of the 1:5 Ozospa solution)
T = 4 (2 hours after adding 10 ml of the 1:5 Ozospa solution on Day 2)
T = 5 (At 3 days, prior to adding 10 ml of the 1:5 Ozospa solution)
T = 6 (2 hours after adding 10 ml of the 1:5 Ozospa solution on Day 3)
T = 7 (At 4 days, prior to adding 10 ml of the 1:5 Ozospa solution)
T = 8 (2 hours after adding 10 ml of the 1:5 Ozospa solution on Day 4)

Challenge Test Results:

Limit of detection: 10 colony –forming units (cfu) per millilitre (ml)

Solution	***Streptococcus mutans* ATCC 25175 Population Levels**								
	Day 0	**Day 1**		**Day 2**		**Day 3**		**Day 4**	
	T = 0 cfu/ml	**T = 1 cfu/ml** Prior to 10 mls of fresh sol'n added	**T = 2 cfu/ml** 2 hrs after 10 mls of fresh sol'n added	**T = 3 cfu/ml** Prior to 10 mls of fresh sol'n added	**T = 4 cfu/ml** 2 hrs after 10 mls of fresh sol'n added	**T = 5 cfu/ml** Prior to 10 mls of fresh sol'n added	**T = 6 cfu/ml** 2 hrs after 10 mls of fresh sol'n added	**T = 7 cfu/ml** Prior to 10 mls of fresh sol'n added	**T = 8 cfu/ml** 2 hrs after 10 mls of fresh sol'n added
Neutralising Buffer Solution	2.5×10^6	3.2×10^6	1.4×10^6	1.6×10^6	6.5×10^5	5.5×10^5	2.7×10^5	2.8×10^5	2.1×10^5
15190 1:5 Ozospa product	2.7×10^6	2.0×10^4	1.2×10^4	2.8×10^2	1.7×10^2	70	13	20	20

Challenge Test Conclusions:

- The organism populations would have naturally become diluted by approximately half each time fresh solution was added. Even when this is taken in to account, every 24 hours there appeared to be a much higher overall population drop in the Ozospa solution than in the neutralising buffer solution.

- Simple dilution over the 5-day period accounted for a 1-log reduction in the *Streptococcus mutans* population over the 5 days in the Neutralising Buffer solution, but a 5-log reduction was observed in the *Streptococcus mutans* population present in the Ozospa solution.

- These results strongly suggest that the product Ozospa has had a significant effect on reducing the population of *Streptococcus mutans* over a sustained period of time in a controlled laboratory test regime. This testing protocol does not take in to account what effect residual food, saliva or other competing microorganisms would have on the effectiveness of the product in a 'real life' setting.

I hope this information is of help to you. If you have any queries please do not hesitate to contact me.

Yours faithfully,

Elaine Khor
B.Sc

Adrienne Burnie
B.Sc., NZCMT

The sample was tested as received.