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TEST REPORT

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Applicant	:	Trumer Medicare Sdn Bhd. No. 16-2, Jalan SS19/1G, 47500 Subang Jaya, Selangor. (Mr. KC Yap)
Manufacturer / Company	:	-
Sample	:	Nano Colloidal Argentum
Method of Test	:	Ocular Irritection® Test
Description of Sample	:	Received one sample for testing with the following identifications: a) Colour : Clear b) Shape/Form : Liquid c) pH : 5.5 d) Quantity : Approximately 100 mL
Date Received	:	21 June 2013
Job No.	:	J207/13
Issue Date	:	26 JUL 2013

Approved signatories,

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1.0 Test timetable

Receipt of sample : 21 June 2013
Study : 22 - 23 July 2013
Report : 26 July 2013

2.0 Test method

2.1 Study Objective

The Nano Colloidal Argentum provided by Trumer Medicare Sdn Bhd was evaluated with the Irritection® Assay System in order to predict its potential to cause ocular irritation.

To achieve this objective, standard volume-dependent dose-response study was performed using the Ocular Irritection® assay system.

2.2 Background

The proprietary Ocular Irritection assay is a standardized and quantitative *in vitro* test which utilizes changes of relevant macromolecules to predict the acute ocular irritancy of chemicals and chemical formulations. This assay is based on the principle that chemical compounds will promote measurable changes in target biomolecules and macromolecular structures. Previous studies have clearly demonstrated that the processes of protein denaturation and disaggregation that are induced in this *in vitro* assay mimic the effects that are produced when these types of irritants are applied to the eye. Consequently, this *in vitro* test may be employed to predict the *in vivo* toxic effects of chemicals and formulations.

Additionally, the Ocular Irritection assay system provides significant benefits when compared to the *in vivo* Draize test method. The quantitative Ocular Irritection *in vitro* assay has been found to be highly reproducible. Of even greater relevance, the Ocular Irritection assay method can be readily employed to evaluate multiple samples at varying volumes or concentrations. Thus, the test serves as an extremely useful screening tool that facilitates all stages of raw material selection, formulation development and final product selection.

2.3 Materials and Methods

The Ocular Irritection assay is a quantitative *in vitro* test method that mimics an acute ocular irritation test. To perform this standardized assay, the test sample is applied to a synthetic biobarrier composed of a semi-permeable membrane. Following application, the sample is absorbed by and permeates through this synthetic biobarrier to gradually come into contact with a proprietary solution containing glycoproteins. Reaction of the test sample with these proteins and macromolecular complexes promotes conformational changes that may be readily detected as an increase in the turbidity of the protein solution. The turbidity may be detected spectrophotometrically at a wavelength of 405 nm.

The irritancy potential of a test sample is expressed as an Irritection Draize Equivalent (IDE) score. This score is defined by comparing the increase in optical density (OD_{405}) produced by the test material to a standard curve that is constructed by measuring the increase in OD_{405} produced by a set of Calibration substances. These Calibrators have been selected for use in this test because their irritancy potential has been previously documented in a series of *in vivo* investigations. As a general guideline, the predicted ocular irritancy classification is shown in Table 1.

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Table 1. Relationship of Irritection Draize Equivalent (IDE) Score to Irritancy Classification for the Ocular Irritection Test Method

Irritection Draize Equivalent (IDE)	Predicted Ocular Irritancy Classification
0.0 - 12.5	Minimal Irritant
12.5 - 30.0	Mild Irritant
30.0 - 51.0	Moderate Irritant
51.0 - 80.0	Severe Irritant

All data were calculated and analyzed via a computer program which determines assay result acceptance based upon qualification parameters defined in the program. Irritancy score that correlates most closely with the *in vivo* irritancy properties of a test article is the highest qualified score calculated by the irritection software. This value is defined as the maximum qualified score.

A standard volume-dependent dose-response study was performed with the Ocular Irritection® test method.

3.0 Results and Discussion

Results and the predicted *in vivo* classifications analyzed via the Irritection software are shown in Table 2.

Table 2. Summary of Ocular Irritection® Results
([a] Maximum Qualified Score)

Sample Description	volume (µL)	IDE Score	Predicted Ocular Irritancy Classification
Nano Colloidal Argentum	50	12.6	Minimal/Mild Irritant
	75	15.0	Mild Irritant
	100	[a]19.8	Mild Irritant

The Nano Colloidal Argentum was classified as minimal ocular irritant with an IDE score of 19.8.

4.0 Conclusion

The **Nano Colloidal Argentum** was evaluated for Ocular Irritection® and classified as **Mild Ocular Irritant.**

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