Title: Safety Evaluation Report – Human Repeat Insult Patch Test and Challenge Test among Sensitive Skin (Ref: 2021/Clintest/R0003)

Coordinator: Skin Tree Center (002673980-A)

Sponsor: Craftiviti Sdn Bhd

Principal Investigator: Gertrude P. Chan, M.D., M.Sc

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1. Introduction

All the work described in this clinical study were conducted in accordance to Good Clinical Practice (CPMP Working Party on Efficacy of Medicinal Products Note for Guidance: Good Clinical Practice for Trials on Medicinal Products in the European Community-1990-CB-55-89-716-EN-C) and in accordance with the guidelines by COLIPA (Walker A.P. et al: Test Guidelines for Assessment of Skin Compatibility of Cosmetic Finished Products in Man. Food and Chemical Toxicology 34, 1996, 651-660). Because it was a study in humans, it was carried out in accordance with the Declaration of Helsinki (2013) and subsequent revisions.

The standard protocol for clinical assessment of irritation potential is the patch testing. It will allow us to assess the primary irritation potential of cosmetic-finished products and ingredients.

The repeat insult patch testing is intended to measure the extent of the skin damage (irritation) resulting from repetitive exposure to the product. The damage is visually measured and subjectively quantified after each exposure. The

skin readings are scored according to the scale in Appendix 1.

2. Testing Facility

The clinical study was carried out at the Clinical Trial Management and Testing Associates facility at the Civic Center Bldg, Rm 1207, Filinvest City, Alabang, Muntinlupa City on 7 – 25 June 2021. The dermatologists in charge were Dr. Gertrude P. Chan, Primary Investigator and Dr. Heidi Chan, Co-Investigator.

3. Test Products

Rinse-off:

A12 – Yein & Young ORGANIC LIQUID CASTILE SOAP

All the investigative products were coded accordingly by the sponsor. The codes were unknown to the investigator /evaluator and to the participants before and during the actual testing.

3. OBJECTIVES:

3.1. To evaluate the capacity of the test products to penetrate the skin of panellists with sensitive skin and to elicit adverse skin reaction by comparing them with the positive control **SS-20** (0.5% Sodium lauryl sulphate).

3.2. To substantiate the manufacturer's claim of "Hypoallergenic" by initiating an Induction phase, Sensitization phase (2 weeks) followed by a challenge phase.

4. METHODOLOGY

4.1. Subject Selection

Thirty-five (35) volunteers, who belong to sensitive skin type, were recruited, for which they were clearly informed, verbally and in writing, regarding the nature of the study, the timetable, constraints and possible risk. They gave their written informed consent before participating in the study. All of the panellists passed the inclusion and exclusion criteria which are as follows:

Inclusion Criteria:

1. Individuals 18-55 years of age;
2. Individuals belonging to sensitive skin type category,

based on the Lactic Acid Stinging Test.
3. Test area should have a healthy skin;
4. Individuals free of any systemic or dermatological disorder which, in the opinion of the investigator would interfere with the study results or increase the risk of adverse reaction;
5. Individuals with uniform-colored skin on the infra scapular area of the back which would allow a discernable erythema;
6. Individuals who has completed a patch study Medical Screening form as well as Personal History form;
7. Individuals who have read, understood, signed and dated an inform consent agreement.

Exclusion Criteria

1. Individuals with any visible skin disease at the study site, which, in the opinion of the investigative; personnel, would interfere with the evaluation;
2. Individuals receiving systemic or topical drugs or medication which, in the opinion of the investigative personnel, would interfere with the study result;
3. Individuals currently under oral steroid treatment for asthma or other medical conditions;
4. Individuals with psoriasis and/or active atopic dermatitis/ eczema;
5. Individuals with a known sensitivity to cosmetics, skin care products, household products or topical products related to products being tested;
6. Females who are pregnant, planning pregnancy or nursing a child.

5. Results:

5.1 Validation of the test

To validate the clinical testing, positive control, 0.5% SLS (**SS20**) and the negative control, water (**SS19**), were run at the same time with the test products.

The Primary Irritation Index after 21-day RIPT were as follows.

SS20 (+Ctrl) = 1.39
SS19 (-Ctrl) = 0.00

Statistical analysis utilizing the Wilcoxon Signed Ranks Test at 0.05 level of significance, was done. The results showed **SS20** was statistically more irritating than **SS19** with a p value of 0.000.

5.2 21-Day Cumulative Irritancy Assay

The Primary Irritation Index (PII) was derived using the responses observed by the trialist. It is a value depicting the average response of the panel as a whole. It is calculated by adding the final irritation scores and divided by the total number of test subjects. This value represents the cumulative exposures.

When the 35 panelists were exposed topically, daily for 21 consecutive days to the product, the Primary Irritation Index (PII) were as follows:

SS20 = 1.39
A12 = 0.61

5.2.2.Data analysis

To compare the irritation potential of the test product **A12** to the positive control **SS20,** Wilcoxon Signed-ranks Test was utilized and the results were as follows: (p value equal or less than 0.05 was considered significant).

Table 1: Data Analysis comparing the products with the positive control

Products versus SS20 (0.5% SLS)	21-day-RIPT
A12	SS20 significantly more irritating, p=0.000

Test product **A12** was significantly less irritating than the positive control after the 21-day Repeat Insult Patch Test.

6. Challenge Tests

After the sensitization/rest period of two weeks, the same panelists were exposed with the test and control products under occlusive dressing for 48 hours. The patch test areas were read and scored after 48, 72 and 96 hours. To further validate the sensitization scores from the first challenge test, another re-challenge test was done.
The sensitization period of at least two weeks will allow the immune system to sensitize or educate the T lymphocytes of the panelists to the allergen or product. If the panelist is positive for sensitization, upon challenge or exposure with the same product, he/she will react positively.

The study of Frosh and Kligman (1) after testing different irritants, showed a 14% incidence of sensitive skin in the normal population.

Based on the published article by Jackson (2), irritant response is universal while sensitization response is individual and specific. In a given population, many will response to an irritant but only some will response to a sensitizer. Clearly irritation is the more frequent reaction from application of products.

In Singapore, a retrospective study by Dr Chee Leok Goh showed that of 34% of the 74,589 new cases seen over a 2-year period, 39% were irritant contact dermatitis while 11% were allergic contact dermatitis.(3)

Considering the above findings/observations, it can be assumed that sensitization score of more than 14% is significant and can be considered to result in higher level of sensitization potential.

From the 3 challenge tests, the product/s should have at least 2 sensitization scores of 14% or lower.

Table 1 Number of sensitization scores from the 3 challenge Tests

Products	1st Challenge	2nd Challenge	3rd Challenge
A12	**6 (17%)**	**5 (14%)**	**3 (9%)**

With a sensitization score of 14% or less, in 2 out of 3 Challenge tests, product **A12** was considered to have low level of sensitization potential and have low probability to induce allergic reactions to consumers with sensitive skin.

7. Conclusions

7.1 21-day Repeat Insult Patch Test – Induction Phase

On the basis of the 21-Day Repeat Insult Patch Test, product **A12 – Yein & Young ORGANIC LIQUID CASTILE SOAP** compared to the positive control, **SS20** is considered to have low level of irritation potential, and safe for use by consumers with sensitive skin.

7.2 Challenge Tests

Product **A12 – Yein & Young ORGANIC LIQUID CASTILE SOAP** passed Induction Phase, the Sensitization Phase and the Challenge Tests is considered to have low level of sensitization potential and have low probability to developed allergic reactions to consumers with sensitive skin.

Submitted By:

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