CENTRUM ESTETICKÉ DERMATOLOGIE, LÉČBY AKNÉ A OBLIČEJOVÝCH DERMATÓZ SYNCARE PLUS, s.r.o.
Nestátní zdravotnické zařízení, REG: 605/2008 JMK
Ambulance Podpěrova 518/6, 621 00 Brno

FINAL REPORT ON THE COSMETIC PRODUCT'S SKIN TOLERANCE TEST

The test was supervised, and the evaluation verified by a dermatologist-venereologist. The procedure and protocol were conducted in accordance with Cosmetics Europe: Product Test Guidelines for the Assessment of Human Skin Compatibility

Test supervisor	MUDr. Jarmila Rulcová
Approval number by the ethics committee	22/2016, approval confirmed by: MUDr. Jaromír Houzar, Chairman of the Ethics Committee
Type of test performed	**Skin tolerance test for sensitive skin**
Protocol number	**107/2024**
Test execution date	05.08.2024 – 21.08.2024

Client	**PUELLAvone s.r.o.** **Company number: 53 991 893** Rovníková 1457/7, Košice – mestská časť Nad jazerom 040 12, Slovakia

1) IDENTIFICATION OF THE PRODUCT

Order Number	Product Code	Name of Tested Sample	Test Method
05/2024	246	**Puella Universal Laundry Sheets**	**JOT Sensitive**

JOT – Simple open epicutaneous test diluted/undiluted for sensitive skin
OUTO – Repeated epicutaneous application closed patch test with occlusion

The tested products were stored at ambient temperature (20 °C +/- 5 °C).

2) OBJECTIVE OF THE TEST

To assess the level of skin tolerance of the product based on the conducted dermal test corresponding to the intended use.

3) VOLUNTEERS IN THE TEST

The test was conducted with a number of volunteers according to the selected type of method used, as detailed below. The characteristics of the tested volunteers are provided in the chart at the end of the test.

Inclusion Criteria

- Healthy volunteer;
- Age 18-65 years;
- The volunteer must not have dehydrated or sensitive skin in the tested area;
- No history of allergic disease;
- No dermatological pathology in the tested area;
- Previous history shows no allergies to cosmetics or other chemical mixtures that come into contact with the skin;
- Woman who is not pregnant or will not become pregnant during the study, woman who is not breastfeeding;
- The volunteer has signed a written informed consent;
- The volunteer is capable of understanding the study requirements.

Exclusion Criteria

- Volunteer with a condition incompatible with the study;
- Volunteer with an active dermatological disease;
- Volunteer with dry and/or sensitive skin in the tested area;
- Currently using anti-inflammatory medications, corticosteroids, antihistamines, or any other treatments that reduce or inhibit inflammatory or allergic reactions; prohibited medications are described in the current internal manual;
- Woman who is pregnant, may become pregnant, or is breastfeeding;
- Volunteer in a washout period between two studies.

4) DESCRIPTION OF THE TEST METHOD USED

[ ] a) **Description of the OUTO method - repeated epicutaneous application closed patch test with occlusion:**
a) This method is used for products that can be left on the skin after application without subsequent washing. The tested product with an occlusive patch is applied to the chosen test site – the volar side of the forearm, arm, or back. Subjects are instructed to keep the test area dry throughout the test duration. The product is reapplied: on day 1, it is left for 24 hours; on days 2 to 5, it is left for 6 hours. It is always placed under the aforementioned occlusive patch, which is replaced after each application of the product. After each patch removal, the skin reaction is evaluated. The product is then wiped off with a cotton swab soaked in aqua for injection. Before applying the next dose of the product, skin reactivity is reassessed. The Primary Skin Irritation Index (IK) is determined. The index is further evaluated on the 8th day from the start of the test, and the final reading of the reaction and evaluation of the test is conducted on the 10th day. In this regard, we have modified the original Frosch-Kligman test.
Number of Volunteers: 20
Application Site: –

[X] b) **Description of the JOT Method - Simple open epicutaneous test for sensitive skin, diluted:**
This method is used for products that do not remain on the skin after application and are rinsed off. The tested product is applied in a diluted form (10% solution) to the selected test site – the volar side of the forearm, arm, or back. The application duration is 30 minutes. After that, the unabsorbed portion is rinsed off with water or gently wiped away with a damp cotton pad soaked in aqua pro injectione. The evaluation is performed immediately after removing the product, as well as 24 and 48 hours later. The reaction is assessed using the Primary Skin Irritation Index (IK). Given the focus of the test on assessing skin reactions for sensitive skin, the number of volunteers has been increased to 20 individuals.

Note: The application of the product: Puella laundry sheet was dissolved in 100 ml of water and subsequently applied to the volar side of the right arm.

Number of volunteers: 20
Application site: volar side of the right arm

☐ c) **Description of the JOT Method - Simple Open Epicutaneous Test for Sensitive Skin - Undiluted:**
This method is used for products that do not remain on the skin after application and are rinsed off. The test substance is applied in accordance with the nature of the tested sample to the selected testing site—volar side of the forearm, arm, or back. The application duration is 30 minutes. Then, any unabsorbed portion is rinsed off with water or lightly wiped away with a damp pad soaked in aqua for injection. Evaluation is performed immediately after the removal of the product, and then again after 24 and 48 hours. The reaction is assessed using the Primary Skin Irritation Index (IK). Given the focus of the test on verifying skin reactions for sensitive skin, the number of volunteers was increased to 20 individuals.

Number of volunteers: 20
Application site: -

5) EVALUATION METHODS

The compatibility of the tested preparation with the skin was assessed according to the method used for the test. The test site was evaluated visually under standard lighting conditions by a qualified person at the specified time intervals.

If any severe irritation occurred during the ongoing test, subjects were instructed to remove the patch, rinse the area with clean water, and consult the study supervisor.

Clinical assessment of skin reactions (erythema and edema) was assigned degrees from 0 to 3 according to the following table:

Assessment	Erythema (Er)	Edema (Ed)
0	No erythema	No edema
0.5	Barely noticeable erythema in part of the area under the patch (light pink discoloration)	Hardly perceptible, palpable edema
1	Mild erythema, pink discoloration over the entire area under the patch	Palpable and visible edema
2	Moderate erythema, clear discoloration over the entire area under the patch	Noticeable edema with or without papules or vesicles
3	Severe erythema, intense discoloration over the entire area under the patch	Significant edema diffusing from the area with or without papules or vesicles

Any other skin reactions (blisters, papules, vesicles, dryness, desquamation, roughness, soapy effect, etc.) were assessed according to the following scale and described subsequently:

Scale	Description of Reaction
0	No reaction
0,5	Very mild reaction
1	Mild reaction
2	Moderate reaction
3	Severe reaction

At the end of the study, the average irritation index (IKI) was calculated according to the following formula:

I_{KI} = Sum of skin reactions (Er + Ed + blisters + papules + vesicles) / Number of volunteers in the study

IKI made it possible to arbitrarily classify the tested preparation using the scale shown in the table below:

Scale	Rating
$I_{KI} \leq 0{,}20$	Compatible with skin surface and non-irritating
$0{,}20 < I_{KI} \leq 0{,}50$	Mildly irritating
$0{,}50 < I_{KI} \leq 2$	Moderately irritating
$2 < I_{KI} \leq 3$	Very irritating

6) RESULTS

The dermal compatibility skin test was completed by all participants in the trial.

Protocol Deviations - No protocol deviations were recorded during the study.
Results - The average irritation index (IKI) of the tested item is: 0
Adverse Effects - No adverse effects were reported during the trial.

7) CONCLUSION

The tested cosmetic product **246 - Puella Laundry Sheets** was evaluated according to the methods mentioned above. Under the given testing conditions, no objective irritant reactions or any subjective negative observations were recorded among the volunteers.

From the perspective of dermal compatibility assessment, the product is recommended for intended use on sensitive skin, provided that the recommended frequency and method of application are followed.

8) SIGNATURE

The study referred to in this report was conducted in accordance with the experimental protocol and proper clinical practice. I confirm that this report accurately reflects the study conducted and the factual results obtained.

Date of Report Issuance: 21.8.2024

Doc. MUDr. Jarmila Rulcová, CSc.

doc. MUDr. Jarmila Rulcová, CSc., dermatovenerolog

(Signature: doc. MUDr. Jarmila Rulcová, CSc., Dermatovenereologist)

Appendix: Chart of Characteristics of Tested Volunteers and Skin Reactions

ARCHIVING

This Final Report, along with the signed Informed Consent of the volunteer, is archived in electronic form by the processor for a period of 10 years. After this period, the data will be destroyed unless explicitly requested otherwise by the trial sponsor. Access to the computer archive is governed by the relevant internal procedures.

Protocol Number: 107/2024 Code: 246 From: 5.8.2024 To: 21.8.2024

Product Name: Puella Laundry Sheets Total Number of Volunteers: 20

Characteristics of Tested Volunteers				Skin Reactions		
Number	Initials	Gender	Age	In-Situ Reactions to the Product	Individual Scores	Notes
1	LT	M	49	0	0,0	
2	PL	Z	42	0	0,0	
3	ŠM	Z	53	0	0,0	
4	KZ	Z	62	0	0,0	
5	KI	Z	60	0	0,0	
6	SV	Z	64	0	0,0	
7	KL	Z	51	0	0,0	
8	ŠM	Z	51	0	0,0	
9	NM	M	48	0	0,0	
10	MB	Z	24	0	0,0	
11	ŠS	Z	31	0	0,0	
12	HN	Z	64	0	0,0	
13	DM	Z	50	0	0,0	
14	BI	M	55	0	0,0	
15	ČT	M	41	0	0,0	
16	TH	Z	64	0	0,0	
17	JK	Z	42	0	0,0	
18	MT	Z	24	0	0,0	
19	PR	M	53	0	0,0	
20	PP	Z	47	0	0,0	

Legend

Total Score Achieved	0
Average Score	0
Standard deviation	0,00

Abbreviations		Abbreviations	
E	Erythema	**V**	Vesicles
O	Edema	**B**	Bullae (blisters)
P	Papules	**n/a**	Unanalysed data

Result (verbal description)

Under the conditions of testing, no objective irritant reactions or subjective negative observations were recorded among the volunteers.

Assigned Degrees of Reaction

0	No erythema
0,5	Barely noticeable
1	Mild erythema with or without edema
2	Moderate erythema, edema with or without papules
3	Intense erythema, edema with or without papules or vesicles
n/a	Unanalyzed data