CENTRUM ESTETICKÉ DERMATOLOGIE, LÉČBY AKNÉ A OBLIČEJOVÝCH DERMATÓZ SYNCARE PLUS, S.R.O.
PODPĚROVA 518/6, 621 00 BRNO
Company ID: 72998579, Tax ID: 16536591

Protocol on the Irritation Potential of the Product – Mixture for Development Purposes

(Project CZ.01.1.02/0.0/0.0/21_374/0026954)

– on the skin after repeated application in volunteers via an epicutaneous closed test

TYPE: HRIPT (Human Repeated Insult Patch Test)

Report (Version 2):	H01/2025
Test number:	1/H/2025
Product (Agent):	Puella Laundry Sheets
Center:	Centrum estetické dermatologie, Syncare Plus, s. r. o., Podpěrova 518/6, 621 00 Brno
Researcher:	Rulcová Jarmila, doc., MUDr., CSc.

Zadavatel testu: Puellavone s.r.o., IČ:

Test number	01/H/2025
Test start date	30. 1. 2025
Test end date	19. 3. 2025

The test was conducted at a healthcare facility in the field of dermatovenereology, which is insured for adverse effects by the insurance company Kooperativa pojišťovna, a. s.

The above-mentioned test was conducted in accordance with the rules of Good Clinical Practice and standardized procedures.

1 . OBJECTIVE

The objective of this test is to confirm the skin tolerance and absence of allergenic potential of a chemical mixture intended for the development of a polymeric biocompatible dental varnish under the following conditions:

Sample: Puella Laundry Sheets

After repeated application to the skin during boundary experimental conditions in volunteers.

1.1. RELEVANCE OF THE TEST

Ethics

The test was conducted in accordance with the principles of the Helsinki Declaration and its later amendments.

Although the predictable risk for volunteers participating in the test is minimal, there is an appropriate balance between the goal of this test, its potential risks, and the possible effects related to the procedures planned in the protocol.

The product applications were carried out at the SynCare Aesthetic Dermatology Clinic – hereinafter referred to as the "Study Center" – under the supervision of a dermatologist, assisted by a co-investigator or responsible technician. Clinical examinations by the dermatologist, with the help of the co-investigator or technician, were conducted during each visit to the Study Center. In case of significant reactivity to the product, the application for the affected volunteers may be interrupted.

The test was conducted in accordance with Good Clinical Practice. The protocol and testing conditions were reviewed by the Ethics Committee of Syncare Plus, s.r.o. before the test was carried out, and an opinion (No. 1.28.07) was issued on June 1, 2017.

All data related to the health of the subjects and clinical data obtained during and after the completion of the test are protected by the physician-patient (*lege artis*) confidentiality. The clinical research center cannot provide the test sponsor/manufacturer with the true identity of the volunteers. The clinical research center will present only fully protected data concerning the

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information about the volunteer.

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Methodology

The skin tolerance of the product was confirmed by a dermatologist, an expert with extensive experience in this field.
The created experimental conditions induce occlusion at the site of application, which increases the permeability of the tested product's components into the skin and subsequently into the body. If any of these components have allergenic potential, this procedure makes it easier to detect.

The methodology used represents a modification of the original procedure by Marzulli and Maibach (Human Repeated Insult Patch Test – delayed contact hypersensitivity: HRIPT) and adheres to the recommended guidelines for this type of study:

- Marzulli F.N., Maibach H.I., Contact allergy : predictive testing in man, Contact Dermatitis , 1976, 2, pp.1-17
- Draize J.H., Appraisal of the safety of chemicals in Food, Drugs and Cosmetics, FDA (ed), USA, 1959, pp. 46-48
- Frosch P.J. & Kligmann A.M., The Duhring Chamber an improved technique for epicutaneous testing of irritant and allergic reactions, Contact Dermatitis 1979, 5, pp 73-81 ,AFSSAPS, Test clinique final de securite d'un produit cosmetique en vue de confirmer son absence de potentiel sensibilisant cutane retarde : recommandations aux promoteurs de recherche et aux prestataires de service, Paris, 2009

The type of patch and the conditions for using the products were adapted to the type of product being tested in accordance with the relevant procedure.

The chosen experimental area allows for easy testing of products, primarily cosmetic and, if applicable, medical devices. The application site of the product was monitored randomly to prevent variability in skin reactions depending on the location.

The control site (with a patch covering an area where Aqua pro injectione, B. Braun Medical, was applied) serves as a comparative control to prevent potential hidden reactions not directly related to the tested products.

Group of Individuals (Volunteers)

Based on experience gained in the field of contact allergy to cosmetic products, solid knowledge of the individual ingredients included in the tested products, and the number of volunteers defined in the protocol, it was possible in the initial testing phase to confirm the absence of allergenic potential in the tested products. The test was conducted on a group of 30 volunteers.

Results

The results are presented primarily in the form of descriptive data and do not require statistical processing.

Since the tested product shows good skin compatibility under these experimental conditions (extrapolation), it should be considered safe for human health when applied under normal usage conditions.

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2. TYPE OF TEST

This monocentric test was conducted as an open public study.

The volunteers simultaneously served as the control group.

3. STUDY CENTER

Ambulance estetické dermatologie Syncare Plus, s. r. o.
Podpěrova 518/6, 621 00 Brno
Phone: 733 377 799
e-mail: ambulance@syncare.cz

4. INVESTIGATORS – Healthcare Professionals

Principal Investigator: doc. MUDr. Jarmila Rulcová, CSc.

5. DURATION OF THE TEST

Start: January 30, 2025 End: March 19, 2025

6. PRODUCTS

Total number of products: One (1) product was tested.

This number of tested products is in accordance with the relevant procedure, which defines the maximum number of products to be tested based on the selected experimental area and patch material.

This report applies only to the following products:

Puella Universal Laundry Sheets

At the same time, one control patch was applied, corresponding to the type of patch material used, containing an ad hoc amount of Aqua pro injectione, B. Braun Medical.

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7. IDENTIFICATION OF THE TESTED PRODUCT

Product name	Puella Laundry Sheets
Reference code	H01/2025
Batch number	GF88256
Form and organoleptic characteristics	Soluble strip containing surfactants and enzymes
Product volume or weight	1 piece (1.75 g), 50 pieces per package

8. INFORMATION ABOUT THE TESTED PRODUCT

The documents related to the tested products, provided with the sample, include:
approval letter from the Ethics Committee, qualitative and quantitative composition, safety data sheet, and order form.

9. VOLUNTEERS

Sixteen (16) volunteers were enrolled in the test. The number of volunteers whose data were used at the end of the test is 16.

There were 0 withdrawals and no exclusions.

The tolerance of the tested product was therefore assessed in sixteen (16) volunteers.

9. 1 Specific Inclusion Criteria for the Test

The specific inclusion criteria for the test, as defined in the protocol, are as follows:

- **Age:** 18 to 65 years
- **Gender:** Male and female
- **Phototype (Fitzpatrick):** I to IV
- **Skin type:** Normal, without pathology

All volunteers met these specific inclusion criteria. Their characteristics are listed in the appendix.

9. 2 Specific Exclusion Criteria for the Test

The specific exclusion criteria for the test are as follows:

- Specific skin conditions or morphologies in the tested area that could influence the evaluation of skin reactions (e.g., pigmentation, scars, excessively developed body hair, an excessive number of freckles and nevi, solar dermatitis, etc.),
- Clinically present signs of skin inflammation and/or inflammatory reactions, scars, or pigment changes as a result of previous tests in the tested area,
- Allergy to colophony or nickel,
- Allergy or reactivity to cosmetic products of the same category as the tested product,
- Skin hyperreactivity,
- Reactivity to adhesive plaster,
- Participation in more than 5 studies involving excessive use (under plaster) within 12 months prior to the study, including up to 3 HRIPT tests,
- Intensive sun exposure within one month prior to the start of the test,
- Expected intense sunlight exposure or UVA exposure (UV lamps) during the testing period,
- Expected bathing (bathtub, sea, or pool), sauna, or Turkish bath during the testing period,
- Intensive or regular training in one or more sports, where temporary interruption would cause difficulties,
- Treatment with retinoic acid or its derivatives during the 3 months prior to the start of the test,
- Treatment with topical corticosteroids in the test area during the 8 days before the start of the test,
- PUVA or UVB treatment during the 1 month prior to the start of the test,
- Expected vaccination during the testing period or vaccination within 3 weeks before the start of the test.

All volunteers complied with these specific exclusion criteria.

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10. METHODOLOGY

Experimental area and sites of application of tested products

The selected experimental area was marked with a skin marker. It is the skin area between the medial border of the scapula *(mago medialis scapulae)* and the midline of the back.
The application site of the products was chosen by a dermatovenerologist or the principal investigator, or possibly the responsible technician. The clinical condition of the skin was taken into account. In particular, it was necessary to avoid friction areas between the skin and clothing.

10.1 Experimental conditions for the application of tested products

Type of patch	Experimental conditions of use	Amount used
Finn Chambers on Scanpor occlusive patch, 100 x 10 chambers	In accordance with the study	0,2 g

Occlusive patch: Finn Chambers on Scanpor®, manufacturer: Smart Practice. These are standardized patches with aluminum chambers, in which the product was placed (20 µL or approximately 20 mg). The chambers with the test sample are held in place by hypoallergenic adhesive: Scanpor® (internal diameter: 8 mm, area 50 mm²).

The amount of the product was applied and spread with the pad of the index finger under light pressure. The finger was covered with a latex glove or sleeve. For liquid products, 0.3 ml was measured; for solid and semi-solid products, 0.3 g. The weighing was performed on a calibrated laboratory scale TSCALE NHB600, 600 g / 0.01 g.

All experimental conditions defined in the protocol were fully respected.

10.2 Test schedule

The application of the tested product and removal of the patch were performed by a dermatologist, co-investigator, responsible technician, or participant. The test itself consists of two phases – induction and stimulation (challenge).

Induction phase: The sample is applied according to the following schedule.

- The first application of the product to the designated site was performed on day D1 (usually Monday).
- The second application of the product to the designated site was performed on day D3 (usually Wednesday).
- The third application of the product to the designated site was performed on day D5 (usually Friday).

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Participants in the test were instructed to come for patch removal on days D2, D4, and D6. If any of these days fell on a public holiday or day of rest, participants were asked to remove the patch themselves 24 hours after application.

Within 24 hours, or within 48 hours if the removal fell on a Saturday, the application sites were examined by the responsible person in accordance with the test protocol, and the assessment was performed using the Draize scoring system (Draize et al., 1994), modified by Phillips et al. (1972), and further modified by McNamee (2008).

This procedure was followed according to the same protocol in the subsequent weeks. If the first application was on day D1, Monday, then typically the final evaluation — reading of the reaction — took place on day D16, also a Monday.

- Checks: skin examination and targeted questions (paragraph IX.6) were conducted:

  a) before applying the patch on day: D1
  b) and on days: D3, D5, D8, D10, D12, D16

Evaluation on D3 was also carried out for the control sample.

The evaluation was performed 16 minutes after the removal of the patch (or later if redness appeared as a result of patch removal).

Application on day, if D1 = Monday	Evaluation on day, if D3 = Wednesday
D1	D3
D3	D5
D5	D8
D8	D10
D10	D12
D12	D16

- Rest period without application: at least 10 days after the last application.

Stimulation (challenge) phase:

- Application of the product to the site designated for the induction phase under a patch. Usually, the application day is the eleventh day after the last application in the induction phase, i.e., day D23. The tested product is applied using the same procedure to a skin site that was not used in the induction phase — the contralateral side of the back.

- Removal of the patch – participants come to our medical facility 24 hours after application, where a qualified professional removes the patch and performs the first reading.

- kontrolní odečty: další odečty jsou provedeny v den D16 (48 hodin), D26 (72 hodin). Poslední odečet v čase D26 (72 hodin) musí být proveden dermatologem.

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Všechny experimentální podmínky, které jsou definovány v protokolu, byly plně respektovány.

Repeated stimulation (rechallenge) phase:
Participants in the test who showed an unclear positive reaction indicating a possible contact allergy during the stimulation phase of the test were invited to take part in a repeated stimulation test. During this HRIPT phase, the tested product and the control were reapplied to the contralateral side of the back skin. The application was done on areas that had not yet been tested. If the result in this part of the test remains unclear, participants undergo an open application test with the exposure site in the cubital fossa (inner elbow), or possibly on the volar side of the forearm. The tested product and control are applied three times daily for 5 days. The first application is always performed at our medical facility.

10. 3 Limitations related to the test

The limitations concerning the volunteers were those defined for this type of methodology in accordance with the relevant procedure.

10. 4 Control of Protocol Compliance

The investigator verified adherence to the established limitations.

Volunteers – test participants – were interviewed at the end of the test. The investigator assessed the significance of any deviations compared to the experimental conditions required at the start of the test.

Evaluation of the obtained responses is provided in the appendices.

All deviations from the protocol were analyzed, and the investigator assessed their impact on the validity of the results.

All test-related restrictions defined in the protocol were respected by the volunteers.

10. 5 Frequency of Testing

Skin examinations and related questioning were conducted by a dermatologist, assisted by a co-investigator or authorized technician.

The examinations were performed visually under standard daylight conditions. All tests were carried out in accordance with the conditions defined in the protocol.

10. 6 Interpretation of Skin Examination and Questionnaire Results

The interpretation of the results from the skin examinations and questionnaires was defined for this type of test in accordance with the relevant procedure.

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HRIPT Score

Erythema

The pathological unit of erythema is evaluated solely based on the degree of skin redness. The score is assessed at each application of the tested product according to the following levels:

0 – No visible erythema
1 – Barely noticeable erythema (light pink with transitions to clearly pink colour)
2 – Clearly noticeable erythema (clearly pink colour, but not intense enough to be classified as very intense)
3 – Intense erythema (very intense redness)

Record of additional signs characteristic of adverse reaction grading

E – edema
P – papule
V – vesicle
B – bulla – recorded if the vesicle is larger than 5 mm

Other recorded signs

S (spreading) – spreading of reaction signs beyond the application boundaries (Must not be confused with cases where the tested material extends beyond the patch area.)
W (weeping, madidace, oozing) – fluid passing to the surface of a vesicle or bulla

Other symbols that may be recorded

T – reaction to the patch
X – patch was not used
R – subject did not remove the patch at the designated time
L-1 – subject reported loss (detachment) of the patch within 12 hours after exposure
L-2 – subject reported loss (detachment) of the patch between 12 and 48 hours after exposure (–) – subject did not show up

All volunteers included in the test were considered for confirming skin tolerance to the tested product if they underwent at least one post-application examination within the designated timeframe.
All volunteers included in the test were considered to confirm the absence of allergenic potential in the tested product (in the absence of allergic reactions during the induction phase) for the duration of their participation in the test.

Interpretation of the results of the skin examination and questioning was carried out using an appropriate procedure in accordance with this type of test.

Possible reactions observed during testing at the “clean” site were compared with the values observed at the “induction” site at the same time. These reactions were either irritation or the detection of an allergy that developed during the induction phase of the product testing.

The nature, intensity, time of onset, duration, and location (induction site and/or clean site) of the skin reaction were taken into account when interpreting the results.

For the assessment of skin tolerance and potential irritation, the interpretation of results—carried out by a dermatovenerologist assisted by the investigator—was unconditional (based on the **experience of the investigator’s research center** in this field and particularly on **data obtained** from products of the same category tested under similar conditions). Therefore, the tested product could be classified as having **very good, good, moderately good, or poor skin tolerance.**
For the evaluation of allergenic potential, the interpretation of results was partly based on the allergy grading scale established by the **ICDRG** (International Contact Dermatitis Research Group), taking into account visible reactions (clinical signs) and possible reactions observed at the control site.

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11. RESULTS

Summary

1) Puella Laundry Sheets

Under the experimental conditions established for the repeated application of the chemical mixture Puella laundry sheets under occlusive patch, only mild irritation was observed in one volunteer during the induction phase, with no allergic reaction. In the stimulation phase, only mild irritation was present during the induction phase and no allergic reaction occurred. **The product demonstrates good skin tolerability, and no allergenic potential was detected in the tested group.**

Claims derived from the conducted test for product labeling:

- **The tested product does not demonstrate allergenic potential in the tested group of volunteers.**

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Number	Sample	Iki Induction	Iki Stimulation	Cumulative Number of Reactions / Number of Readings (Induction Phase)	Cumulative Number of Reactions / Number of Readings (Stimulation Phase)
1	Puella Laundry Sheets	0.89	2.10	1/112	1/48

Signatures and Dates:

Investigator:

I declare that the overall conduct of the test was carried out under my supervision, and I adhered to the fundamental principles of Good Clinical Practice according to the international ICH E6 guideline, step 4, dated 1/5/1996.

doc. MUDr. Jarmila Rulcová, CSc.

Brno, 31. 3. 2025

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Health Care Facility Insurance Number: Kooperativa – 8602681845
Facility ID (IČZ): 7299857

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Record of Objective Reactions and Other Observations During **Induction Phase**

1) Sample: **Puella Laundry Sheets**

Volunteers: Test subjects			Reaction record					
No.	Initials	D3	D3 Control	D5	D8	D10	D12	D16
1	R. S.	/	/	/	/	/	/	/
2	R. S.	/	/	/	/	/	/	/
3	A. B.	/	/	/	/	/	/	/
4	D. S.	/	/	/	/	/	/	/
5	B. S.	/	/	/	/	/	/	/
6	A. J.	/	/	/	/	/	/	/
7	P. J.	/	I	/	/	/	/	/
8	M. Č.	/	/	/	/	/	/	/
9	P. S.	1	/	/	/	/	/	/
10	D. Č.	/	/	/	/	/	/	/
11	M. H.	/	/	/	/	/	/	/
12	K. S.	/	/	/	/	/	/	/
13	T. S.	/	/	/	/	/	/	/
14	K. S.	/	/	/	/	/	/	/
15	M. O.	/	/	/	/	/	/	/
16	K. O.	/	/	/	/	/	/	/

Record of objective reactions and other observations made during the **stimulation (challenge) phase.**

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1) **Sample:** Puella Laundry Sheets

Volunteers – Test Subjects				
No.	Name (Initials)	D24	D16	D26
1	R. S.	/	/	/
2	R. S.	/	/	/
3	A. B.	/	/	/
4	D. S.	/	/	/
5	B. S.	1	/	/
6	A. J.	/	/	/
7	P. J.	/	/	/
8	M. Č.	/	/	/
9	P. S.	/	/	/
10	D. Č.	/	/	/
11	M. H.	/	/	/
12	K. S.	/	/	/
13	T. S.	/	/	/
14	K. S.	/	/	/
15	M. O.	/	/	/
16	K. O.	/	/	/

Záznam objektivních reakcí a další záznamy, které byly pozorovány v průběhu **indukční fáze**

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KRAJSKÝ ÚŘAD JIHOMORAVSKÉHO KRAJE
Odbor zdravotnictví
Žerotínovo náměstí 3, 601 82 Brno

File No.: 23030/2023/ev. no. 2020/2014 Reference No.: S-JMK 21809/2023/OZ/Sam Brno, February 10, 2023

DECISION

on the amendment of authorization to provide health services
File No.: JMK 131314/2012/ev. no. 2020/2014
dated November 12, 2014

The Regional Office of the South Moravian Region, Department of Health, as the materially and locally competent authority pursuant to Section 15, Paragraph 1, Letter a) of Act No. 372/2011 Coll., on Health Services and Conditions for Their Provision (Health Services Act), as amended, has **decided** under the provisions of Section 20a, Paragraph 1, Letter b) of the same Act, and pursuant to Act No. 500/2004 Coll., the Administrative Procedure Code, as amended, based on the application submitted on February 8, 2023, by the healthcare provider **Syncare Plus, s.r.o., Company ID No. 255 36 591, as follows:**

In the authorization issued by the Department of Health of the Regional Office of the South Moravian Region under file number 2020/2014 dated November 12, 2014, the place of service provision is **changed** from the address Pekařská 397/3, 602 00 Brno to the address **Podpěrova 518/6, 621 00 Brno.**

Effective date of the change: **on the date this decision becomes legally valid.tí**

Furthermore, the authorization to provide health services is stated in its current valid wording as follows:

Provider name:	**Syncare Plus, s.r.o.**
Registered office:	Vranovská 349/70, 614 00 Brno
Company ID (IČO):	255 36 591
Statutory representative:	**Renata Svobodová, Managing Director** Date of birth: August 11, 1975 Permanent residence: Zeiberlichova 84/14, 644 00 Brno
Field of healthcare:	**Dermatovenerology**
Form of healthcare:	Specialized outpatient care
Professional representative:	**doc. MUDr. Jarmila Rulcová, CSc.** Date of birth: March 12, 1946 Permanent residence: Vackova 2175/72b, 612 00 Brno

Address of the place where healthcare services are provided: Podpěrova 518/6, 621 00 Brno

Duration of the authorization: Unlimited (granted for an indefinite period)