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HUMAN REPEAT INSULT PATCH TEST (HRIPT): ASSESSMENT OF PRIMARY AND CUMULATIVE SKIN IRRITATION AND SKIN SENSITIZATION OF A PRODUCT, UNDER CONTROLLED AND MAXIMIZED CONDITIONS (HRIPT TEST)

Study Number: 23-401

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Sponsor: Covalon Technologies Ltd.
1660 Tech Ave., Unit 5
Mississauga, ON, Canada, L4W 5S7

Sponsor Representative: [redacted]

Estimated Study Start Date: TBD

Estimated Study End Date: TBD

Sponsor's Test Article Code	ALS Accession Number	Lot/Batch Number
ColActive Plus Collagen Matrix Dressing	TBD	44575-2-1

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1. ABBREVIATION AND DEFINITION LIST

µg/cm^2	Micrograms Per Square Centimeter
AE	Adverse Event
Ave.	Avenue
cm	Centimeter
Dr.	Doctor
e.g	For Example
GCP	Good Clinical Practices.
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
LTDA	Limited
PDF	Portable Document Format
SAE	Serious Adverse Event
SOP	Standard Operating Procedure
Test Article/Product	These terms may appear interchangeably in the protocol and are defined as the test article/product used in the study
Subject/Participant	These terms may appear interchangeably in the protocol and are defined as the individual(s) that will participate in the study.

2. OBJECTIVE

The objectives of this study are to determine by repetitive epidermal contact, the primary or cumulative irritation and the allergic contact sensitization potential of the ColActive Plus Collagen Matrix Dressing, under maximized conditions, with controlled product quantity and application site. The data will be used as part of a regulatory submission to the FDA.

3. QUALITY REVIEW AND VERIFICATION

This study will be conducted in adherence to Good Clinical Practice Regulations (21 CFR 50: Protection of Human Subjects-Informed Consent). These procedures are designed to ensure adherence to the spirit of Good Clinical Practices (GCPs), as described in:

- International Council for Harmonization (ICH) of Technical Requirements for Pharmaceuticals for Human Use, ICH Harmonized Tripartite Guidelines for Good Clinical Practice 2023 [ICH E6 (R3)].
- US 21 CFR dealing with clinical studies (including part 50 concerning informed consent).
- Declaration of Helsinki, concerning medical research in humans (Recommendations Guiding Physicians in Biomedical Research Involving Human Subjects, Helsinki 1964, amended Tokyo 1975, Venice 1983, Hong Kong 1989, Somerset West 1996, Edinburgh 2000, Washington 2002, Tokyo 2004, Seoul 2008, and Brazil 2013).

The Investigator agrees when signing the protocol to adhere to the instructions and procedures described in it and, thereby, to adhere to the GCPs to which it conforms. The study data and final report will be reviewed and signed by Quality Assurance staff. The Investigator will permit trial related monitoring, audits and regulatory inspections and will provide copies of, and direct access to, source data/documents.

4. BACKGROUND [1, 2]

Allergic contact dermatitis (sensitization) results when a substance contacting the skin has undergone an immunological alteration in its reactivity. This altered reactivity is the result of prior exposure of the material to the skin, which may lead to the hallmark visual symptoms of erythema, edema, papules.

Primary (acute) irritation is a complex biologic syndrome, with a diverse pathophysiology, natural history and clinical appearance. When exposure is sufficient and the offending agent/test material is potent, classic symptoms of acute skin irritation are seen which include hallmark visual symptoms of erythema, edema, and papules. The irritant reaction quickly peaks and then begins to heal upon removal of irritant. The onset is acute and often after one single exposure. Predictive testing is widely performed to determine the irritant potential of test agents.

This procedure is used as a predictive test for contact sensitization in humans. The procedure involves the application of a discontinuous series of occlusive patches to human skin over a three-week induction period. Induction is followed by an approximate two-week rest period. Challenge consists of a single application to naive skin.

5. TEST PRODUCT

ColActive Plus Collagen Matrix Dressing is an advanced wound dressing made from fish-derived collagen, sodium alginate, carboxymethylcellulose (CMC), and ethylenediaminetetraacetic acid (EDTA). ColActive Plus is a topically applied wound dressing that will transform into a soft gel sheet when in contact with wound exudates. [redacted]

An adequate amount of test article will be supplied by the Sponsor in containers suitable for use by the testing facility. Enough test products will be supplied to last for the maximum number of subjects for the entire study duration. Responsibility of the identity, purity, strength, safety, composition, and stability of the test articles will remain with the Sponsor. Additionally, by signing this protocol the Sponsor confirms that the test articles being evaluated are composed of common cosmetic ingredients with a known, favorable, safety profile and do not pose a

known health risk when used as intended. The test articles will be stored in a secured location with the temperature monitored until use.

Upon arrival at ALS the test product will be assigned a unique laboratory code number and entered into a daily log identifying the lot number, sample description, sponsor, date received, and tests requested.

Sample will be retained for a minimum of 30 days beyond submission of final report. Sample disposition will be conducted in compliance with appropriate federal, state and local ordinances.

6. SUBJECT SELECTION CRITERIA

Approximately 75 healthy subjects satisfying all criteria listed below will be enrolled to complete the study with a minimum of 50 subjects.

6.1 Inclusion Criteria

Individuals included in the study will be enrolled based upon the following criteria.

1. Age: 18-70
2. Sex: Male & Female
3. Fitzpatrick: I-V, minimum 10% of panel Fitzpatrick V.
4. Individuals who will be able to read, understand and give an informed consent relating to the study they are participating in.
5. Individuals who will be free of any dermatological or systemic disorder, which in the Investigator's opinion, could interfere with the study results.
6. Individuals who will be in general good health and who will complete a preliminary medical history form mandated by the testing facility.
7. Individuals who will be able to and agree to cooperate with the Investigator and clinical staff.
8. Individuals who will agree to have test products applied in accordance with the protocol and are able to complete the full course of the study.
9. Individuals who have not participated in a similar study in the past 30 days.
10. Individuals who agree to refrain from sun tanning/bathing and prolonged exposure to sunlight (outdoors).
11. Female volunteers who are willing to undergo a urine pregnancy test.
12. Individuals who agree to not change their current brand of personal care products such as soaps, body washes, laundry detergents, body sprays, body spritzes, etc. while participating on the study.

6.2 Exclusion Criteria

Individuals meeting any of the following criteria will be excluded from study participation.

1. Individuals who are currently taking any medications (topical or systemic) that may mask or interfere with the test results (specifically, corticosteroids, topical and/or systemic [except nasal steroids], non steroidal anti-inflammatory drugs [e.g. ibuprofen, Advil, Motrin, aspirin > 325mg/day], antihistamines, and topical/oral immunosuppressive medications). Subjects must refrain from using any topical/oral anti-inflammatory medications during the length of the study (6 weeks).
2. Individuals who have a history of any acute or chronic disease that might interfere with or increase the risk on study participation. (e.g., systemic lupus erythematosus, rheumatoid arthritis, HIV positive).
3. Individuals who are diagnosed with chronic skin allergies (atopic dermatitis/eczema) or recently treated skin cancer within the last 12 months.
4. Individuals who have damaged skin in close proximity to test sites (e.g., sunburn, uneven skin pigmentation, tattoos, scars, excessive hair, active acne papules or other disfigurations).
5. Individuals who control their diabetes using insulin.
6. Individuals with any history, which in the Investigator's opinion, indicates the potential for harm to the subject or places the validity of the study in jeopardy.
7. Female volunteers who indicate that they are pregnant or are planning to become pregnant or nursing.
8. Individuals with a known history of hypersensitivity to any cosmetics, personal care products, fragrances, and/or adhesives.
9. Employees of ALS.

10. Individuals with a known fish allergy.

7. INFORMED CONSENT FORM AND MEDICAL HISTORY FORM

An informed consent will be obtained from each subject consistent with requirements in 21 CFR 50 prior to initiating the study describing reasons for the study, possible adverse effects, associated risks and potential benefits of the treatment and their limits of liability. Subjects will sign and date the informed consent document to indicate their authorization to proceed and acknowledge their understanding of the contents. Each subject will be sent home with a signed and dated copy of the informed consent form. Each subject will be assigned a subject identification number and medical history information will be collected. This information, along with the signed consent forms will be available for inspection on the premises of ALS only.

8. EXPERIMENTAL TECHNIQUES AND METHODS

Skin response to the test product will be evaluated according to the Berger and Bowman[6] Scale listed below.

Induction Phase (Berger and Bowman1 Scale)

Numeric Scores

0 = No evidence of irritation
1 = Minimal erythema, barely perceptible
2 = Moderate erythema, readily visible; or minimal edema; or minimal papular response
3 = Strong erythema; or erythema and papules
4 = Definite edema
5 = Erythema, edema and papules
6 = Vesicular eruption
7 = Strong reaction spreading beyond test site

Letter Grades (always upper case)

A = Slight glazed appearance
B = Marked glazing
C = Glazing with peeling and cracking
F = Glazing with fissures
G = Film of dried serous exudate covering all or a portion of the patch site
H = Small petechial erosions and/or scabs

Challenge Phase

Inflammatory Responses

0 = No visible reaction
+ = Slight, confluent or patchy erythema
1 = Mild erythema (pink)
2 = Moderate erythema (definite redness)
3 = Strong erythema (very intense redness)

Definition of letter grades appended to a numerical grade:

E = Edema - swelling, spongy feeling when palpated
P = Papule - red, solid, pinpoint elevation
V = Vesicle - small elevation containing fluid
B = Bulla reaction - fluid-filled lesion (blister)
S = Spreading - evidence of the reaction beyond the Webril® pad area
W = Weeping - result of a vesicular or bulla reaction - serous exudate
I = Induration - solid, elevated, hardened, thickened skin

Superficial Effects

g = Glazing

y = Peeling
c = Scab, dried film of serous exudate of vesicular or bulla reaction
d = Hyperpigmentation (reddish-brown discoloration of test site)
h = Hypopigmentation (loss of visible pigmentation at test site)
f = Fissuring - grooves in the superficial layers of the skin

Each of the scores represents the presence of a clinically significant effect that is localized in a representative portion of the patch area, defined as 25% or more of the patch site. Questionable (barely perceptible, minimal or involving less than 25% of the patch site) reactions as well as the + designation are inconclusive and are not included in calculation.

9. PROCEDURE

9.1 Consent

Subjects will be given an informed consent form (ICF) to read and sign. They will have all of their study related questions answered by the Investigator or designated staff. Subjects who qualify based on the inclusion and exclusion criteria will be enrolled into the study.

9.2 Method of Application:

Occlusive – Test products to be studied under occlusive conditions will be applied on a 25-millimeter Hill Top Chamber®, consisting of an outer ring and an inner, flexible flange. Within the chamber is a non-woven Webril ® pad. The chamber is surrounded by a Durapore® semi-occlusive, hypoallergenic adhesive tape.

9.3 Test Products:

The test product will be applied on the 25-millimeter Hill Top Chamber®, which will be applied directly to the test site. Enough product will be used to cover the chamber.

Positive Control:
0.5% sodium lauryl sulfate (Sigma Chemicals, St. Louis, Missouri) in distilled water will be applied as a positive control. Positive control is applied on the same type of occlusive patch as the test product and is applied to the intrascapular region of the subjects' back. Positive control remains on the subjects' skin for 48 hours. The patch is removed, and the positive control site will be evaluated according to the Berger and Bowman[6] Scale by a trained ALS staff member. Positive reactions are notated and reported in final report. Positive Control will only be applied for Induction 1.

Negative Control:
Distilled water will be applied as a negative control. Negative control is applied on the same type of occlusive patch as the test product and is applied intrascapular regions of the subjects' back. Negative control remains on the subject skin for 48-72 hours. The patch is removed and will be evaluated according to Berger and Bowman[6] Scale by a trained ALS staff member. Positive reactions are notated and reported in final report. Subjects who observe a positive reaction are dropped from the study. The negative control will be applied throughout the entire study along with test product.

9.4 Test Product Preparation

Test Product Preparation will be performed as noted below unless otherwise requested by Sponsor.
Prepare ColActive Plus Collagen Matrix Dressing samples using the following method:

Step 1: ColActive Plus Collagen Matrix Dressing: cut with sterile scissors to fit the chamber of the patch (25-millimeter diameter).
Step 2: Place the test article directly on the Hill Top Chamber®.
*Step 3: 0.8mL of distilled water will be dispensed to saturate the test product.**

Step 4: Place a Hill Top Chamber® on the test site.
**Visual inspection will be done to confirm the entire test article has come in contact with distilled water and test product has transformed into a gel.*

9.5 Test Sites/ Procedure:

The patches will be applied directly to the skin of the intrascapular regions of the back, to the right and/or left of the midline, and/or upper arms.

Subjects will be given a Panelist Instructions for the Safety Patch Test. Subjects will be dismissed with instructions to keep the test area dry while the patches are in place and to not expose the test area to direct sunlight. Subjects will be instructed to notify clinical staff during the course of the study should they begin using any new medications, cease taking any medications or have any change in their medical condition.

Patches will remain in place for a minimum of 48 hours. Subjects will be instructed to not remove the patches prior to their next scheduled visit. Subjects patched on Friday will have patches remain on until the following Monday (72 hours). The procedure will be repeated until a series of 9 consecutive 48-hour exposures have been made over 3 consecutive weeks. This is considered the induction phase.

Before each re-application, the test sites will be visually evaluated for any reaction.

Following the induction phase, subject will be given a minimum of 10-day rest period.

A challenge or test dose will be applied once to a previously unexposed test site. Clinical staff will remove the patches 48 hours post-application. Reactions will be scored 30 minutes post-removal, 24-hours post-removal, and 48-hours post-removal. The skin grader will be blinded to the treatment assignments and any previous scores.

Skin response to the test product will be evaluated according to the Berger and Bowman[6] Scale (Section 8. Experimental Techniques and Methods).

The Sponsor will be notified within 1-2 business days of any subject who:
•exhibits an erythema score of 2 or greater
•exhibits papules, vesicles, bullae and/or spreading beyond the patch site, regardless of the degree of erythema
•requires follow-up

If any subject exhibits a grade 2 during induction phase, the test site will be changed. If the subject shows another grade 2 of the same test product during induction phase at the new test site, the test product will be discontinued for safety concerns. If any subject shows a grade 3 or greater during induction phase, the test site will be discontinued for safety concerns.

If any subject shows a significant reaction at the 30 minutes post-removal, 24-hours post-removal, or 48-hours post-removal evaluation during the challenge phase (Grade 1, 2, and 3 only), the Investigator may decide to schedule a re-challenge patch test for confirmatory purposes. This follow-up investigation will be conducted approximately 4-8 weeks after the completion of the challenge phase and will be identical to the challenge phase.

In the event of a re-challenge patch the original test substance should be applied under the same testing conditions (concentration and patch type) to confirm delayed contact hyper-reactivity. Related substances (product reformulations, individual ingredients, ingredient groups, etc.) can also be applied to aid in determining the causative relationship but must be pre-approved prior to application.

10. SUBJECT DISCONTINUATION

The removal of a study subject by the Principal Investigator could occur due to the following reasons:

1. Adverse Event
2. Concomitant treatments or illnesses incompatible with the study

3. Withdrawal of consent by the participant
    a. All subjects will be informed of the fact that they may, of their own free will, withdraw their consent to participate in the study, if they so wish.
4. Loss of follow-up
5. Emergence of a non-inclusion criterion
6. Researchers' decision
7. Protocol violation

Those subjects who, for some reason, are discontinued from the study will not be replaced. Data from these subjects will not be analyzed unless the reason for their discontinuation was related to the investigational product.
The Investigator should carefully track and document all premature discontinuations and their reasons on study documentation and also, when necessary, on the "adverse event" form, as well as on the study documentation.

11. ADVERSE EVENTS

The most commonly observed reactions in this study are:
1. Itching, redness, rash, peeling, swelling and in rare cases blistering.
2. There is also a possibility of getting a reaction from the tape possible resulting in hyperpigmentation.

There also may be risks and discomforts, which are not yet known.

An adverse event (AE) is any unfavorable medical occurrence experienced by the subject, whether or not the event may be related to the test article.

Each adverse event must be promptly recorded and sufficiently documented by the Study Director in the source documentation and case report form even if the adverse event is assessed by the Study Director as unlikely to be related to the study. Adverse events are graded on a scale of severity (mild, moderate, severe, or lift threatening) and on a scale of relationship to the product (unknown, unrelated, unlikely, possible, probable, or definite). All adverse events will be reported to the Sponsor within five business days. All adverse events will be followed up until resolved, stabilized, the subject is lost to follow-up or the event is otherwise explained. All follow-up information should be reported to the Sponsor.

A serious adverse event (SAE) as defined in the CFR 312.32 is "any experience that is fatal or life threatening, is permanently disabling, requires inpatient hospitalization, or is a congenital anomaly, cancer, or overdose". All serious adverse events will be reported to the Sponsor within 24 hours of ALS Beauty & Personal Care notification.

If, according to the Investigator, medical care is warranted, appropriate referrals will be made. ALS Beauty & Personal Care will follow adverse events until resolution.

12. PROTOCOL AMENDMENT

Any changes to the study protocol will be approved in writing by the client and ALS then submitted to the IRB for approval prior to implementation in the study, unless immediate change is necessary to eliminate hazard to the subject(s). Any violations in the study conduct will be documented as protocol deviations. Protocol amendments will be signed by the Investigator and Sponsor representative.

13. STUDY REPORT

A final report will be issued to the sponsor within 4 weeks of study completion.

14. DATA REPORTING

Skin responses to the test product for each subject will be presented in a table in the final report. Product reporting for skin irritation will be based on scores after the first 48hour patch removal. Product reporting for skin sensitization (contact allergy) will be based on scores after the 72hour patch removal of challenge phase.

15. RECORDS RETENTION

All original samples, raw data sheets, technician's notebooks, correspondence files, a copy of final report and remaining specimens will be maintained on the premises of the clinic in limited access marked storage files. A duplicate copy of the final report will be separately archived at ALS. All records pertaining to this study will be retained by ALS, for a period of not less than six (6) years following the submission of the final report.

16. ACCEPTANCE OF PROTOCOL

2/27/2024

Covalon Technologies Ltd. Date

2/14/2024

Principal Investigator Date

REFERENCES

1. 21 CFR. Ch.1. Part 50, Subpart B.
2. Weltfriend S, Ramon M, and Maibach H. Irritation Dermatitis, In Dermatotoxicology, 6th edition, CRC press, 2004, pp.181-235.
3. Contact Dermatitis, Dermatology, Volume 1, 2nd edition, by S Moschella MD, H Hurley MD, W.B Saunders, Company, 1985.
4. Fischer T, and Maibach HI. Finn chamber patch test technique. Contact Dermatitis .11:137-40. 1984.
5. Rietschel, R.L., Fowler, J.F., Ed., Fisher's Contact Dermatitis (fourth Ed.). Baltimore, Williams & Wilkins, 1995.
6. Berger, R.S., Bowman, J.P. (1982). A reappraisal of the 21-day cumulative irritation test in man. J. Toxicol. Ot. & Ocular Toxicol. 1(2);109-115.

APPENDIX I:

QUESTIONNAIRE FOR SUBJECT RECRUITMENT HRIPT
Study Number: 23-401

Subject ID: _____ **Fitz:** _____ **Subject Initials:** _____
Age: _____**(18-70) Date of Birth:** _____ **Race:** _____ **Sex:** _____

Test Site: Upper Arms and Back	**Circle Response**
1. Do you understand that this is a pre-screening recruitment and does not guarantee you will be enrolled on the study? The study is enrolled on a first come first serve basis.	**YES** NO
2. Do you have a history of hypersensitivity or allergic reactions to any cosmetics, personal care products, fragrances, adhesives and/or a known allergy to fish?	YES **NO**
3. Are you willing to have your back photographed?	**YES** NO
4. Have you participated in any study involving the same test site in the past 30 days?	YES **NO**
5. Do you have damaged skin on or in close proximity to test sites (e.g., sunburn, excessive hair, back acne, uneven skin pigmentation, tattoos, scars or other disfigurations)?	YES **NO**
6. Do you agree to refrain from swimming, sun bathing and prolonged exposure to sunlight (outdoors) during the length of the study?	**YES** NO
7. Have you been diagnosed with any of the following that might interfere with or increase the risk on study participation?	
a. active or chronic skin allergies (atopic dermatitis/eczema)	YES **NO**
b. acute or chronic diseases	YES **NO**
c. recently treated skin cancer within the last 12 months	YES **NO**
d. are you insulin dependent for diabetes?	YES **NO**
10. Are you pregnant, or nursing or planning to get pregnant?	YES NO MALE
10a. For Females ONLY, please list your method of birth control: _____	
11. Do you agree to refrain from using any topical/oral anti-inflammatory medications (i.e. Tylenol, Aspirin, Ibuprofen, Naproxen) or/and anti-histamines and/or immunosuppressive medication during the length of the study (6 weeks)?	**YES** NO
12. Are you willing to wear the patches the entire study as instructed?	**YES** NO
13. Do you agree to protect the patch during the length of the study (6 weeks) from water and/or damage?	**YES** NO

Recruited by: _____ **Verified by:** _____

Initial/Date **Initial/Date**

APPENDIX II:

23-401 A QUESTIONNAIRE FOR SUBJECT ENROLLMENT

Subject ID: _____ **Subject Initials: _____**

1) Are you aware that this study involves research? **YES** / NO (circle one)

2) How long is your study participation? _____

3) For the first 9 patching visits, where will the patches containing test product be applied? _____

4) For the visit 11, where will the patches containing test product be applied? _____

5) Female volunteers:

Are you pregnant, or nursing or planning to get pregnant? YES / NO / MALE (circle one)

If of child bearing potential, list means of birth control subject agrees to use while participating on study.

_____

6) What are some possible side reactions expected from this study? _____

7) How many total visits will you make to the facility? _____

8) What is the expected duration of the testing visits? _____

9) You have been given a study calendar for the required visit dates and the open patch hours. Are you able to make all required dates and open hours listed?
YES / NO (circle one)

10) Do you understand that you will be discontinued from the study without full compensation if you fail to complete all required study visits?
YES / NO (circle one)

11) Do you understand the reminder calls and emails are a courtesy and not a requirement of the facility, you will be responsible to making the required visits listed on the calendar?
YES / NO (circle one)

12) Have all your questions about the study process been answered? **YES** / NO (circle one)

L: _____ b*_____ Fitzpatrick Score: _____ (I-V)

_____ _____
ALS Staff Initial Date Subject Initial Date