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SKIN PRICK TEST FOR SKIN SENSITIZATION (CONTACT ALLERGY) OF TEST PRODUCT COLACTIVE PLUS COLLAGEN MATRIX DRESSING

Study Number: 23-233

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

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	44675-2-1

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1. ABBREVIATION AND DEFINITION LIST

AE	Adverse Event
Ave.	Avenue
cm	Centimeter
GCP	Good Clinical Practices.
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
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

To determine the sensitization (contact allergy) potential of test product ColActive Plus Collagen Matrix Dressing after a skin prick test to the skin of human subjects.

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. 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.

5. SUBJECT SELECTION CRITERIA

Approximately 30 healthy subjects satisfying all criteria listed below will be enrolled to complete the study with a minimum of 20 subjects.

5.1 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. Individuals who are willing to refrain from wetting, wiping, cleanse, and covering the test sites (volar surface of the forearms) between the baseline and up to 48-hour time points.
12. Female volunteers who are willing to undergo a urine pregnancy test.
13. Individuals willing to refrain from vitamin C (Emergen-C), orange juice and vitamin water for the study duration and two days prior to the SPT test.

5.2 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).
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. Individuals that have a history of dermatographism.
10. Individuals have a history of frequent skin irritation.
11. Employees of ALS.
12. Individuals with a known fish allergy.

6. 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.

7. EXPERIMENTAL TECHNIQUES AND METHODS

This will be a single center, with no randomization or blinding, study design in 30 healthy adult subjects, age 18-70 years. Based on prior experience approximately 30 subjects will be enrolled to ensure 20 subjects complete the study.

Skin response to the test product and both positive and negative controls will be evaluated and measured by an allergist. If the measured diameter is ≥3mm in response to the test product with expected reactions to both the positive and negative controls, the site will be considered a positive allergen.

Initial reading will take place after 15 minutes looking for the presence or absence of a wheal and flare at all three sites and measuring the diameter of both the wheal and flare if present. The wheal at the positive control must exceed a wheal at the negative control by 3 mm. A second reading is conducted about 6 hours after the SPT administration and similar measurements are taken. The subjects return to the office 1-2 days after the initial administration for a third test reading and measurements. A positive reaction is present when there is a measurable wheal of >3 mm. Small wheals are confirmed by palpation. The skin prick reaction is quantified by measuring the mean diameter of the wheal, using a ruler marked in mm. If the result is a circular wheal, one measurement of the diameter (in mm) is sufficient; if ovoid or irregular, two diameters are measured (smallest and largest) and the numbers are added and divided by 2 (mean diameter). A flare alone is measured but is not of any clinical significance. The flare, area of erythema, may also be recorded by the same method.

Table 1
Sample grading scale for skin-prick testing

Grade	Wheal Size
0 or Negative	Less than 3 mm
+1	3 mm to 5 mm
+2	6 mm to 10 mm
+3	11 mm to 15 mm
+4	Greater than 15 mm or pseudopods

8. BACKGROUND

Skin prick testing (SPT) is a standard method for diagnosing allergic sensitization. SPT interpretation utilizes the presence and degree of cutaneous reactivity as a surrogate marker for sensitization within target organs, i.e., eyes, nose, lung, gut, and skin. When relevant allergens are introduced into the skin, specific IgE bound to the surface receptors on mast cells are cross-linked, mast cells degranulate, and histamine and other mediators are released. This produces a wheal and flare response which can be quantitated. Many different allergens can be tested simultaneously because the resultant reaction to a specific allergen is localized to the immediate area of the SPT.

9. PROCEDURE

9.1 Consent

Subjects will be given an informed consent form (ICF), inclusive of a HIPAA disclosure and California Subject Bill of Rights, 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:

UniTest PC device through a drop of allergen extract. All applications will be to the volar forearm.

9.3 Test Product/Equipment:

The test product will be provided by the sponsor. Test product will be applied using a UniTest PC device to the test site (volar forearm). Aliquot all test articles into their respective sterile reservoir to serve as a basin during application.

9.4 Test Product Preparation / Application:

Test Product Preparation will be performed as noted below unless otherwise requested by Sponsor.

Step 1: ColActive Plus Collagen Matrix Dressing: cut with sterile scissors into a 25-millimeter diameter.
*Step 2: 0.8mL of distilled water will be dispensed to saturate the test product.**
Step 3: Aliquot test product into a reservoir to allow for the UniTest PC device to be dipped into the test product.
Step 4: Use the UniTest PC device to apply the test product to the subject volar forearm skin.
**Visual inspection will be done to confirm saturation of the dressing with distilled water and test product has transformed into a gel.*

9.5 Test Sites:

The test product ColActive Plus Collagen Matrix Dressing, Positive control and Negative control will be applied directly to the volar forearm skin of the subject. The sites will be a minimum of 2cm from each other to minimize cross contamination.

Positive Control:
Use the UniTest PC device and apply the histamine positive control to subjects volar forearm.

Negative Control:
Use the UniTest PC device and apply the saline with glycerin (bacteriostatic) negative control, to subjects volar forearm.

Test sites should be more than 5 cm from the wrist and 3 cm from the antecubital fossa and marked by numbers on the skin to identify the test article, negative control and positive control.

9.6 Procedure:

The test area will be cleaned with an alcohol pad. A series of marks will be made using a skin pen on the subjects' volar forearm.

An aliquot of the test article will be placed in a reservoir. The UniTest PC device will be dipped into the reservoir and then pricked into volar forearm skin of panelist/subject. A new skin prick device will be used for each prick and will be performed in identical order for each panelist/subject. The UniTest PC device will be pressed through the drop of allergen extract and held against the skin for at least 1 second. There will be equal pressure applied for each test. The epithelial layer of the skin will be penetrated without inducing bleeding. A new UniTest PC device will be utilized for each allergen.

Excess solution from drops will be blotted using a clean Kim-wipe tissue. A timer will be used, sites will be read at 15-20 minutes following application.

Positive and negative controls will be measured first. The negative control should exclude presence of an allergen reaction. The positive control should include presence of an allergen reaction. Both controls must show correct reaction in order to proceed with study.*
*Erythema present in the test site of the negative control: According to the consulting allergist, it is acceptable for the negative control to elicit an allergic response as long as the wheal diameter of the positive control exceeds the wheal size of the negative control by at least 3mm. This is to account for individuals who have sensitive skin.

The test sites will be visually evaluated for any reaction by an allergist. The allergist will be blinded to any previous scores the entire study. A trained ALS staff member will scribe for the allergist. Subjects will be brought into the same

sufficiently lit (fluorescent bulbs) room each visit. Subjects will be sitting and will be instructed to expose test site. Grading will be performed during daylight hours between 8am-5pm

The wheal of each test will be measured. A positive being a wheal of ≥3 mm. (Section 7. Experimental Techniques and Methods).

Subjects will be given a panelist instruction handout. Subjects will be dismissed with instructions to keep the test area dry and to not expose the test area to direct sunlight. Subjects will also be instructed not to wet, wipe, cleanse, or cover the test sites. 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. Subjects will be instructed to remain in the testing facility.

At approximately 6-hour (±30 minutes) following the skin prick, test sites will be evaluated by an allergist. The allergist will be blinded to any previous scores. Subjects will be brought into the same sufficiently lit (fluorescent bulbs) room each visit. Subjects will be sitting and will be instructed to expose test site. Grading will be performed during daylight hours between 8am-5pm.

Following the 6-hour grading, subject will be dismissed from the testing facility and instructed to return approximately 24-48 hours post-application. Subjects will also be instructed not to wet, wipe, cleanse, and cover the test sites.

At approximately 24-48 hours following the skin prick, test sites will be evaluated by the allergist. The allergist will be blinded to any previous scores. Subjects will be brought into the same sufficiently lit (fluorescent bulbs) room each visit. Subjects will be standing and will be instructed to expose test site. Grading will be performed during daylight hours between 8am-5pm.

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. Lost to 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 the reasons on study documentation and also, when necessary, on the "adverse event" form.

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 a reaction from the tape, which may result 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. STATISTICAL RATIONALE AND DATA REPORTING

Evaluation of results consists of only one physiological measure (that of wheal/flare) measured at 3 time points: T1-15 minutes after SPT, T2=6hr after SPT and T3=24-48 hrs. after SPT. Our power analysis calculation indicates at a confidence level of 99% and confidence interval of 1.35, we will need a sample size of n=20. No statistical analysis will be performed. No significant effect or interaction effects are anticipated hence the small sample size. Skin responses to the test product and controls for each subject will be presented in a table in the final report.

Product reporting for skin sensitization will be based on statistically significant positive scores at each interval. Any subject that has erythema present in the negative control eliciting an allergic response will be included as long as the wheal diameter of the positive control exceeds the wheal size of the negative control by at least 3mm. This is to account for individuals who have sensitive skin. This data will be noted in the clinical findings.

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.

REFERENCES

1. 21 CFR. Ch.1. Part 50, Subpart B.

2. Heinzerling, L., Mari, A., Bergmann, K. *et al.* The skin prick test – European standards. *Clin Transl Allergy* 3, 3 (2013). https://doi.org/10.1186/2045-7022-3-3.

3. Heinzerling L, Frew AJ, Bindslev-Jensen C, Bonini S, Bousquet J, Bresciani M, Carlsen KH, Van CP, Darsow U, Fokkens WJ, Haahtela T, Van HH, Jessberger B, Kowalski ML, Kopp T, Lahoz CN, Lodrup Carlsen KC, Papadopoulos NG, Ring J, Schmid-Grendelmeier P, Vignola AM, Wohrl S, Zuberbier T: Standard skin prick testing and sensitization to inhalant allergens across Europe--a survey from the GALEN network. Allergy. 2005, 60 (10): 1287-1300.

APPENDIX I:

QUESTIONNAIRE FOR SUBJECT RECRUITMENT SPT
Study Number: 23-233

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

Test Site: Inner Forearms	**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. Have you used any of the following topical/oral anti-inflammatory medications (i.e. Tylenol, Aspirin, Ibuprofen, Naproxen) or/and anti-histamines and/or immunosuppressive medication, anti-depressants, sedatives, and/or anxiolytics at least 2-weeks prior to the start of the study?	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 (3 Days?	**YES** NO
12. Do you have a history of dermatographism? (also known as dermatographia) is a common, benign skin condition in which even a small amount of pressure, like scratching, causes the skin to swell along the line where it was applied.	YES **NO**
13. Do you agree to protect the test site during the length of the study (3 days) from water and/or damage? No cleansing of site allowed.	**YES** NO

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

Initial/Date **Initial/Date**

APPENDIX II:

23-233 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) Where will the test product be applied? _____

4) How many test sites will be used? _____

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) Do you understand the study will require a use of a UniTest PC device on the test sites, which may be painful for individuals?

   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) 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