MASCOT SPINCONTROL
clinical research centre

SPINCONTROL®
au coeur de la peau...

CONFIDENTIAL REPORT

Ref: D01-6Q01-AH-DR21

Version: 01

Dated: 02/02/2022

EVALUATION OF THE IRRITATION POTENTIAL OF

HAIR CARE FORMULATION

THROUGH:

- **Dermatological Evaluation –Single Application Patch Test Method**

TEST PRODUCT REFERENCE:

- **Manswag Hair Serum (AF20-445) : Product A**

Study Sponsor:
ALNA BIOTECH PRIVATE LIMITED
PLOT NO 21, HSIIDC INDUSTRIAL ESTATE,
ALIPUR BARWALA, Panchkula,
Haryana, 136109

MASCOT SPINCONTROL INDIA PVT. LTD.
3rd Floor, Kohinoor Estate,
Sun Mill Compound,
Lower Parel,
Mumbai – 400013, INDIA.

FEBRUARY 2022

CONTENTS

1. EXPERIMENTATION SITE, PARTICIPANTS

1.1 EXPERIMENTATION SITE

MASCOT-SPINCONTROL India Pvt. Ltd.
Kohinoor Estate, 3rd Floor,
Sun Mill Compound,
Lower Parel West,
Mumbai – 400013, INDIA.
Telephone: +91-22-43349191/192
E-mail: info@mascotspincontrol.in

SPONSOR

1.2 STUDY SPONSOR

ALNA BIOTECH PRIVATE LIMITED
PLOT NO 21, HSIIDC INDUSTRIAL ESTATE,
ALIPUR BARWALA, Panchkula,
Haryana, 136109

1.3 STUDY MONITOR

Vijay Kimtata Signature:
ALNA BIOTECH PRIVATE LIMITED
PLOT NO 21, HSIIDC INDUSTRIAL ESTATE, Date:
ALIPUR BARWALA, Panchkula,
Haryana, 136109
E-mail: vijaykimtata.vk@gmail.com

MASCOT SPINCONTROL

1.4 STUDY DIRECTOR

Mr. Mohit Lalvani
MD Signature:
MASCOT SPINCONTROL
Telephone: +91-22-43349191/ 92 Date:
E-mail: mohit@mascotspincontrol.in

1.5 PRINCIPAL INVESTIGATOR

Dr. Raji Patil
Dermatologist Signature:
Reg. No. 200401330
MASCOT SPINCONTROL Date:
Telephone: +91-22-43349191/ 92
E-mail: raji@mascotspincontrol.in

1.6 CO-INVESTIGATOR

Dr. Rajeshree Mayekar
Dermatologist — Signature:
MASCOT SPINCONTROL
Telephone: +91-22-28772314 — Date:

1.7 QUALITY ASSURANCE MANAGER

Ms. Shraddha Jadhav
Assistant General Manager - Quality Assurance — Signature:
MASCOT SPINCONTROL
Telephone: +91-22-43349191/ 92 — Date:
E-mail: shraddhajadhav@mascotspincontrol.in

2. SUMMARY OF THE STUDY

2.1 OBJECTIVE

The objective of this study was to evaluate the irritation potential on healthy human subjects of Hair care Formulation coded:

- **Manswag Hair Serum (AF20-445) : Product A**

The evaluation was performed using:

- **Dermatological Evaluation: Single Application Patch Test Method***
  (*Primary irritation patch test method)

2.2 POPULATION

Twenty four (24) subjects were selected for the study.

The subjects selected for this study were healthy females and males with aged between **18** and **45** years old. In which **12 were female and 12 were male subjects.**

These subjects were selected according to the inclusion/ non-inclusion criteria listed in paragraph 3.1.

2.3 STUDY DURATION

Duration: 8 days [3days and T8 (T+1 week after 0 hour of patch removal) visit was scheduled to monitor follow up reactions].

Scheduled Procedures:

	Screening	T0 (before patch application) Patch Application Day	T1 day (0 hour after the patch removal) Patch Removal Day	T2 days (24 hours after the patch removal)	T8 days (T+1 week after 0 hour of patch removal)
Registration	■				
Protocol Briefing	■				
Consent	■				
ICF		■			
Inclusion and Non-Inclusion criteria by the Dermatologist	■	■			
History Questionnaire	■				
Routine Check up	■				
Clinical Observation	■				
Site Identification		■			
Proscriptions and Restrictions			■	■	■
Concomitant Medication		■	■	■	■
Patch Application		■			
Patch Removal			■		
Dermatological Evaluation reading of patch tests				■	■
AE/SAE Monitoring		■	■	■	■
					End of the study

Study Schedule:

Screening & T0 (before patch application)	T1 (0 hour after the patch removal)	T2 (24 hours after the patch removal)	T8 (T+1 week after 0 hour of patch removal)
18/01/2022	**19/01/2022**	**20/01/2022**	**26/01/2022**

2.4 STUDY DESIGN

- Single application, closed, occlusive patch test.
- Non comparative, single center study.
- Subjects served as their own references.

3. STUDY PROTOCOL

3.1 SUBJECT SELECTION

Mascot Spincontrol's subject panel is composed of subjects selected on the basis of a questionnaire filled in by the Principal investigator for subjects, prior to the study that provides details of their medical history, possible allergies, skin-care, hair-care and make-up habits, as well as a certain amount of administrative information.

The selection procedures are elaborated in order to guarantee that the subjects receive all possible information about the aims of the study and the consequences of their participation.
This selection procedure includes:

- A preliminary interview, during which the following points are explained to the subjects: the study's modalities, its practical considerations, possible payment, as well as any possible cosmetic benefits, inconveniences or potential risks.
- The information form which is specific to the study, including all essential information is then given to the subject to read.
- The consent form which is read and filled in freely and intentionally, approved, and signed by the subject to substantiate the fact that they freely accept the conditions of the study which has been described to them.
- The Informed Consent form which is filled in freely and intentionally by the subject after it had been fully explained to them, in the event of any claims for damages, enables them to benefit from the terms of the insurance policies taken out by Clinical Research Organization as soon as the subject is accepted into the study by the Principal Investigator/ Co-Investigator.

The subject respected the following conditions: (as well as those already mentioned)

- Available for the entire duration of the study
- Motivated to freely participate in the study
- Able to justify a permanent address
- Able to understand Hindi, Marathi, Gujarati and/or English language: i.e. only Hindi, Marathi, Gujarati and/or English speaking subjects capable of reading the consent documents and able to accept the participation conditions.
- No individual sentenced to imprisonment by a court decision or by an administrative decision, or hospitalized without consent, or admitted in a medical or social establishment.
- No minor as well as individual of age benefiting from a legal protection measure or enable to express his/her consent.

The subjects selected for the study were chosen under the supervision of the Principal Investigator and co-investigator, on the basis of the inclusion/non-inclusion criteria listed below.
A selection of 24 subjects were made for this study.
The results given included all of the present and assessable subjects at each examination.

3.1.1 Inclusion criteria

The study was conducted on subjects who fulfilled the following criteria:

Standard criteria

- Female and male Asian Indian subjects.
- Healthy human subjects (no infectious and evolutive pathology which could make the subject vulnerable and stop the study, no pathology which could interfere with the study, no symptom in the process of an exploratory check up)
- Between 18 and 65 years of age.
- Skin is healthy on the studied anatomic unit (free of eczema, wounds, inflammatory scar….)

3.1.2 Non-inclusion criteria

Standard criteria

- For female: Being pregnant or breastfeeding or having stopped to breastfeed in the past three months
- Having refused to give his/her assent by not signing the consent form
- Taking part in another study liable to interfere with this study
- Being diabetic.
- Being asthmatic.
- Following a chronic medicinal treatment comprising any of the following products: aspirin-based products, anti-inflammatories, anti-histamines, corticotherapy, taken by general or local routes (the only medication permitted is paracetamol).
- Having cutaneous hypersensitivity (except in the case of studies with evaluation of sensitive skin).
- Having a diagnosed or highly probable allergy to one or several compounds of the cosmetic products.
- Having undergone a surgery requiring a general anaesthesia of more than one hour in the past 6 months.
- Having changed his/her cosmetic habits in the 14 days preceding the start of the study on the studied anatomic unit.
- The day of the patch application: no cosmetic product must be used (test site clean with water only).
- Refusing to follow the restrictions below during the study:
- For female: Do not become pregnant nor breastfeed.
- Do not take part in another study liable to interfere with this study
- Do not take medicinal treatment comprising any of the following products: aspirin-based products, anti-inflammatories, and anti-histamines, corticotherapy, taken by general or local routes (the only medication permitted is paracetamol).
- Do not change his/her cosmetic habits apart from the particular conditions mentioned in the protocol, on the studied anatomic unit.

Specific criteria

- Having eczema, psoriasis, lichen plan, vitiligo whatever the considered area
- Having disorder of the healing (whatever the considered area)
- Having a rhinitis, allergic conjunctivitis or rhino sinusitis
- Having an allergy to perfumes and/or conservatives in cosmetic products
- Having an allergy to plaster
- Having a food allergy
- Having a cardiovascular pathology (taking a beta blocker treatment)
- Having immunosuppressive drugs, such as cyclophosphamide, methotrexate, azathioprine, etc.
- Taking a retinoid based treatment by general or oral route
- Taking specific treatment on the back.
- Having taken an anti-histaminic treatment in the last 2 weeks preceding the start of the study
- Having miliaria (prickly heat) on the back.
- Presenting too many naevus on the back
- Having high pilosity on the back.
- Refusing to follow the restrictions below during the study:

- During the first 24 hours (after patch application), neither cosmetic products nor water must be applied on the back.
- Till the follow up period of until T8 days, only water is accepted from the first reading i.e. T2 days (24 hours after the patch removal).
- Do not practice an intensive sport activity during the first 24 hours (until the removal of the patches)
- Do not expose the back to the sun.

3.2 THE PRODUCT

3.2.1 Presentation of the product

The test product was supplied free of charge by the study sponsor.

Reference of the product	Code	Batch/ LT	Constituent form	Mfg. Date	Expiry Date	Packaging	Capacity
Manswag Hair Serum (AF20-445)	**A**	**AF20-445**	**Serum**	**01/2021**	**12/2022**	**Pump Bottle**	**50ml**

The study sponsor was in charge of product manufacturing and packaging. He / She was responsible for product identification, purity determination, composition, innocuousness, and any other characteristics of each product to be tested prior to the beginning of the study.

The study sponsor was responsible for supplying the appropriate amount of product needed to carry out the study.

For this study, the study sponsor supplied:
The appropriate quantity of the product required to treat all of the subjects; A sufficient quantity of the product for any additional subjects participating in the study;
One product per reference and per batch was retained in the sample cabinet of MASCOT SPINCONTROL.

Product was stored in an ambient temperature away from light.
At the end of the study, the product used by the volunteers or the left over product can be sent back to the sponsor if he has asked for it on the document attached to the quotation or by mail.
On the other hand, the investigator proceeds to eliminate the remaining products according to the method of their choice described in their procedures.

The cost of the product destruction by the investigator was charged to the sponsor

3.2.2 Patch preparation

The patches were prepared in the morning of the application, one hour before the visit i.e. at T0 in acclimatized room 20°C -25°C.

The product was stored in IP storage room (T°C between 20°C -25°C., Humidity between 40 RH and 60 RH).

Product	Code	Patch No.	Application area	Frequency of application	Application duration	Conservation
Manswag Hair Serum (AF20-445)	**A**	1	Between scapulae and waist	Once	24 hours	At an ambient temperature
Negative Control (0.9% Isotonic saline solution)	-	2	Between scapulae and waist	Once	24 hours	At an ambient temperature
Positive Control (1% w/w SLS)	-	3	Between scapulae and waist	Once	24 hours	At an ambient temperature

3.2.2.1 Patch Preparation for Test Product

a. Procedure for Patch Preparation of Patch Preparation of Manswag Hair Serum (AF20-445): Product A as per BIS Standard clause 4.3.1.2, IS 4011:2018, 3rd Revision:

- 0.04 ml (40 µl) of test sample was measured with the help of Micropipette.
- The test product was transferred on previously numbered Aluminium Finn Chamber with an appropriate sized disc of Whatman no. 3 filter paper with the help of Micropipette.
- The Finn chambers with the product was then loaded filter paper discs be taped onto the back of subjects

b. Patch preparation for Negative control as per BIS Standard clause 4.3.1.2.4, IS 4011:2018, 3rd Revision:

- 40 µl of 0.9% Isotonic Saline Solution was transferred to previously numbered Aluminium Finn Chamber with an appropriate sized disc of Whatman no. 3 filter paper with the help of Micropipette.
- The Finn chamber with the 0.9% Isotonic Saline Solution was then taped onto the back of subject.

c. Patch preparation for Positive control as per BIS Standard clause 4.3.1.2.4, IS 4011:2018, 3rd Revision:

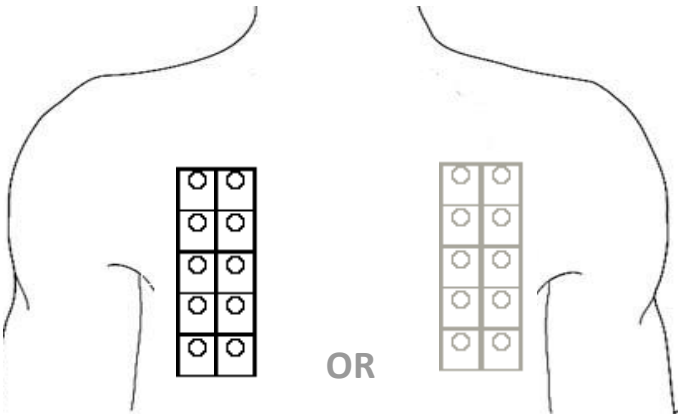
- Sodium Lauryl Sulphate solution: 1% w/w solution of SLS in distilled water was prepared.
- 0.04 ml or 40 µl of the SLS solution was loaded onto a Whatman filter paper disc, placed in clean Aluminium Finn Chamber.
- The Finn chamber with the loaded 1% w/w SLS solution was then taped onto the back of subject.

Patch application

The patch application was carried out by the CRA:

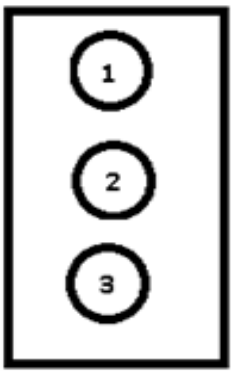
For subject:

- The Dermatologist determined the site that poses to be the most appropriate, based on moles, body hair; freckles of the selected area was sound, without excessive hair growth
- Patches were not applied on naevi.
- Location of application: Between scapula and waist.
- The application of the patches were made as follows:



For CRA

- Hands were washed with disinfectant.
- Began application of the strip by the bottom, the rooms were pressed up to release the air.
- When the tape was fully in place, each patch was gently pressed containing a test product or Control, to ensure an even distribution of the substance.
- Pressure was applied to the tape and especially its edges to ensure good fixation. The person being tested avoided sudden movements.
- The applications of patches were strengthened by applying micropore tape on all the four sides of patch.



3.3 STUDY DESIGN

- This was a single application closed, occlusive patch test.
- Non comparative, single center study.
- Subjects served as their own reference.

3.4 STUDY PROCEDURE

3.4.1 Dermatological Evaluation

➢ **Principle**

The patch test under occlusion is a method used to check safety in terms of irritation potential of any cosmetic or cosmeceutical formulation which is to be applied topically on healthy human subjects.

Irritants are the substances that may damage the skin. The damage will depend upon the nature, concentration and duration of exposure. Irritation is manifested as inflammatory responses such as erythema (redness) and oedema (swelling), vesiculation and finally to an intense suppurate reaction without the involvement of immune system

The evaluation of the different products was compared with 1% (w/w) sodium lauryl sulphate as positive control & 0.9% Isotonic saline solution as negative control.

The kinetic of the evaluation was as follows:

T0 = (before patch application)	Patch application
T1 day = (0 hour after the patch removal)	Patch removal
T2 days = (24 hours after patch removal)	Patch reading by the Dermatologist
T8* days = (T+1 week after 0 hour of patch removal)	Checking of the evolution of the positive cases

➢ **<u>Methodology of patch application</u>**

The patch application was carried out at T0 visit by the CRA.

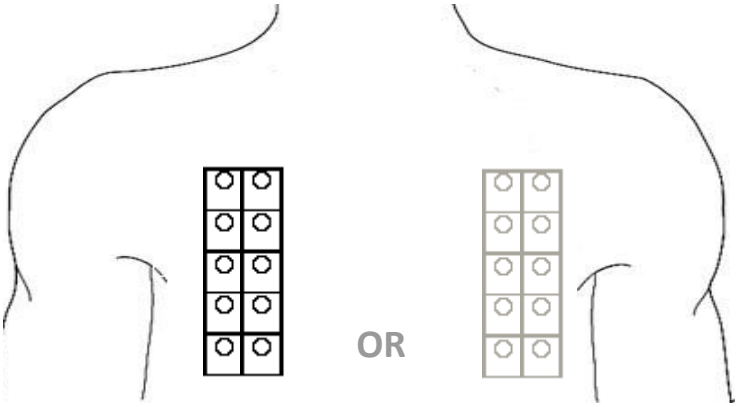
- Hands were washed with disinfectant.
- Began application of the strip from the bottom, the rooms were pressed up to release the air.
- When the tape was fully in place, each patch was gently pressed containing a test product or control, to ensure an even distribution of the substance.
- Pressure was applied to the tape and especially its edges to ensure good fixation. The person being tested avoided sudden movements.
- The applications of patches were strengthened by applying micropore tape on all the four sides of patch.

- <u>Studied areas and location</u>

The patches were applied on the top of the back near the shoulder blade.

The dermatologist <u>determined the best area to apply the patch, depending on the naevi</u>, the pilosity and the freckles. The selected area was healthy, without an excessive pilosity. Patches were not applied on a naevi.

The patches were applied on the right or left part of the back: the side was mentioned in the CRF by the dermatologist.



- Position of the subject

It was recommended that the subject sat with the back slightly bent forward.

- **Methodology of patch removal**

The CRA removed the patch from the bottom to the top. The area was gently wiped off with a soft tissue paper.

- **Methodology of patch reading by the Dermatologist**

The skin reaction was assessed under a constant artificial daylight source.

The Dermatologist scored the reactions namely erythema (including dryness, scaliness and wrinkles) on a 0-4-point scale and oedema on another 0 – 4 point scale as per Draize Scale (Clause 4.3.1.3 Observation and scoring for Skin Irritation Test, Draize scale for scoring the treatment sites- IS 4011:2018 Methods of test for safety evaluation of cosmetics – 3rd Revision).

Score for Erythema/ dryness/ wrinkles	Reaction	Score for Oedema	Reaction
0	No reaction	0	No reaction
1	Very slight erythema/dryness with shiny appearance	1	Very slight Oedema
2	Slight erythema/ dryness/wrinkles	2	Slight Oedema
3	Moderate erythema/ dryness/wrinkles	3	Moderate Oedema
4	Severe erythema/ wrinkles/scales	4	Severe Oedema

In case of positive reaction, a photograph of the patches were captured on T2 days. Another photograph at T+8 days was enabled to evaluate the evolution of the sign.

3.5 EXAMINATION SCHEDULE

The effect of the product was evaluated over a 3 - day's period and follow up visit at T8 (T+1 week after 0 hour of patch removal). The scheduled measurement procedures were as follows:

Screening

- Registration
- Protocol Briefing
- Reading and signature of the consent form
- History Questionnaire
- Routine Check up
- Clinical Observations
- Checking of the inclusion/non-inclusion criteria by the Dermatologist

***At T0* (before patch application)**

- Acclimatization at temperature 20°C - 25°C for 20 mins.
- Acknowledgement, reading and signature of the ICF
- Concomitant Medication
- Checking of the inclusion/non-inclusion criteria by the Dermatologist
- Site Identification by Dermatologist
- Patch Application by CRA.
- AE/SAE Monitoring

***At T1* (0 hour after the patch removal)**

- Acclimatization at temperature 20°C - 25°C for 20 mins
- Proscriptions and restrictions
- Concomitant Medication
- Patch Removal by CRA.
- AE/SAE Monitoring

***At T2* (24 hours after the patch removal)**

- Acclimatization at temperature 20°C - 25°C for 20 mins
- Proscriptions and restrictions
- Concomitant Medication
- Dermatological Evaluation: patch test reading
- AE/SAE Monitoring

***At T8* (T+1 week after 0 hour of patch removal)**

- Acclimatization at temperature 20°C - 25°C for 20 mins
- Proscriptions and restrictions
- Concomitant Medication
- Dermatological Evaluation: patch test reading
- AE/SAE Monitoring
- Filling of Study completion form by the Dermatologist

3.6 DATA ANALYSIS AND STATISTICS OF TECHNICAL DATA

- Carried out by Study In charge at Mascot Spincontrol India.

The assessment was only based on the mean score obtained 24 hours after the patch removal (T2) for the technique dermatological evaluation.

Mean Score for Irritation = $\frac{\text{Total score (Erythema+ Oedema) for each sample}}{\text{Total no. of Subjects}}$

Mean score calculation for test product coded: Manswag Hair Serum (AF20-445): Product A.
Observation and scoring for Skin Irritation Test, Draize scale for scoring the treatment sites (IS 4011:2018 Methods of test for safety evaluation of cosmetics – 3rd Revision- Clause 4.3.1.3 Skin Irritation Test) was used to calculate the mean score of observations made for assessing the investigational site for Skin Irritation as presented in Mean Score for Irritation:

Mean Score	Classification
2.0 / 8.0	Non- Irritant
Up to 4.0 / 8.0	Mild Irritant
Above 4.0 / 8.0	Irritant

Average score produced by each of the test sample was compared with that produced by the positive control & negative control.

Note: Positive control must give combined mean score of greater than 2.
Negative control must give combined mean score of less than 2.

If positive control gives a combined mean score less than 2.0 and/or negative control gives a combined mean score of greater than 2.0, then the test needs to be repeated on another group of newly recruited volunteers.

4. ETHICAL AND LEGAL CONSIDERATIONS

4.1 STUDY PERSONNEL

The Principal investigator assured that the Study In charge and everyone who participates in this study have the required qualifications and abilities to carry it out.

4.2 DATA ARCHIVING

The documents are archived for a period as per recommendation of sponsor or 5 years. Dual archiving is ensured by using both paper and IT storage media.
Paper files are archived by Mascot Spincontrol until the end of the archiving period.

Electronics files are archived in 2 CD ROMs (DVD's), disks are stored for 5 years. In Mascot Spincontrol premises keep one copy of the protocol signed by the principal investigator & by the study sponsor as well as the filled case report form, questionnaire and all associated documents, consent forms & all project related documents of any type for a 5 years period following delivery of the final report.
All these documents are accessible upon request for inspection by the study sponsor, their representative or by administrative authorities.
The Principal investigator informs the study sponsor of his intention to proceed with their destruction after the 5 years period.

4.3 INSURANCE POLICY

The damages caused by the failure of the Principal Investigator or a third party shall be imputable to Mascot Spincontrol.
Adequate insurance cover of the subject for liability arising from any serious event or death during the conduct of the study will be taken by Mascot Spincontrol India Pvt. Ltd. through an insurance contract with The Oriental Insurance Company Limited, Mumbai, India. (Insurance policy number 121200/48/2022/7027).

4.4 ANONYMITY OF THE SUBJECTS

The subjects are identified for the study sponsor using a five-character alphanumeric code and a number. The investigator makes a commitment not to raise the anonymity of the subjects.
The study sponsor cannot have access to the confidential data relative to the subjects registered in the data base of Mascot Spincontrol.

4.5 CONSENT TO PARTICIPATE IN THE STUDY

An information form is given to each subject providing full details about the study and:

- its objectives, methods, and duration;
- possible expected aesthetic benefits, constraints, and potential risks;
- The non-inclusion criteria, the amount of the payment, the right of access to data files and their later destruction.

This information enables the subjects to sign their participation consent form freely and unequivocally, in the knowledge that they are fully aware of the testing details.

4.6 USE OF IMAGE

If the study involves the use of photographs, the volunteers are informed, in the consent form, that their image without direct identification may be used by Mascot Spincontrol all over the world, with no time limit on this usage. The volunteers are also informed that Mascot Spincontrol may also provide images to the sponsor for publishing or duplication.

4.7 CONFIDENTIALITY

All the information, data, results and audio-visual recordings of informed consent process of the study are confidential. Everyone having access to such data are informed of their confidentiality.

Any medical information concerning a subject's state of health and the results of the clinical examinations carried out during the recruitment, selection and admission phases before a study is subject to the medical secrecy regulations, in no case should such information be communicated to the study sponsor using a subject's identity.

4.8 QUALITY ASSURANCE

Our quality system has been developed to meet guidelines relevant to our type of activity for ingredients and cosmetic product efficiency and tolerance testing.

As such, our Quality System is in full compliance with ICH-E6 -Good Clinical Practice (GCP) guidelines in our test companies: Mascot Spincontrol (India) and Spincontrol Tours (France).

The entire dossier of a study (protocol, results, report, and any other study-related documents) is subject to a Quality Management audit which conforms to the regulatory texts and procedures in force. Verifications of data generated in this study are performed in accordance with the Quality Assurance of the studies documents.

The investigator cooperates in ensuring any additional auditing required by the study sponsor to ensure that the study progresses in accordance with regards the protocol and the current procedures.

Sr. No.	Audit Report	Date of Auditing
1.	Audit of study protocol	04/01/2022
2.	Audit of the CRF's	17/01/2022
3.	Audit report of the Trial Master File	27/01/2022
4.	Audit of the Raw Data & Results	28/01/2022
5.	Audit of the Study Report	02/02/2022

4.9 REGULATIONS

This study is carried out in conformity with the most recent recommendations of the World Medical Association (Declaration of Helsinki 1964, amended in Fortaleza, Brazil, 2013).

This study complies with the "Schedules of the Drugs and Cosmetics Act".

4.10 PRACTICAL CONSIDERATIONS

A preliminary agreement between the Investigator and the study sponsor, concerned by the present contract, is necessary for any publication or communication directly concerning the two parties. They must both take the initiative to inform each other if a change is to occur.

5. RESULTS

This report is based on the exploitation of the results regarding the irritation potential of Hair Care formulation by Primary Irritation Patch Test Method.

5.1 PROTOCOL DEVIATIONS

The protocol has been respected as a whole.

5.2 ABSENCES

- **Subject n° 008 KAMUT was absent on T+2 day visit.**

As the interpretation of results is based on T+2 day visit, the data of this subjects will not be exploited in the global study results

5.3 POPULATION CONSIDERED IN THE EXPRESSION OF THE RESULTS

At T0, 24 subjects were recruited:

Considering the information previously mentioned in the paragraph (5.1) the number of subjects considered in the expression of the results, at each examination time, presented in the following table:

Technique	T0 (before patch application)	T1 day (0 hour after the patch removal)	T2 days (24 hours after the patch removal)	T8 (T+1 week after 0 hour of patch removal)
Dermatological Evaluation	24	24	23	23

From above techniques and time points data for T2 visit (24 hrs. of patch removal) – Dermatological evaluation was considered for the mean score calculation of patch test.

5.4 DESCRIPTION OF THE EXPLOITED PANEL

The exploited panel consisted of 23 healthy females and males subjects aged between 18 and 45 years old (Mean age in years: 29.9 Standard deviation in years: 10.4 and median age in years: 32 see detail in Appendix 1) of Asian (Indian) skin type.

5.5 DERMATOLOGICAL EVALUATION

The detailed results of the dermatological evaluation are presented in appendix 2.
The studied parameters are:

1. Erythema
2. Oedema

5.4.1 Observed results at T2 days (24 Hours after the Patch Removal)

The following table summarizes the total and mean scores obtained on the exploited panel, for the erythema and oedema parameters, as well as the conclusion concerning the irritation potential of each tested material (if applicable), 24 hours after the patch removal, on the back

	Results for T2 Days (24 Hours After the Patch Removal) Visit for Dermatological Evaluation				
Test material	**Total Score for Erythema**	**Total Score for Oedema**	**Total Score for Erythema + Oedema**	**Mean Score (Irritation)**	**Conclusion on the Irritation Assessment**
Manswag Hair Serum (AF20-445)	1.0	0.0	1.0	0.0	Non-irritant
Negative Control (0.9% Isotonic saline solution)	0.0	0.0	0.0	0.0	-
Positive Control (1% w/w SLS)	35.0	16.0	51.0	2.2	-

5.4.2 Analysis

At T2 days (24 hours after patch removal) visit mean score of erythema and oedema by dermatologist is found as follows:

- 0.0 for **Manswag Hair Serum (AF20-445): Product A**
- No irritative type response at T2 day (24 hours after patch removal) was observed by dermatologist.
- No reaction was observed for the negative control (i.e. 0.9% Isotonic Saline Solution).
- The Mean Score for positive control 1% (w/w) SLS solution is 2.2.

6. DISCUSSION AND CONCLUSION

In our experimental conditions, based on the incident of the response and comparison of the mean scores with positive and negative control of erythema and oedema observed for the single application of closed patch for 24 hours of the Hair Care Formulation for product **Manswag Hair Serum (AF20-445): Product A** according to the Primary irritation patch test method on panel of 23 healthy human subjects (11 males + 12 females) aged between 18 and 45 years old, leads to the following results through dermatological evaluation at 24 hours after the patch removal.

Test product coded **Manswag Hair Serum (AF20-445): Product A** was **dermatologically tested for safety**

&

can be considered as **Non-Irritant to skin**.

7. APPENDICES:

APPENDIX 1:

CHARACTERISTICS OF THE PANEL

CHARACTERISTICS OF THE PANEL

D01-6Q01-AH-DR21				
DEMOGRAPHICS				
PRODUCT REF.:				
1.Patch No. 01- Product A: Manswag Hair Serum (AF20-445)				
2.Patch No. 02- Negative Control - 0.9% Isotonic Saline Solution				
3.Patch No. 03- Positive Control- 1% SLS				
Sr. No.	**Subject Code**	**Subject No.**	**Age**	**Sex**
1	TATPR	001	40	FEMALE
2	JAIDI	002	36	FEMALE
3	TATSW	003	21	FEMALE
4	JAMSA	004	18	MALE
5	KAMAJ	005	19	MALE
6	CHAVI	006	18	MALE
7	MEHRU	007	19	MALE
8	KAMUT	008	NE	NE
9	MIRPA	009	44	FEMALE
10	BABSA	010	45	MALE
11	SHIR1	011	20	MALE
12	PRAPA	012	19	MALE
13	BHAKA	013	19	MALE
14	PRAGE	014	44	FEMALE
15	MORKU	015	23	MALE
16	TURSA	016	38	FEMALE
17	GAUAD	017	19	MALE
18	PANBI	018	36	FEMALE
19	MALUD	019	42	FEMALE
20	DARSO	020	32	FEMALE
21	YADAA	021	34	FEMALE
22	VARGE	022	42	FEMALE
23	VISDE	023	23	MALE
24	RAJSA	024	37	FEMALE
MEAN AGE (in years)			**29.9**	**Male :11**
MINIMUM AGE (in years)			**18**	**Female :12**
MAXIMUM AGE(in years)			**45**	
STANDARD DEVIATION (in years)			**10.4**	
MEDIAN AGE (in years)			**32**	
TOTAL SUBJECTS			**23**	

NE: NON EXPLOITED

APPENDIX 2:

RESULTS OF THE STUDY

RESULTS OF THE STUDY

D01-6Q01-AH-DR21					
3.4.1 Dermatological Evaluation AT T2 (24 hours after the patch removal)					
PRODUCT REF.: **1.Patch No. 01- Product A: Manswag Hair Serum** **2.Patch No. 02- Negative Control - 0.9% Isotonic Saline Solution** **3.Patch No. 03- Positive Control- 1% SLS**					
GRADING FOR ERYTHEMA /DRYNESS/ WRINKLES					
Sr. No.	**Subject Code**	**Subject No**	**Patch No.1**	**Patch No. 2**	**Patch No.3**
1	TATPR	001	0	0	1
2	JAIDI	002	0	0	1
3	TATSW	003	0	0	2
4	JAMSA	004	0	0	1
5	KAMAJ	005	0	0	1
6	CHAVI	006	0	0	2
7	MEHRU	007	0	0	1
8	KAMUT	008	NE	NE	NE
9	MIRPA	009	0	0	1
10	BABSA	010	0	0	3
11	SHIR1	011	0	0	1
12	PRAPA	012	0	0	1
13	BHAKA	013	0	0	4
14	PRAGE	014	0	0	1
15	MORKU	015	0	0	1
16	TURSA	016	0	0	1
17	GAUAD	017	0	0	3
18	PANBI	018	0	0	1
19	MALUD	019	0	0	1
20	DARSO	020	0	0	1
21	YADAA	021	0	0	1
22	VARGE	022	0	0	3
23	VISDE	023	0	0	1
24	RAJSA	024	1	0	2
Total Score For Erythema (E)			**1.0**	**0.0**	**35.0**

Scale:

Score For Erythema	Reactions
0	No reaction
1	Very slight erythema/dryness with shiny appearance
2	Slight Erythema/ Dryness/ Wrinkles
3	Moderate erythema/ dryness /wrinkles
4	Severe erythema/ wrinkles/scales

NE: NON EXPLOITED

RESULTS OF THE STUDY

D01-6Q01-AH-DR21					
3.4.1 Dermatological Evaluation AT T2 (24 hours after the patch removal)					
PRODUCT REF.: **1.Patch No. 01- Product A: Manswag Hair Serum** **2.Patch No. 02- Negative Control - 0.9% Isotonic Saline Solution** **3.Patch No. 03- Positive Control- 1% SLS**					
GRADING FOR OEDEMA					
Sr. No.	**Subject Code**	**Subject No**	**Patch No.1**	**Patch No. 2**	**Patch No.3**
1	TATPR	001	0	0	0
2	JAIDI	002	0	0	0
3	TATSW	003	0	0	1
4	JAMSA	004	0	0	0
5	KAMAJ	005	0	0	0
6	CHAVI	006	0	0	1
7	MEHRU	007	0	0	0
8	KAMUT	008	NE	NE	NE
9	MIRPA	009	0	0	0
10	BABSA	010	0	0	2
11	SHIR1	011	0	0	0
12	PRAPA	012	0	0	0
13	BHAKA	013	0	0	3
14	PRAGE	014	0	0	0
15	MORKU	015	0	0	0
16	TURSA	016	0	0	0
17	GAUAD	017	0	0	3
18	PANBI	018	0	0	0
19	MALUD	019	0	0	1
20	DARSO	020	0	0	0
21	YADAA	021	0	0	0
22	VARGE	022	0	0	3
23	VISDE	023	0	0	1
24	RAJSA	024	0	0	1
Total Score For Oedema (O)			**0.0**	**0.0**	**16.0**

Scale:	
Score For Oedema	**Reaction**
0	No Reaction
1	Very slight Oedema
2	Slight Oedema
3	Moderate Oedema
4	Severe Oedema

NE: NON EXPLOITED

RESULTS OF THE STUDY

D01-6Q01-AH-DR21									
3.4.1 Dermatological Evaluation at T2 (24 hours after the patch removal) and T8 (T+1 week after 0 hours of patch removal)									
PRODUCT REF.: 1.Patch No. 01- Product A: Manswag Hair Serum 2.Patch No. 02- Negative Control - 0.9% Isotonic Saline Solution 3.Patch No. 03- Positive Control- 1% SLS									
GRADING FOR ERYTHEMA (E) AND OEDEMA (O)									
Sr. No.	Sub. Code	Sub. No.	Reaction	Patch No.1		Patch No.2		Patch No.3	
				T2	T8	T2	T8	T2	T8
1	TATPR	001	E	0	0	0	0	1	0
			O	0	0	0	0	0	0
2	JAIDI	002	E	0	0	0	0	1	0
			O	0	0	0	0	0	0
3	TATSW	003	E	0	0	0	0	2	1
			O	0	0	0	0	1	0
4	JAMSA	004	E	0	0	0	0	1	0
			O	0	0	0	0	0	0
5	KAMAJ	005	E	0	0	0	0	1	0
			O	0	0	0	0	0	0
6	CHAVI	006	E	0	0	0	0	2	1
			O	0	0	0	0	1	0
7	MEHRU	007	E	0	0	0	0	1	0
			O	0	0	0	0	0	0
8	KAMUT	008	E	NE	NE	NE	NE	NE	NE
			O	NE	NE	NE	NE	NE	NE
9	MIRPA	009	E	0	0	0	0	1	0
			O	0	0	0	0	0	0
10	BABSA	010	E	0	0	0	0	3	2
			O	0	0	0	0	2	1
11	SHIR1	011	E	0	0	0	0	1	0
			O	0	0	0	0	0	0
12	PRAPA	012	E	0	0	0	0	1	0
			O	0	0	0	0	0	0
13	BHAKA	013	E	0	0	0	0	4	3
			O	0	0	0	0	3	1
14	PRAGE	014	E	0	0	0	0	1	0
			O	0	0	0	0	0	0
15	MORKU	015	E	0	0	0	0	1	0
			O	0	0	0	0	0	0
16	TURSA	016	E	0	0	0	0	1	0
			O	0	0	0	0	0	0
17	GAUAD	017	E	0	0	0	0	3	1
			O	0	0	0	0	3	0
18	PANBI	018	E	0	0	0	0	1	0
			O	0	0	0	0	0	0
19	MALUD	019	E	0	0	0	0	1	0
			O	0	0	0	0	1	0
20	DARSO	020	E	0	0	0	0	1	0
			O	0	0	0	0	0	0
21	YADAA	021	E	0	0	0	0	1	0
			O	0	0	0	0	0	0
22	VARGE	022	E	0	0	0	0	3	2
			O	0	0	0	0	3	1
23	VISDE	023	E	0	0	0	0	1	0
			O	0	0	0	0	1	0
24	RAJSA	024	E	1	0	0	0	2	0
			O	0	0	0	0	1	0

NE: NON EXPLOITED

RESULTS OF THE STUDY

D01-6Q01-AH-DR21
MEAN SCORE CALCULATION
3.4.1 Dermatological Evaluation AT T2 (24 hours after the patch removal)
PRODUCT REF.: 1.Patch No. 01- Product A: Manswag Hair Serum (AF20-445) 2.Patch No. 02- Negative Control - 0.9% Isotonic Saline Solution 3.Patch No. 03- Positive Control- 1% SLS

Mean Score	Classification
2.0 / 8.0	Non- Irritant
Up to 4.0 / 8.0	Mild - Irritant
Above 4.0 / 8.0	Irritant

Mean Score for Irritation = $\frac{\text{Total score (Erythema+ Oedema) for each sample}}{\text{Total number of Subjects}}$

Parameter	Patch No.1	Patch No.2	Patch No.3
Total score for erythema	1.0	0.0	35.0
Total score for oedema	0.0	0.0	16.0
Total score for Erythema + Oedema	1.0	0.0	51.0
Erythema + Oedema / 23	0.0	0.0	2.2
Conclusion	Non-irritant	-	-

APPENDIX 3:

QUALITY ASSURANCE STATEMENT

QUALITY ASSURANCE STATEMENT

This study (D01-6Q01-AH-DR21) has been regularly monitored by the quality assurance department by way of periodic audits as recommended by Good Clinical Practice and applicable regulations. The dates of these audits and the subsequent reports to the management are listed here:

Audit Schedule :

Sr. No.	Audit Report	Audit Report Number	Date of Audits
1.	Audit of study protocol	D01-6Q01-AH-DR21-AU01	04/01/2022
2.	Audit of the CRF's	D01-6Q01-AH-DR21-AU02	17/01/2022
3.	Audit report of the Trial Master File	D01-6Q01-AH-DR21-AU03	27/01/2022
4.	Audit of the Raw Data & Results	D01-6Q01-AH-DR21-AU04	28/01/2022
5.	Audit of the Study Report	D01-6Q01-AH-DR21-AU05	02/02/2022

This report has been audited by the quality assurance department and is found to be an accurate description of such methods and procedures as were used during the conduct of the study and an accurate reflection of the raw data.

Signature: _____
Auditor(s)

Signature: _____
Auditor(s)

Signature: _____
Quality Assurance Manager

APPENDIX 4:

COPY OF STUDY PROTOCOL

SUMMARY: D01-6Q01-AH-DR21

Test Product: Manswag Hair Serum (AF20-445): Product A

DESCRIPTION OF THE STUDY

EVALUATION OF THE IRRITATION POTENTIAL OF HAIR CARE FORMULATION THROUGH:

- **Dermatological Evaluation –Single Application Patch Test Method**

NATURE OF THE TESTED PRODUCT AND METHODOLOGY:

Product Reference	Manswag Hair Serum (AF20-445): Product A
Study design	Single application Closed, Occlusive patch study, Non comparative, single center study, Subjects served as their own reference.
Total duration of the study	8 days [3 days and T8 (T+1 week after 0 hour of patch removal) visit was scheduled to monitor follow up reactions].
Kinetics	T0 (before patch application), T1 day (0 hour after the patch removal), T2 days (24 hours after the patch removal) and T8 days (T+1 week after 0 hour of Patch removal).
Product application	Single application of test product, Positive control (1% SLS) & Negative control (0.9% Isotonic Saline Solution), on the back, under occlusion during 24 hours.
Number of volunteers	23 (12 females and 11 males)
Special selection criteria	Healthy skin on the studied anatomic unit (free of eczema, wounds, inflammatory scar….)

RESULTS AND CONCLUSION

In our experimental conditions, based on the incident of the response and comparison of the mean scores with positive and negative control of erythema and oedema observed for the single application of closed patch for 24 hours of the Hair Care Formulation for product coded **Manswag Hair Serum (AF20-445): Product A** according to the Primary irritation patch test method; on panel of 23 healthy human subjects (12 females + 11 males) aged between 18 and 45 years old, leads to the following results through dermatological evaluation at 24 hours after the patch removal.

Test product coded **Manswag Hair Serum (AF20-445): Product A** was **dermatologically tested for safety**

&

can be considered as **Non-Irritant to skin.**

Note: Based on result & conclusion client can only claim Non-irritant & Dermatologically tested for safety.