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Standardization and Validation Study for Methods Used for Patch Testing

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Abstract: Standardization is the best approach to maximize compatibility, interoperability safety, repeatability and verify the quality of the process. The process of standardization can itself be standardized. This study report describes the standardization process for Patch Testing Studies. The objective of the study was to standardize and validate the patch test methods and controls (positive control and negative control) and irritation scoring by 24 Hours patch testing studies under complete occlusion or any patch testing studies. Safety was assessed throughout the study by monitoring of adverse events. The Single-center, evaluator-blinded clinical study of patch testing was conducted on ten healthy adult human subjects with varied skin types (Oily, Dry, Normal and Combination). Patch testing is done by patches to back (between the scapula and waist) of the subjects and leaving them on for 24 hours. The patches are then removed and the areas examined for signs of a reaction up to 168±2 hours. The results of negative and positive controls met the required criteria. Minimum irritation score was calculated. No complaints of adverse effects or worsening of skin conditions were reported on a single application of the product during and till the completion of the study. The study demonstrates various standardization methods used for patch testing. From this validation study, we have set a benchmark for patch testing which produces consistent, compatible, accurate, qualitative and reproducible results for testing methods of patch testing.

INTRODUCTION

Dermatology is a growing field that covers skin ailments, related medicine and cosmetology. The development in this field is useful for assessing the efficacy and safety of topical products. [1-3] To identify the quality and efficacy, it is necessary to perform the standardization and validation study of the test procedures. The objective of this validation study was to standardize the methods and validate the positive and negative controls and evaluation techniques that are used during the conduct of the Primary Irritation Patch Test (PIPT) by 24 Hours Patch Test under complete occlusion or any patch testing studies. Our study established the documentary evidence, demonstrating that procedural steps, processes and clinical activities carried out in patch testing for dermal safety studies help in maintaining the desired level of compliance and consistency.

METHODOLOGY

A single-center, evaluator-blinded clinical study was performed on ten (pilot validation in house study) healthy adult human subjects (Table 1) with varied skin types (Oily, Dry, Normal and Combination). The method is based on the Bureau of Indian Standards (BIS) method 4011:2018, third revision (July 2018) and BIS 13424:2001, first revision (Oct 2001) modified in terms of site of application and CHD 25: Soaps and Other Surface-Active Agents Sectional Committee [first revision and IS 13424:2001 Safety evaluation of bathing bars and toilet soaps.

Subjects were pre-screened and enrolled at our laboratory. The subjects' demographics, (i.e., age, gender, predominant race, height, weight) and a brief medical history were documented. Safety was assessed throughout the study by monitoring adverse events. Subjects with active dermatitis or subjects with self-reported immunological disorders such as HIV positive, AIDS, systemic lupus erythematous or with known allergy or sensitization to medical adhesives, bandages etcetera. were excluded from the study. The study was started after approval from ACEAS Independent Ethics Committee on 27 October 2021. This clinical trial has been registered at CTRI [Clinical Trial Registry of India] with the trial registered number is CTRI/2021/12/038364. After explaining the procedure in detail to the patient, informed consent was taken.

First of all, lactic acid stinging test was performed by using 10% v/v aqueous solution of lactic acid. The site of application should not be inflamed/broken and should be free of cuts, scratches, tattoos, scars, any birthmarks and abrasions. 2-3 drops sample solution of lactic acid and saline solution were applied on the study participant's right Nasolabial fold and the contralateral fold, i.e. on a left side respectively using cotton bud applicator as negative controls. Subjects were asked to score skin sensation parameters, i.e. prickling, itching, burning sensation, stinging sensation, numbness as per the intensity of the reaction induced and scores were recorded on a log sheet of the lactic acid stinging test.

Once the subject's skin examination and inclusion and exclusion criteria had been evaluated, the subject number was allotted on Visit 01(Day 01). The application site was cleaned using water and wiped with a non-abrasive gauze pad, an hour before patch application. Appropriate sites were then determined on the back of the subject and a line was drawn horizontally using a dermal marker just above the site for patch placement. Sites application and numbering were done keeping space of 1 inch from the application site. After that, the patch application for all the subject's started from the left side of the back from Site S1 to S4 and then the right side of the back from Site S5 to Site S8 (Figure 1). Total 2 test products were applied on Site S1 and Site S2. In addition to 2 test products, 2 concentrations of positive controls (Figure 2) and 1 negative control (Table 2) were used and were kept in contact with the skin under occlusion for 24 hours (+ 2 hours).

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Table 1: Summary of Demographic Details of Subjects

Summary of Demographic Characteristics		
Variables		(N=10)
Gender, n (%)	Males	5 (50%)
	Females	5 (50%)
Race, n (%)	Asian	10 (100%)
Age (years)	N	10
	Mean (SD)	32 (1.5)
	Median	24
	Min, Max	19, 60
Fitzpatrick Skin Type	N	10
	Type III	10 (100%)
Skin Type	N	10
	Normal	3 (30%)
	Dry	3 (30%)
	Oily	2 (20%)
	Combination	2 (20%)

Subjects are counted in N numbers, where N=10

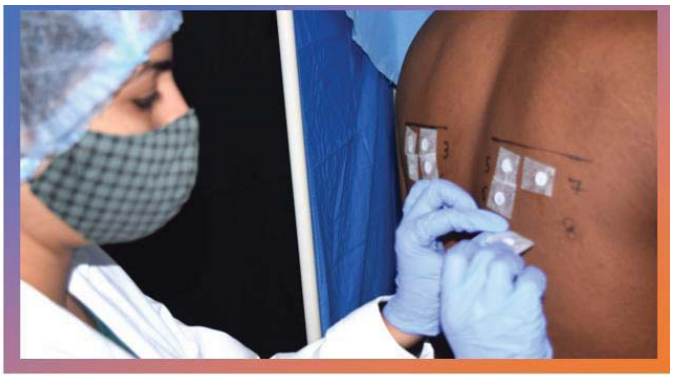
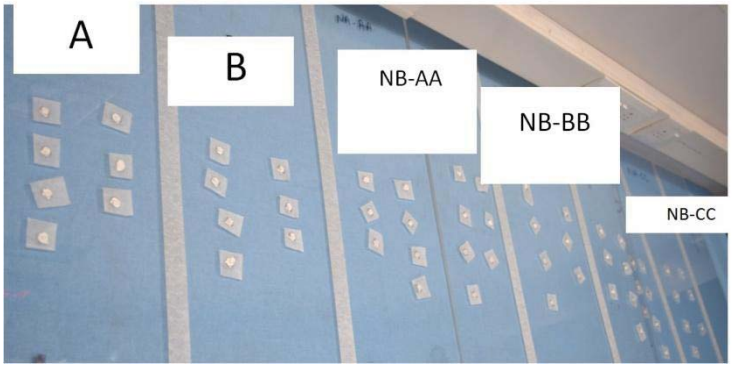


Figure 1: Application of patches on subjects and site marking



Product A and B are marketed products, NB-AA and NB-BB are Sodium Lauryl Sulphate (SLS) 1% and 3% w/v respectively while NB-CC is saline solution 0.9%

Figure 2: Patch representation

The patch test units were removed after 24 hours and readings were taken. The application sites were cleaned using water with a wet cotton gauge pad to remove the remaining residue of test products. Application sites, i.e., Site 1, Site 2 and so on were demarcated using dermal markers before irritation scoring without affecting application sites. Application sites were demarcated using dermal marker before irritation scoring without touching the application sites.

Application sites (Subject's back) were evaluated for scoring the reaction, namely, erythema, dryness and wrinkles on a 0–4-point scale separately for each parameter and oedema on another 0-4 points scale as per the Draize Scale after 30+5 minutes of patch removal, 24±2 hours and a telephonic follow-up was taken at 168±2 hours after patch removal for any signs of dryness and redness at patch application sites. The total study duration was 9 days from the patch application day. Dermatologist Validated Evaluator visually assessed the skin condition at each test site. All scoring of all reactions to the test and control products during the study was done by a single dermatologist validated master scorer to avoid any subjective variability of scoring. Mean Score for Irritation (MIS) was calculated.

Table 2: Test and Control Samples Details with its Concentration and Dispensing Form

Serial No.	Test Product Code	Dispensing form	Sample Composition	Type of Patch	Amount Dispensed
1	A	Semi-Solid	Neat	Occluded	0.04 mL
2	B	Semi-Solid	Neat	Occluded	0.04 mL
3	NB-AA	Liquid	1% w/v	Occluded	0.04 mL
4	NB-BB	Semi-Solid	3% w/v	Occluded	0.04 mL
5	NB-CC	Semi-Solid	0.9% w/v	Occluded	0.04 mL

Note: Product A and B are marketed products, NB-AA and NB-BB are Sodium Lauryl Sulphate (SLS) 1% and 3% w/v respectively while NB-CC is saline solution 0.9% w/v

Table 3: Data Analysis of Mean Irritation Score [MIS] for each Tested Formulation

Serial No.	Test Product	Site	Mean Irritation Score [Day 02]	Mean Irritation Score [Day 03]	Irritancy Level
1	A	S01	0.00	0.00	Non-Irritant
2	B	S02	0.00	0.00	Non-Irritant
3	NB-AA	S03	2.15	2.00	Irritant
		S04	2.00	2.05	Irritant
4	NB-BB	S05	3.00	3.00	Irritant
		S06	3.05	3.05	Irritant
5	NB-CC	S07	0.15	0.00	Non-Irritant
		S08	0.10	0.00	Non-Irritant

Note: Product A and B are marketed products, NB-AA and NB-BB are Sodium Lauryl Sulphate (SLS) 1% and 3% w/v respectively while NB-CC is saline solution 0.9%

The light intensity of an Irritation scoring area, digital photography area was recorded using LUX Meter (i.e. Light meter) before and after the start of activity intensity of ceiling light was measured. Measuring was started after 15 mins of the lights being turned on, as it would take some time to heat a wire filament to such a high temperature, that it glows with visible light (incandescence). The filament was protected from oxidation with a glass or fused quartz bulb, filled with inert gas or a vacuum. Corner 1, Corner 2, Corner 3, Corner 4 and the Center of the room/area readings were taken keeping around 6-6.5 feet distance away from the ceiling. An average reading was calculated and documented. The accepted average range for light levels is 250-500 Lux.

Digital photographs of the application sites and surrounding areas were taken using Nikon D3300 DSLR (24.2 megapixels) camera with 18-55 mm lens. Subject's back region was the center of the frame at a distance of 02 feet. An additional flashlight was on during photography. All photographs were captured at 300 dpi.

RESULTS

The age of the patients in the study group ranged from 18 to 65 years. In this study, there were 5 Males and 5 Females; the age of the subjects ranged from 18 years with the average being 32 years. Subjective stinging sensations were recorded at 2 mins, 5 mins and 15 mins by using a 4-point scale (0=No sensation, 1=Mild sensation, 2=Moderate sensation and 3=Severe sensation). For study participant which has sensitive skin, the lactic acid induced a "stinging sensation" in 1 or 2 minutes. This is reaching a peak in 5 minutes, only to become insignificant in 15 minutes. Skin allergy was not observed in any subjects.

All subjects were assessed on Day 09 after the application of test articles for any signs of irritation such as dryness, redness, swelling, at the patch application site, there were no complaints from subjects on signs like erythema, dryness, oedema, or wrinkles. The product irritancy level was classified and reported based on the classification of the Mean Irritation Score. Irritation Scoring was carried out in our activity as per Draize Scale for Scoring the Treatment Sites. For Scoring the Treatment Sites Four parameters namely Erythema, Dryness, Wrinkles and Oedema were scored separately on the irritation scoring sheet. For calculation of the Mean Irritation Score of erythema, dryness and wrinkles the highest score was taken. Mean Score for Irritation (MIS) = Total score (highest score from Erythema, dryness, wrinkles + Oedema) for each sample / Total no. of Subjects.

Scoring of irritation was conducted using normal daylight by the single scorer and same light condition at the same area for all scoring. Variation of not more than 50 Lux from daily measured light was observed during the conduct. Mean Irritation Score (MIS) and standard deviation were calculated using the standard statistical formula to provide variability of data for each product Detailed irritation score observed during our study has been shown in Table 3. Marketed products, used in this study are non-irritant as compared to negative controls. No complaints of adverse effects or worsening of skin conditions were reported on the single application of the products during and till the completion of the study.

DISCUSSION

Several studies have reported that skincare products (moisturizing and cleansing cream/lotion/milk) account for the majority of cases of contact allergy to cosmetics. [4-6] But drug patch tests can be especially helpful in determining the culprit drug in a variety of ailments such as eczematous drug reaction, systemic contact dermatitis

and maculopapular drug rash. [7] Here, at the end of the patch study, both the concentration of the positive control i.e. 1% w/v and 3% w/v SLS solution showed high irritancy levels at Day 3 as compared to other test products and negative control. Test products and negative controls are non-irritant and no irritation persists after completion of the study. The results of negative and positive controls were meet the criteria and hence the product is safe to use. No complaints of adverse effects or worsening of skin conditions were reported on the single application of the product during and till the completion of the study.

CONCLUSION

To conclude, the authors have explained the standardization and validation of different patch test methods, the importance of patch testing and the careful use of cosmetics. [8] Our standardization and validation study provides us diagnostic approach and comprehensive knowledge of allergic and contact dermatitis. The main clinical relevance of our study was to give standards to prepare and conduct study with positive and negative controls that to be used for primary irritation patch testing (PIPT) to determine test cosmetics/personal care topical products/Rx drugs - irritation level in comparison of negative and positive control. In addition to that, our study standardizes and validates the test parameters such as sensitivity test, site marking during patch test, patch application, patch removal, site cleaning, digital photography, irritation scoring, method of preparation for negative and positive controls (1% w/v and 3% w/v), mean irritation score and Light intensity of Irritation scoring area. We have set a benchmark for patch testing studies that produced consistent, compatible, accurate, qualitative and reproducible results, controlled testing methods of patch testing. In addition to that, this study standardize and validated parameters such as sensitivity test, site marking during patch test, mean irritation score and light intensity of Irritation scoring area. Digital photographs were taken for all activities performed, by which one can approach further clinical trials for allergic reactions of the skin or contact dermatitis.

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