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VALIDATION AND STANDARDIZATION OF TEST METHODS AND EVALUATORS FOR TESTING OF HAIR CARE RANGE OF PRODUCTS.

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ABSTRACT

Background: The effectiveness of medicines and cosmetics on hair growth can be evaluated with the aid of many clinical hair procedures. However, there are very less qualitative approaches that are seldom available to evaluate standardization and validation and validated in any significant way. **Aims:** The purpose of this validation test was to assess the standardization of these methods used during the conduct of a clinical safety and efficacy study for hair care products by various techniques and procedural steps. It is also been a useful guide to laboratories planning hair testing facilities so that they may fully understand the methodology involved with establishing quality-enhanced hair testing services. **Methods:** This standardization and validation study was carried out by the trainee evaluators' of NovoBliss Research who voluntarily wish to take part in this study. There are multiple techniques for establishing documentary evidence demonstrating that standardised procedural steps, processes and methods are carried out in testing for hairs care range of products to maintain the desired level of compliance. Validation of methods such as assessment of the general appearance of hair, assessment of scalp hair, assessment of hair strength, Grey severity score, 60s hair combing method, hair pull test, and trichogram test, has been performed to establish documentary evidence. **Results:** Scores of Dermatologist and Evaluators were analysed to assess the variability between the scoring of dermatologist and Evaluators. With at least 80% agreement in scoring the designated Evaluators were considered as Dermatologist Trained and Validated Evaluators for the general appearance of hair and scalp skin. Above methods were validated and statistical data were interpreted. **Conclusion:** In order to verify that the designated evaluators are Dermatologist Trained and Validated to perform the efficacy of the method claims substantiation for Hair Growth | Care products; we established the standards to perform test methods with the least minimal variability for phototrichogram, microscopical evaluation, and statistically significant correlations between the evaluators for above-mentioned techniques.

INTRODUCTION

Hair is the framework of choice in forensic toxicology when retrospective analysis is needed. Various clinical hair techniques can assist in assessing the efficacy of drugs and cosmetic agents on hair growth. Great advances have been made during the recent decades in the methodology of hair growth trials in dermatology and cosmetology. Clinical evaluations benefit from a number of additional specific techniques that enhance the perception of hair growth, shedding and alopecia. Hair loss is the most frequent and distressing clinical complaint encountered by dermatologists in clinical practice.Few types of hair loss are linked specifically to correctable causes while others are not[1]. Hair loss may be categorized as non-cicatricial alopecia, cicatricial alopecia, or hair shaft abnormalities. To decrease hair loss, scalp care is essential as it determines the health and condition of the hair and prevents diseases of the scalp and hair. Inspection of the scalp (capillitium) or dermatoscopic examination reveals whether there is a visible reduction of the amount of hair (alopecia) and, if so, in what pattern [2-3]. Any inflammatory redness or scaling should be noted, as psoriasis and eczema can cause effluvium. For years, hair diseases are evaluated with many techniques but there is no standardization approaches are available.

There are several ways to establish written proof that testing for the hair care line of products by following standardised procedural procedures, processes, and methodologies in order to maintain the necessary degree of compliance. It is crucial for ensuring that the process will consistently deliver the desired results in addition to final product testing and compliance. The specifications for the processes' results are used to determine the desired outcomes.

Rationale:

The standardization and validation study has been done in past for establishing documentary evidence demonstrating only a few steps, processes and methods were carried out in testing for hair care safety and efficacy studies to maintain the desired level of compliance by NovoBliss Research on 10 subjects in the year 2021[4]. Initial training was provided by the Principal Investigator and Dermatologist to the evaluators (5 evaluators were trained). The study was done to have training from the Dermatologist and upon getting training; the inter- and intra-observer assessments were established between the Dermatologist and the Dermatologist's Trained Evaluators. However, the study has its own limitation including a small sample size. A study with a bigger sample size can enhance the clinical study's scientific value. Therefore, NovoBliss Research conducted the validation study for the general appearance of hair, assessment of scalp hair, assessment of hair strength, 60s hair combing method, hair pull test and trichogram test in order to make the validation study more credible and acceptable to scientists. This study's aim is to standardise and evaluate the procedures, processes, and evaluation techniques used in *in-vivo* clinical safety and efficacy trials for a variety of hair care products.

MATERIALS AND METHODOLOGY

Ethics And Informed Consent:

The clinical investigation, including the Informed Consent Document (ICD), Advertisement, was reviewed by ACEAS Independent Ethics Committee in accordance with ICMR ethical guidelines, applicable federal government codes ICH-GCP, New drugs and clinical trials rule 2019. ACEAS Independent Ethics Committee is registered at CDSCO and OHRP US DHHS. CDSCO registration#

ECR/281/Indt/GJ/2017/RR-21 and OHRP US DHHS registration number is IRB00011046. The test was performed in accordance with the principles stated in the Declaration of Helsinki and its subsequent amendments and the Good Clinical Practice Guideline. Signed and dated informed consent was obtained from all the subjects before enrolment in the test. The study is registered under CTRI with Registration number CTRI/2023/02/050050 and ClinicalTrials.gov identifier number NCT05763888.

Study Subjects:

Referring to the convenience sampling method which is a type of non-probability sampling, a total of 40 subjects were enrolled. This technique involves selecting a research sample based on convenience & accessibility, drawing the sample from the part of the population close to hand. In this study, the purpose is to standardize and validate the methods and evaluators' and there is no usage of medicines/test articles/test treatment or test products, specific sample calculation of sample size estimation is not required.

Study Design:

There were a total of 40 subjects in the age group of 18 to 75 years, selected according to inclusion and exclusion criteria in the study to complete the required numbers. The study was conducted in month of April 2023. The exclusion criteria include the subjects who were having a history of allergy to any ink, Subjects who have participated any clinical research study related to hair care products, subjects having history of diabetes, Subjects have history of mastectomy for cancer involving removal of lymph nodes within the past year, or treatment of any type of cancer within the last 6 months, subjects having known history of any skin diseases of the scalp, visibly inflamed scalp or active cancer.

The inclusion criteria include males and non-pregnant/non-lactating females (preferably an equal number of males and females), females of childbearing potential must have a self-reported negative urine pregnancy, subjects who is in good general health as determined by the Investigator on the basis of medical history, a subject who is willing and able to follow and allow study evaluators to performed study test methods, the subject is willing and able to follow the study directions, to participate in the study, returning for all specified visits, subjects must be able to understand and provide written informed consent to participate in the study.

At the time of screening, subjects were given a screening number. The screening number appeared on the Informed Consent Document (ICD), Log Sheets, and all study documents relating to all subjects. Screen-passed subjects were considered for the study. Once eligibility was confirmed, each eligible subject was sequentially assigned a subject number. Acclimatization of subjects to the study clinic environment was performed by giving them rest for at least 15 minutes so that their blood circulation could be normalized after possible physical exertion due to travel to the study site. Subjects' well-being, demography (age, race, weight, and gender), along with medical history and current medication (prescription and over-the-counter) use over the past four weeks were recorded followed by inclusion and exclusion criteria verification (Tables 1 and 2). All participants received a modest monetary payment for their enrolment in this study. The assessment schedule and process for different testing were explained to the volunteers (Table 3).

Table 1 Demographic And Baseline Characteristics

Gender		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Female	17	42.5	42.5	42.5
	Male	23	57.5	57.5	100.0
	Total	40	100.0	100.0	

Note: In this study, there were 23 Males and 17Females; age of the subjects ranged from 30 to 49 years with the average being 25 years. N= 40 subjects taken.

Table 2 Descriptive Statistics Of Subjects

	Mean	Median	Std. Deviation	Minimum	Maximum
Age	37.93	37.50	5.171	30	49
Height	164.0600	164.0000	10.18586	145.00	184.00
Weight	65.4925	64.2000	11.21883	42.00	89.00

Note: In this study, there were 23 Males and 17 Females; age of the subjects ranged from 30 to 49 years with the average being 25 years. N= 40 subjects taken

Table 3: Results For Grey Severity Score

Evaluator(s)	N (Subjects)	Sum	Mean	Std. Deviation
Total Score (AM)	40	435	10.88	2.388
Total Score (NP)	40	457	11.43	2.263
Total Score (MP)	40	437	10.92	2.055
Total Score (MY)	40	413	10.33	1.289
Total Score (NJ)	40	436	10.90	1.736
Total Score (KP)	40	433	10.83	1.920
Total Score (KB)	40	414	10.35	1.369
Total Score (BJ)	40	409	10.23	2.434
Valid N (listwise)	40			

The Dermatologists had provided training to five evaluators on skin, hair, and scalp anatomy, scoring for Hair volume, hair density, hair reflection, hair plasticity, hair shininess, hair smoothness, and the appearance of scalp skin (before scoring on actual subjects), Hair growth phases, differentiation about various hair growth phases with hair morphological changes and various validation techniques. Scores of dermatologists and evaluators were analysed to assess the variability between the scoring of dermatologist and evaluators. With at least 80% agreement in scoring the designated evaluators were considered as Dermatologist Trained and Validated evaluators for the general appearance of hair and scalp skin.

Assessments of Grey Hair- Greying Severity Scoring:

It is a new, numeric, objective, and reproducible tool for the evaluation of premature canities and severity of Greying hairs. An evaluation was done by Grey Severity Score by counting non-Grey hair and Grey hairs by dividing the entire scalp surface into 5 zones, i.e. frontal region, vertex, right and left temporal regions, and the occipital. In each of these zones, identify areas showing maximum greying by doing visual examination. The Trainee evaluator shaved a small area i.e. 1x1 cm ($1cm^2$) on the subject's scalp mid-vertex area. The tattoo was made using a permanent ink marker and a digital photograph of the head crown was taken to confirm the tattoo area. These five squares were then photographed and projected on the computer screen using CASLite Nova to count the numbers of white and black hair. The microscope magnification level was set to 60X and the image was captured (Shaved area/tattoo marked area) (Follicle should be clearly visible in the image). Based on the count of non-Grey hair and Grey hairs, a ratio of grey hair was calculated.

Based on the hair count from an image captured by CASLite Nova, a score was assigned to each zone according to the percentage of Grey hair in each square as per the below-listed table. The score was calculated as per % of Grey hair/cm^2 as per This was calculated and scored as Score 1 (assigned to under 10% Grey hair/cm^2); Score 2 (10%–30% Grey hair/cm^2); and Score 3 (more than 30% grey hair/cm^2) The GSS was finally calculated for each patient by taking a sum of the scores at the five representative sites. Thus the maximum attainable score for a patient was 15 (3 × 5). The objective scores were further graded as Mild (a score of 0–5); Moderate (score of 6–10); and Severe (score of 11–15).

Scalp Sebum by Adsorbent Tissue Test:

It is an effective way of determining hair sebum level with the

help of the tissue paper test. For this test, tissue paper has been kept on the predefined site i.e. small area on the subject's scalp 30 cm from the tip of the nose to the vertex-possibly centre of the vertex area. 2x2 cm^2 tissue paper was used to perform the test. After 5 seconds of application of hair care product, tissue paper has been removed, A careful evaluation of the tissue paper was performed to measure scalp sebum level. If the tissue paper looks dry after this test means completely penetration is occurred. If some traces of sebum is found on the tissue paper, means hair care product was not completely absorbed. 0-5 mark scoring has been performed to determine the sebum level on the scalp. Where 1=represents no sebum, 2= slightly sebum, 3=moderately sebum level, 4= seborrhoea. The process and its step have been standardized.

Hydration of Scalp by Delfin's MoistureMeterEpiD

Measurement of water changes at the epidermal level provides important information to assist with understanding scalp healthiness and the effect of products and ingredients on the scalp. The MoistureMeterEpiD is an all-in-one measurement unit that is composed of an integrated probe, a built-in contact force sensor and a display. The LCD display shows non-invasively measured values in the percentage of local tissue water (0 to 100 %) effectively in the epidermis. The MoistureMeterEpiD may be used either as a stand-alone device or measurement data may be collected wirelessly to the DMC software. The DMC software allows users to set up individual projects, store and view measurement data and plot the results or export them to other programs for editing. The MoistureMeterEpiD generates a high frequency, low power electromagnetic (EM) wave into the skin. The reflected EM wave is analyzed and the obtained value is a tissue dielectric constant, which is proportional to the water content of the measured site. This TDC (tissue dielectric constant) value is converted to water percentage and displayed. The value increases with increasing hydration.

Evaluator selected a small area i.e. 1x1 cm (1 cm^2) on the subject's scalp 30 cm from the tip of the nose to the vertex-possibly centre of the vertex area. A permanent ink marker was used to standardize the location of the assessment. The following procedure was done to measure the hydration of the scalp by using MoistureMeterEpiD. Selection of the instrument MoistureMeterEpiD was done and a reading for hydration of the scalp was taken. The readings were saved automatically in the software. In this validation study, the trained evaluator was trained by taking readings from 40 volunteers by performing the above-mentioned process to measure and understand scalp hydration recording.

Scanning Electron Microscope (SEM) of Hair Cuticle

It helps to measure parameters such as surface damage of hair, and photodamage on the outermost cuticle layer of human hair and its morphology. The hair shaft damage as seen on SEM can be assessed using a standardized scoring system. The damage to the hair shaft under SEM was graded. Grade 0 represents virgin intact hair with a regular overlay of the cuticle. Grade 1 expressed an irregular overlay of the cuticle without cracks or holes. Grade 2 represents severe lift-up of the cuticle with cracks or holes but without exposure to the cortex. Grade 3 expresses partial exposure of the cortex. Grade 4 denotes the complete disappearance of the cortex. The method has been validated and standardized.

Dermascope or Trichoscopy

Hair samples of subjects were collected by trained evaluators and analyzed after cutting at a point approximately 5 cm from the hair root. Isolated Grey hairs were discarded. After naked eye examination of the hair shafts, all hair samples were examined under a handheld Dermoscope (DermLite Multispectral, ×10 magnification – white light and blue light were used or similar) and a light microscope at × 10 and × 40, to check if any discernible changes were found.

60-Seconds Hair Count (Hair Combing Method):

Study volunteers were informed not to wash their hair 24 hours before assessment. The volunteers were asked to flip their hair upside down and comb it for 60 seconds over a sheet of contrasting colour to their hairs. Starting with the comb on the back then the top of the scalp and moving the comb forward to the front of the scalp. The same designated comb or brush for each volunteer used. Each trained evaluator counted the number of hairs in the comb or brush and on the sheet. The hair with a hair bulb (i.e. hair fallen from root) and without a hair bulb was counted and stored in separate zip lock bags with proper labelling. The total hair count was recorded. The hair count was verified by the dermatologist. The results of the dermatologist and trained evaluators were analysed to assess the variability between them.[5]

Hair Pull Test:

Approximately 20-60 hairs shafts were taken between the thumb and index finger close to the surface of the scalp skin and pulled firmly, but not forcibly away from the scalp with constant strength along with the hair shaft up to the upper hair tip (Fig 1). Hairs were epilated under this procedure and counted. The pull-test was recorded as a 'negative' (no active hair loss) between none and three epilated hairs, "slightly positive" between three and six epilated hairs, and "clearly positive" above six hairs (>10% of tested hairs). The evaluation of 40 volunteers' was done as per the above method and as guided by the Dermatologist and trained evaluators. During the clinical study, maximum efforts were followed to have single-trained evaluators perform a pull test for all subjects to avoid any subjective variability.[6-7]

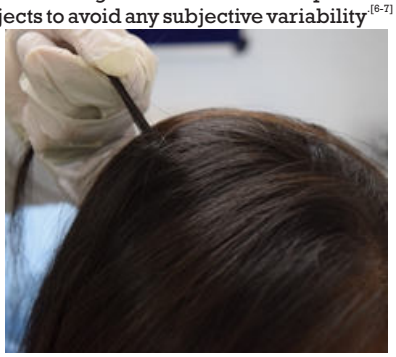

Figure: 1 Hair Pull Test

Trichogram (Hair Pluck Test):

The test was performed on 40 volunteers. The Trichogram was performed on the scalp that has been unwashed and untreated scalp for 5 days. A bundle of about 10-30 hairs was carefully ranged as 10 hairs represented a reliable quantity to provide enough information as guided by the Dermatologist. The lock of hairs was tightly plucked with the forceps/rubber-protected jaws as close as possible to the scalp, to avoid dystrophic and broken hairs and to pluck miniaturized hairs as well. A vigorous massage on the area was given immediately to get relief the discomfort caused by the sampling. Hairs were arranged side by side on a glass slide and taped with transparent adhesive tape. Bulbs were examined at low magnification (40x magnification) with a light microscope or on a screen of a microfilm reader. Several anagen and telogen hairs were recorded and the A:T ratio was calculated. After plucking the slides were prepared per the procedure mentioned above and the slides were reviewed. Results (Images) and slides were preserved for reference. The dermatologist verified the results/readings upon reviewing the same glass slide.[6]

Phototrichogram Test (Tattoo Method) and Growth Rate Measurement:

On Day 01, shaved a small area i.e. 1×1 (1cm^2) on the volunteer's scalp 30 cm from the tip of the nose to the vertex-possibly centre of the vertex area using a ruler. A permanent ink marker was used to standardize the location of the

assessment and readings were taken using the CASLiteNova Hair analyser instrument for hair growth measurement/ average hair length. The microscope magnification level of 60X was set. The image of shaved area/tattoo-marked area was captured by clicking on the capture option, ensuring the follicle should be visible in the image. Random 3 hairs were selected. After 3 days, an image of the same area was taken and again random 3 hairs were selected. The minimum, maximum, and average length were obtained from the analyser which provides a per-day growth rate. Readings of 40 volunteers had been taken by performing the phototrichogram technique to measure and understand hair growth rate calculation. 1st reading was taken on Day 1 and 2nd reading after 3 days on Day 4. The reading of Day 4 has been subtracted from a reading of Day 1 to get the daily hair growth rate of the volunteer(Fig 3).[8]

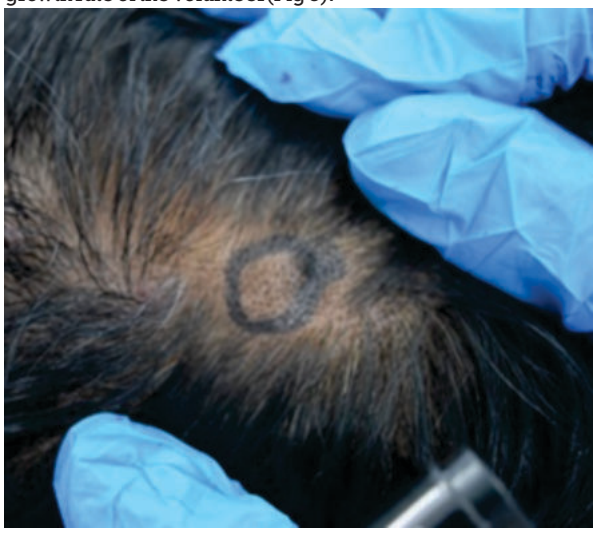

Figure 2. Tattoo Marking

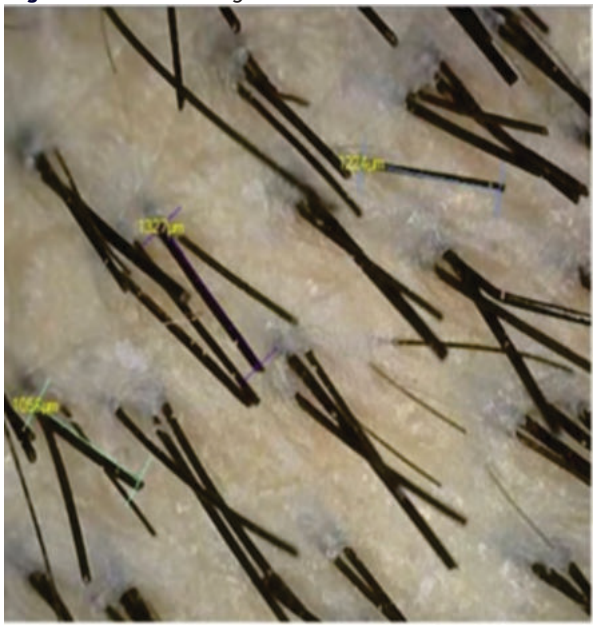

Figure 3. Hair Growth Measurement

Hair Thickness:
Hair readings of all 40 volunteers were taken using the CASLite Nova Hair analyser instrument for hair thickness from the Tattoo marked area. Hair thickness was obtained by phototrichogram and with microscope magnification (200X). Images were captured ensuring that the hair follicle and scalp were visible by clicking on the capture option. The process was repeated for a minimum of 3 hairs to get us the 'Average Hair Thickness'(Fig 4).

Figure 4. Hair Thickness Measurement

Hair Density: Readings were taken using the CASLite Nova Hair analyser instrument for hair density from the same tattoo area to find Hair Density/cm^2. The microscope magnification was set to Level 60X. Images were captured ensuring that the hair follicle and scalp were visible. Terminal and vellus hairs were selected from the marked area and images were captured. Hair density/cm^2 was calculated. Each operator had taken readings of participants by performing the phototrichogram technique to measure hair density(Fig 5). Hair density was also measured by the Ponytail method.

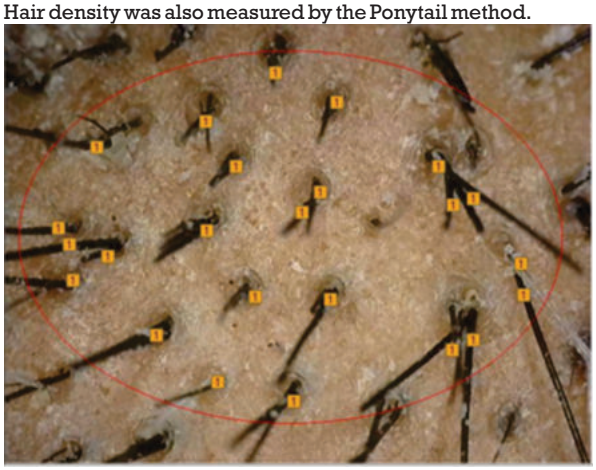

Figure 5. Hair Density Measurement

Scalp Condition:
Readings were taken using CASLite Nova Hair analyser instrument for a scalp condition. The 'Condition of scalp' tab on the phototrichogram menu was clicked to find scalp condition. The microscope magnification was set to Level on 60X. Images were captured. The image to pre-uploaded sample images of scalp condition was compared. Each evaluator had taken readings of participants by performing the phototrichogram technique for measurement and understanding the scalp condition observation.

Instrument for Images:
Digital photographs of the application sites and surrounding areas were taken using Nikon D3300 DSLR (24.2 megapixels) camera with an 18-55 mm lens. All photographs were captured in 300 dpi and maintaining the same distance and same lightening condition by measuring the light intensity using Lux Meter.

Statistical Analysis
Demographic characteristics and results of the study were summarized with descriptive statistics including average, and standard deviation (SD) for continuous variables and frequency and percentages for categorical variables. Any

AEs were summarized with a number and a percentage.

At the time of evaluation, the most suitable method was chosen to best represent the study objective. The statistical analysis was done by using SPSS software with a 5% level of significance (Version: 4.0 or higher). Chi-Square test, Paired Correlation and Paired t-test or applicable test was used to analyse the scores between trainee evaluators and dermatologists.

Categorical variables have been expressed by frequency and percentage along with the graphical presentation whenever required. Whenever required, appropriate statistical tests were applied.

There was no withdrawn subject in the study.

RESULTS

Forty volunteers were enrolled and all forty completed the study out of 40 screened subjects (Table 1 and 2).

General appearances of hair results were obtained by applying chi-square test to check the correlation between Dermatologist (AM) and General physician (NP) to trained evaluators. The general appearance of hair result p-value was found <0.01 with no minimal variability. No significant differences were seen between trained evaluators and dermatologist with regard to normal clinical examination by Scanning Electron Microscope (SEM) and light microscopy findings. A minimal difference or variability was observed.

The total score with standard deviation and statistical description for the Grey severity score has been explained for all 40 subjects (Table 3). Before hair loss testing standardization, different parameters for hair such as itchiness, dryness, scaliness, roughness and redness were studied and evaluated by trained evaluators (Table 4). Validation and standardization of SEM testing methods have been standardized for the identification of hair shaft damage and hair morphology determination. For 60s hair count methods, Positive correlations were found between the Dermatologist and all five evaluators and these correlations were highly significant for all evaluators. The Pearson correlation was near "+1" for trained evaluators and the correlation was significant indicating that there was a perfect positive correlation between the dermatologist, principal investigator and all evaluators. The value of Pearson correlation and Chi-Square p-value were complementary, giving the value of significance of the correlation. Hence, it could be considered that all 5 evaluators were Dermatologist Trained and validated evaluators for performing the 60-Seconds Hair combing method (Table 5).

For the Hair Pull Test chi-square test has performed and All their p-value for the chi-square test for the pull test of independence were found significant for pull test results (Table 6). The Trichogram or hair pluck test is a method that expresses the number and the proportion of hairs in the different phases of the hair cycle. Here, the results of evaluators' readings the Pearson correlation was found near "+1 or +0.5" for evaluators which indicates that there was a positive correlation between Dermatologist and Evaluators. Detailed results and statistics have been explained for the moisture meter in Table 7.

Phototrichogram evaluations for Hair Density, Thickness, and Hair Growth Rate (Table 8 and 9) revealed that all pairs p-values were >0.05 which shows that there was no statistically significant difference in the mean value of evaluators. P-value >0.05 means that there was no statistically significant difference between the two means which showed that they all 3 were performing and taking phototrichogram measurements with minimal variability.

Table 4. General Appearance Of Hair - Statistics And Correlation

Descriptive Statistics					
	N	Minimum	Maximum	Mean	Std. Deviation
Itchiness(AM)	40	0	1	.05	.221
Dryness(AM)	40	0	1	.20	.405
Redness(AM)	40	0	1	.02	.158
Roughness(AM)	40	0	1	.20	.405
Scaliness(AM)	40	0	1	.18	.385
Itchiness(NP)	40	0	1	.10	.304
Dryness(NP)	40	0	1	.25	.439
Redness(NP)	40	0	1	.03	.158
Roughness(NP)	40	0	1	.27	.452
Scaliness(NP)	40	0	1	.25	.439
Itchiness(MP)	40	0	1	.10	.304
Dryness(MP)	40	0	1	.25	.439
Redness(MP)	40	0	1	.02	.158
Roughness(MP)	40	0	1	.25	.439
Scaliness(MP)	40	0	1	.25	.439
Itchiness(MP)	40	0	1	.15	.362
Dryness(MP)	40	0	1	.30	.464
Redness(MP)	40	0	1	.08	.267
Roughness(MP)	40	0	1	.33	.474
Scaliness(MP)	40	0	1	.32	.474
Itchiness(NJ)	40	0	1	.15	.362
Dryness(NJ)	40	0	1	.30	.464
Redness(NJ)	40	0	1	.08	.267
Roughness(NJ)	40	0	1	.33	.474
Scaliness(NJ)	40	0	1	.32	.474
Itchiness(KP)	40	0	1	.18	.385
Dryness(KP)	40	0	1	.37	.490
Redness(KP)	40	0	1	.08	.267
Roughness(KP)	40	0	1	.40	.496
Scaliness(KP)	40	0	1	.30	.464
Itchiness(KB)	40	0	1	.20	.405
Dryness(KB)	40	0	1	.42	.501
Redness(KB)	40	0	1	.18	.385
Roughness(KB)	40	0	1	.32	.474
Scaliness(KB)	40	0	1	.08	.267
Itchiness(BJ)	40	0	1	.20	.405
Dryness(BJ)	40	0	1	.42	.501
Redness(BJ)	40	0	1	.18	.385
Roughness(BJ)	40	0	1	.32	.474
Scaliness(BJ)	40	0	1	.08	.267
Valid N (listwise)	40				

Note: where N represents a number of volunteers

Table 5: 60S Hair count

Paired Samples Statistics					
		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Total Hair Count(AM)	30.85	40	19.820	3.134
	Total Hair Count(NP)	30.80	40	19.860	3.140
Pair 2	Number of Hair with bulb(AM)	11.28	40	7.334	1.160
	Number of Hair with bulb(NP)	11.23	40	7.322	1.158
Pair 3	Number of Hair without bulb	19.58	40	15.495	2.450
	Number of Hair without bulb(NP)	19.58	40	15.630	2.471
Pair 4	Total Hair Count(AM)	30.85	40	19.820	3.134
	Total Hair Count(MP)	30.75	40	19.761	3.124
Pair 5	Number of Hair with bulb(AM)	11.28	40	7.334	1.160
	Number of Hair with bulb(MP)	11.25	40	7.362	1.164

Pair 6	Number of Hair without bulb	19.58	40	15.495	2.450
	Number of Hair without bulb(MP)	19.50	40	15.470	2.446
Pair 7	Total Hair Count(AM)	30.85	40	19.820	3.134
	Total Hair Count(MY)	30.78	40	19.713	3.117
Pair 8	Number of Hair with bulb(AM)	11.28	40	7.334	1.160
	Number of Hair with bulb(MY)	11.28	40	7.331	1.159
Pair 9	Number of Hair without bulb	19.58	40	15.495	2.450
	Number of Hair without bulb(MY)	19.50	40	15.362	2.429
Pair 10	Total Hair Count(AM)	30.85	40	19.820	3.134
	Total Hair Count(NJ)	30.78	40	19.705	3.116
Pair 11	Number of Hair with bulb(AM)	11.28	40	7.334	1.160
	Number of Hair with bulb(NJ)	11.28	40	7.306	1.155
Pair 12	Number of Hair without bulb	19.58	40	15.495	2.450
	Number of Hair without bulb(NJ)	19.50	40	15.312	2.421
Pair 13	Total Hair Count(AM)	30.85	40	19.820	3.134
	Total Hair Count(KP)	30.78	40	19.767	3.125
Pair 14	Number of Hair with bulb(AM)	11.28	40	7.334	1.160
	Number of Hair with bulb(KP)	11.25	40	7.316	1.157
Pair 15	Number of Hair without bulb	19.58	40	15.495	2.450
	Number of Hair without bulb(KP)	19.53	40	15.464	2.445
Pair 16	Total Hair Count(AM)	30.85	40	19.820	3.134
	Total Hair Count(KB)	30.75	40	19.825	3.135
Pair 17	Number of Hair with bulb(AM)	11.28	40	7.334	1.160
	Number of Hair with bulb(KB)	11.23	40	7.262	1.148
Pair 18	Number of Hair without bulb	19.58	40	15.495	2.450
	Number of Hair without bulb(KB)	19.53	40	15.517	2.453
Pair 19	Total Hair Count(AM)	30.85	40	19.820	3.134
	Total Hair Count(BJ)	30.75	40	19.825	3.135
Pair 20	Number of Hair with bulb(AM)	11.28	40	7.334	1.160
	Number of Hair with bulb(BJ)	11.25	40	7.302	1.155
Pair 21	Number of Hair without bulb	19.58	40	15.495	2.450
	Number of Hair without bulb(BJ)	19.50	40	15.512	2.453

Table 6. Pull test method

Chi-Square Tests			
	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	53.333[a]	4	<.001
Likelihood Ratio	42.098	4	<.001
N of Valid Cases	40		
a. 6 cells (66.7%) have expected count less than 5. The minimum expected count is .88.			

Table 7. Results and statistics for MoistureMeter EpiD

Paired Samples Correlations					
		N	Correlation	Significance	
				One-Sided p	Two-Sided p
Pair 1	MoistureMeterEpid(AM) & MoistureMeterEpid(NP)	40	.584	<.001	<.001
Pair 2	MoistureMeterEpid(AM) & MoistureMeterEpid(MP)	40	.125	.221	.441
Pair 3	MoistureMeterEpid(AM) & MoistureMeterEpid(MY)	40	.317	.023	.046
Pair 4	MoistureMeterEpid(AM) & MoistureMeterEpid(NJ)	40	.840	<.001	<.001
Pair 5	MoistureMeterEpid(AM) & MoistureMeterEpid(KP)	40	.166	.154	.307
Pair 6	MoistureMeterEpid(AM) & MoistureMeterEpid(KB)	40	.034	.418	.836
Pair 7	MoistureMeterEpid(AM) & MoistureMeterEpid(BJ)	40	.927	<.001	<.001

Table 8. Phototrichogram Test

Paired Samples Statistics					
		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Calculation of Hair Growth Rate(AM)	302.50	40	85.282	13.484
	Calculation of Hair Growth Rate(DR Nayan)	300.55	40	84.875	13.420
Pair 2	Hair Density(AM)	267.83	40	45.254	7.155
	Hair Density(NP)	264.55	40	47.972	7.585
Pair 3	Hair Thickness(AM)	16.33	40	2.258	.357
	Hair Thickness(NP)	16.10	40	1.932	.306
Pair 4	Growth Rate Day-01 (AM)	491.68	40	114.834	18.157
	Growth Rate Day-01NP)	490.05	40	114.125	18.045
Pair 5	Growth Rate Day-01 (AM)	491.68	40	114.834	18.157
	Growth Rate Day-01 (MP)	489.65	40	115.383	18.244
Pair 6	Calculation of Hair Growth Rate(AM)	302.50	40	85.282	13.484
	Calculation of Hair Growth Rate(MP)	309.98	40	85.975	13.594
Pair 7	Hair Density(AM)	267.83	40	45.254	7.155
	Hair Density(MP)	263.58	40	47.205	7.464
Pair 8	Hair Thickness(AM)	16.33	40	2.258	.357
	Hair Thickness(MP)	16.35	40	2.214	.350
Pair 9	Growth Rate Day-01 (AM)	491.68	40	114.834	18.157
	Growth Rate Day-01(MY)	490.98	40	115.041	18.190
Pair 10	Calculation of Hair Growth Rate(AM)	302.50	40	85.282	13.484
	Calculation of Hair Growth Rate(MY)	300.65	40	87.531	13.840
Pair 11	Hair Density(AM)	267.83	40	45.254	7.155
	Hair Density(MY)	266.78	40	43.018	6.802
Pair 12	Hair Thickness(AM)	16.33	40	2.258	.357
	Hair Thickness(MY)	16.70	40	2.377	.376
Pair 13	Growth Rate Day-01 (AM)	491.68	40	114.834	18.157
	Growth Rate Day-01(NJ)	489.50	40	111.417	17.617
Pair 14	Calculation of Hair Growth Rate(AM)	302.50	40	85.282	13.484
	Calculation of Hair Growth Rate(NJ)	302.05	40	109.326	17.286
Pair 15	Hair Density(AM)	267.83	40	45.254	7.155
	Hair Density(NJ)	277.43	40	48.378	7.649
Pair 16	Hair Thickness(AM)	16.33	40	2.258	.357
	Hair Thickness(NJ)	16.55	40	2.961	.468
Pair 17	Growth Rate Day-01 (AM)	491.68	40	114.834	18.157

	Growth Rate Day-01(KP)	490.65	40	115.767	18.304
Pair 18	Calculation of Hair Growth Rate(AM)	302.50	40	85.282	13.484
	Calculation of Hair Growth Rate(KP)	277.90	40	85.783	13.563
Pair 19	Hair Density(AM)	267.83	40	45.254	7.155
	Hair Density(KP)	270.95	40	45.215	7.149
Pair 20	Hair Thickness(AM)	16.33	40	2.258	.357
	Hair Thickness(KP)	16.78	40	2.370	.375
Pair 21	Growth Rate Day-01 (AM)	491.68	40	114.834	18.157
	Growth Rate Day-01(KB)	481.40	40	101.389	16.031
Pair 22	Calculation of Hair Growth Rate(AM)	302.50	40	85.282	13.484
	Calculation of Hair Growth Rate(KB)	338.85	40	134.782	21.311
Pair 23	Hair Density(AM)	267.83	40	45.254	7.155
	Hair Density(KB)	267.33	40	48.998	7.747
Pair 24	Hair Thickness(AM)	16.33	40	2.258	.357
	Hair Thickness(KB)	17.93	40	3.316	.524
Pair 25	Growth Rate Day-01 (AM)	491.68	40	114.834	18.157
	Growth Rate Day-01(BJ)	488.03	40	106.596	16.854
Pair 26	Calculation of Hair Growth Rate(AM)	302.50	40	85.282	13.484
	Calculation of Hair Growth Rate(BJ)	305.58	40	119.138	18.837
Pair 27	Hair Density(AM)	267.83	40	45.254	7.155
	Hair Density(BJ)	272.85	40	47.616	7.529
Pair 28	Hair Thickness(AM)	16.33	40	2.258	.357
	Hair Thickness(BJ)	17.40	40	3.350	.530

Table 9: Paired Samples Test For Hair Growth Measurement

Paired Samples Correlations					
		N	Correlation	Significance	
				One-Sided p	Two-Sided p
Pair 1	Calculation of Hair Growth Rate(AM) & Calculation of Hair Growth Rate(NP)	40	.990	<.001	<.001
Pair 2	Hair Density(AM) & Hair Density(NP)	40	.937	<.001	<.001
Pair 3	Hair Thickness(AM) & Hair Thickness(NP)	40	.827	<.001	<.001
Pair 4	Growth Rate Day-01 (AM) & Growth Rate Day-01 (NP)	40	.998	<.001	<.001
Pair 5	Growth Rate Day-01 (AM) & Growth Rate Day-01 (MP)	40	.997	<.001	<.001
Pair 6	Calculation of Hair Growth Rate(AM) & Calculation of Hair Growth Rate(MP)	40	.943	<.001	<.001
Pair 7	Hair Density(AM) & Hair Density(MP)	40	.918	<.001	<.001
Pair 8	Hair Thickness(AM) & Hair Thickness(MP)	40	.972	<.001	<.001
Pair 9	Growth Rate Day-01 (AM) & Growth Rate Day-01(MY)	40	.993	<.001	<.001
Pair 10	Calculation of Hair Growth Rate(AM) & Calculation of Hair Growth Rate(MY)	40	.779	<.001	<.001
Pair 11	Hair Density(AM) & Hair Density(MY)	40	.694	<.001	<.001
Pair 12	Hair Thickness(AM) & Hair Thickness(MY)	40	.635	<.001	<.001
Pair 13	Growth Rate Day-01 (AM) & Growth Rate Day-01(NJ)	40	.997	<.001	<.001
Pair 14	Calculation of Hair Growth Rate(AM) & Calculation of Hair Growth Rate(NJ)	40	.424	.003	.006
Pair 15	Hair Density(AM) &	40	.573	<.001	<.001
Pair 16	Hair Thickness(AM) & Hair Thickness(NJ)	40	.690	<.001	<.001
Pair 17	Growth Rate Day-01 (AM) & Growth Rate Day-01(KP)	40	.999	<.001	<.001
Pair 18	Calculation of Hair Growth Rate(AM) & Calculation of Hair Growth Rate(KP)	40	.657	<.001	<.001
Pair 19	Hair Density(AM) & Hair Density(KP)	40	.989	<.001	<.001
Pair 20	Hair Thickness(AM) & Hair Thickness(KP)	40	.858	<.001	<.001
Pair 21	Growth Rate Day-01 (AM) & Growth Rate Day-01(KB)	40	.968	<.001	<.001
Pair 22	Calculation of Hair Growth Rate(AM) & Calculation of Hair Growth Rate(KB)	40	.132	.209	.417
Pair 23	Hair Density(AM) & Hair Density(KB)	40	.255	.056	.113
Pair 24	Hair Thickness(AM) & Hair Thickness(KB)	40	.284	.038	.076
Pair 25	Growth Rate Day-01 (AM) & Growth Rate Day-01(BJ)	40	.989	<.001	<.001
Pair 26	Calculation of Hair Growth Rate(AM) & Calculation of Hair Growth Rate(BJ)	40	.658	<.001	<.001
Pair 27	Hair Density(AM) & Hair Density(BJ)	40	.753	<.001	<.001
Pair 28	Hair Thickness(AM) & Hair Thickness(BJ)	40	.423	.003	.007

Note: p-Value <0.05 it means it is a statistical significance. p-Value >0.05 means there is no statistical significance. There is a positive correlation found between evaluators, dermatologist and principal investigator.

Safety Monitoring:

Throughout the study, every effort was made to remain alert to possible adverse events or serious adverse events. Safety monitoring and reporting were done as per the local regulations about that area (Country) and in-house NovoBliss Standard Operating Procedure (SOP). No adverse event was recorded.

CONCLUSION

In order to verify that there is a positive correlation found between dermatologist, Principal Investigator and the evaluators are validated to perform the methods of efficacy claims substantiation for Hair Growth products. In addition, our study established the standards and validation to establish documentary evidence demonstrating that standardized procedural steps, processes and methods which were carried out in testing for hair care safety and efficacy studies to maintain the desired level of compliance. The study provided more scientific value to measure the standardization of techniques. We established a benchmark

for Hair Care - Hair Growth Products Safety and Efficacy studies from this In-House standardization & validation study, which can produce consistent, compatible, accurate, qualitative, and reproducible results, controlled testing methods for the successful execution of a clinical hair care product efficacy trial.

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