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Clinical Research

A NOVEL EXPLORATORY APPROACH OF VALIDATION AND STANDARDIZATION FOR TEST METHODS AND EVALUATORS TO CONDUCT SAFETY AND EFFICACY CLINICAL TESTING OF HAIR GROWTH PRODUCTS

KEY WORDS:
Standardization, Trichogram, Telogen, Dermatologist, Phototrichogram, Hair growth

Maheshvari Patel M.Pharm, Director and Head, Clinical Trials Department, NovoBliss Research Pvt Ltd., Ahmedabad, India.

Dr. Nayan Patel MBBS, Principal Investigator, Clinical Trials Department, NovoBliss Research Pvt Ltd., Ahmedabad, India.

Dr. Bhavna Nangha Ph. D (Pharmacy), Quality Assurance Department, NovoBliss Research Pvt Ltd., Ahmedabad, India.

Dr. Apexa M. Merja* M.D. Dermatologist, (Expert/ trainer) NovoBliss Research Pvt Ltd., Ahmedabad, India. *Corresponding Author

ABSTRACT

Background: There is an explosion of cosmeceuticals and hair products in the modern world. Despite the high prevalence and impact on quality of life, there are no gold standards for qualitative and quantitative methods are established available for standardization and validation purposes. **Aims:** This study aimed to standardize the hair tests and methodology for all researchers. It demonstrates the standardize and validate the methods (Non-invasive and Semi-Invasive methods) such as the 60-second hair combing test, pull test, pluck test, trichogram review, phototrichogram test, standardizing the hair growth rate measurement, scoring of hair quality general appearance and scalp condition - evaluations techniques and procedural steps. **Methods:** This study standardized and validated different variables and all the above-mentioned techniques and procedural steps which were assessed by phototrichogram and equipment such as CASLite Nova on healthy adult human subjects. **Results:** The positive correlation was shown between the Dermatologist and the Dermatologist's Trained Evaluators' and these correlations were highly significant for all three evaluators. During phototrichogram evaluations, all pairs p-values were found >0.05 (except pairs 15 and 17), showing that there was no statistically significant difference in the mean value of the evaluator. All the p-value for the pull test of independence is not significant for pull test results and showed that all three evaluators (evaluator#1, 2, 3) values are dependent and related to each other's showing a correlation among them. For the pluck test, the results of the evaluators' readings the Pearson correlation is near to "+1 or +0.5" for Evaluators #1 and 2 indicating that there was a positive correlation between the Dermatologist and Evaluators #1 and 2. **Conclusions:** We established the standards to perform test methods with the least minimal variability for phototrichogram, microscopical evaluation, and statistically significant correlations between the evaluator for the above-mentioned techniques, and statistically significant correlation between the dermatologist and evaluators to confirm that the designated evaluator is Dermatologist Trained and Validated to perform the methods efficacy claims substantiation for Hair Growth products. This standardization will assist the post graduate students as well as practitioners in the field of research and dermatology.

Introduction

Human beings are born with approximately 100,000 terminal hair follicles on the scalp that are predetermined to grow long and thick hair.[1] Hair loss is the most frequent and distressing clinical complaint encountered by dermatologists in clinical practice. 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. Any inflammatory redness or scaling should be noted, as psoriasis and eczema can cause effluvium. [2-3] For decades, hair diseases are evaluated but there is no standardization aspects are available. The objective of this validation test was to evaluate standardizing some non-invasive and semi-invasive methods such as the 60-second combing test, pull test, pluck test, phototrichogram test, trichogram review, standardizing the hair growth rate measurement, scoring of hair quality, general hair appearance and scalp condition - evaluations techniques and procedural steps which were used during the conduct of clinical safety and efficacy study for hair care products evaluation on healthy adult human subjects.

Methodology

Ethics and Informed Consent: The clinical investigation, including the Informed Consent Document (ICD), Advertisement, were reviewed by ACEAS Independent Ethics Committee in accordance with ICMR ethical guidelines, applicable federal government codes ICH-GCP, New drugs and clinical trials rules 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. The trial was registered prospectively in the clinical trial registry of India (CTRI) with the registration number CTRI/2021/12/039014. Signed and dated informed consent was obtained from all the subjects before enrolment in the test.

Sample size estimation: In the study, we used Convenience Sampling which is Non-probability Sampling. This technique involves selecting a research sample based on convenience and accessibility which draws the sample from the part of the population close to hand. In our study no requirement to calculate the sample size estimation. In this study, we are not using any medicines. Therefore, we calculated the Ratio of the sample. In this study total of 12 subjects were screened and among them, 11 subjects were enrolled, and all 11 subjects completed the study.

Study design

There was a total of 11 subjects in the age group of 18 to 65 years, selected according to inclusion and exclusion criteria in the study to complete the required numbers. The exclusion criteria include the subjects who were having a history of allergy to any ink, with medication that may affect skin response based on past medical history, have a history of

diabetes, have a 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, who have a history of diabetes, who have participated any clinical research study related to hair care products, who have known history of any skin diseases including eczema, atopic dermatitis or active cancer.

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)

The clinical study was 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 (trained evaluators). As per the present scenario of the clinical trials of longer treatment duration with multiple visits, the same dermatologists' availability is not feasible for multiple time points. In such cases, many times clinical readings are getting missed and not having accurate data in a timely manner with predefined datasets. Therefore, to avoid such a scenario we, as NovoBliss Contract Research Organization decided to develop a novel aspect in view of having accurate, timely readings from the trial patients/subjects of cosmetics, and personal healthcare products, by the "Dermatologist's Trained Evaluators" and those to be doing subjective scoring referring to FDA guidelines allowing to have trained evaluators for skin irritation, sensitization scoring, skin blanching, many other skin attributes. The primary reason for not having specialist doctors (MD Dermatologists) as observers, is due to their very minimal availability in the cosmetic's clinical trials to their busy schedule for personal healthcare products such as hair care products includes - hair growth products, hair fall reduction shampoo, conditioners, hair colours, serum etc. Also, the overall budget for such clinical efficacy studies is too less, hence, we have taken lead in minimizing the overall cost of the clinical trials and having in-depth hair, scalp anatomy and physiology from MD Dermatologist to MBBS doctors, Registered Nurses, Clinical Pharmacist, Microbiologists – who can do subjective scoring upon getting training from MD Dermatologist. Also, to assess agreement in the level of assessment between MD dermatologists and Trainee observers (From the Medical and Paramedical fields) – we had done a training program with an examination to verify inter- and intra-observer assessments. So, ultimately the Dermatologist's Trained Evaluators' did observation under the supervision of the Dermatologist and in absence of the Dermatologist, a trained evaluator can do the scoring. So, we can get all the readings from all patients in a timely manner which is very important from a statistical analysis point of view and to check product safety and efficacy. The evaluator was properly trained evaluator on skin, hair, and scalp anatomy, scoring for Hair volume, hair density, hair reflexion, hair plasticity, hair shininess, hair smoothness, the appearance of scalp skin (before doing scoring on actual subjects), Hair growth phases, differentiation about various hair growth phases with hair morphological changes and various validation techniques. Each designated study evaluator assessed the general appearance of hair and scalp for 11 subjects in a separate scoring station (Tables 4 and 5). Scores of the dermatologist and study evaluator were analysed to assess the correlations between the scoring of dermatologist and study evaluator.

60-Seconds Hair Count (Hair Combing Method): The 60-seconds hair count is aimed to find out a range of shedding hair during a 60- seconds hair combing period. 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 was used. Each study 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, stored in separate zip lock bags with proper labelling. The total hair count was recorded. The hair count was verified by the dermatologist (Fig 1). The results of the dermatologist and study evaluator were analysed to assess the variability between the dermatologist and the study evaluator. [4]

Traction test or Pull Test: This test allows for obtaining a semi-quantitative clinical impression about the applicability of scalp hair and assessing the severity and location of hair loss. Approximately 60 hair 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. 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 10 volunteers' was done as per the above method and as guided by the Dermatologist and study evaluator. During the clinical study maximum efforts was followed to have a single evaluator perform a pull-test for all subjects to avoid any subjective variability. [5-6]

Trichogram (Hair Pluck Test): This method was analysed to determine the anagen and telogen phase of hair based on their specific morphological characteristics and counting of anagen, catagen, telogen, and dystrophic hair. The test was performed on 10 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 (Fig 2). 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 from 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 (Fig 3) and the A:T ratio was calculated. After plucking the slides were prepared per the procedure mentioned above and slides were reviewed (Fig 3). Results (Images) and slides were preserved for reference. The dermatologist verified the results/readings upon reviewing the same glass slide. [6]

Hair Growth Rate by Phototrichogram Test (Tattoo Method): The phototrichogram is a noninvasive, reproducible method that is based on the manual marking of shaved scalp/hairs on images taken at close to target areas on the scalp skin which

shows hair loss or pre-defined area in case of cosmetics evaluation in healthy volunteers scalp. There are some intra and inter-individual variation in this method itself which has been overcome by several modifications. When these variations can be reduced by standardized measurement procedures, this method represents a very satisfactory qualitative and quantitative technique to study hair growth, diameter, anagen/telogen rate, and vellus-/terminal-hair-rate in clinical hair loss trials.

On Day 01, study evaluators shaved a small area i.e. 1×1 (1cm2) on volunteer's scalp 30 cm from the tip of the nose to the vertex-possibly centre of the vertex area using a ruler (Fig 4). 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. 3 hairs were selected. After 3 days, an image of the same area was taken and 3 same hairs were measured. This was done by keeping the Casalite nova on the right border of tattoo. Minimum, maximum, and average length was obtained from the analyser which provides a per-day growth rate (Fig 5). Readings of 10 volunteers had been taken by performing the phototrichogram technique to measure and understand hair growth rate calculation. 1streading was taken on Day 1 and 2ndreading 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.[7]

Hair Thickness: Hair readings of all 10 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 same 3 hairs to get us the 'Average Hair Thickness'(Fig 6).

Hair Density : Readings were taken using the CASLite Nova Hair analyser instrument for hair density from the same tattoo area to find Hair Density/cm2. The microscope magnification was set to Level on 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/cm2 was calculated. Each operator had taken readings of 10 volunteers by performing the phototrichogram technique to measure hair density(Fig 6).

Scalp Condition: Readings were taken using CASLite Nova Hair analyser instrument for a scalp condition. '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 the pre-uploaded sample images of scalp condition was compared. Each operator had taken readings of 10 volunteers by performing the phototrichogram technique to measure and understand scalp condition recording.

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.

Statistical Analysis and measurement of the level of significance

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. There were no AEs reported in this test. At the time of evaluation, the most suitable method i.e. chi-square test, paired Correlation and paired t-test has been chosen to best represent the study objective. The statistical analysis was done by using SPSS (Statistical Package for the Social Sciences) with a 5% level of significance (Version: 15.0 or higher). None of the subjects was withdrawn from the study.

The correlation value range is -1 to 1. If the correlation value is very near +1 then perfect positive correlation: Meaning is one value is increased and then the corresponding value is also increased in the same magnitude and same direction. Near +0.5 than Partial positive correlation: One value increase than corresponding other value will increase by half magnitude and same direction. Near 0 then there is no correlation. Near -0.5 partial negative correlation: One value increases then the corresponding value is decreased. Near -1 than perfect negative correlation: One value is increased then the corresponding value decreased.

Results

Eleven healthy adults (6 males and 5 females) volunteers were enrolled and all eleven completed the study out of 12 screened subjects wherein, one was deemed a screen fail.

General appearances of hair results were obtained by applying Fisher Exact test to check the correlation between Dermatologist (AM) to Evaluator#1, 2, 3, and 4. General Appearance of Hair result p-value was found <0.05 except (Evaluator#3 Hair Reflexion, Plasticity, Evaluator#1 Hair Plasticity, Evaluator#3 Hair Plasticity, Hair Smoothness to Dermatologist respectively). Evaluator#4 results in p-value were <0.05 which shows a statistically significant correlation between Dermatologist and other evaluator members i.e., evaluator#1, 2, 3 were also Dermatologist Trained Evaluators for General Appearance of Hair evaluation.

60-Seconds Hair Count (Hair Combing Method): Positive correlation was found between the Dermatologist (AM) and all three Evaluators, These correlations were highly significant for all three evaluators. The Pearson correlation was near to "+1" for evaluator#1, 2, 3 and the correlation was significant indicating that there was a perfect positive correlation between the dermatologist and Evaluator#1, 2, 3. 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 3, evaluator#1, 2, 3 were Dermatologist Trained and Validated Evaluators for performing 60-Seconds Hair combing method (Table 6 and 7).

Hair Pull Test: All their p-value for the chi-square test for the pull test of independence was not significant for pull test results. It was found that pair1, pair2, and pair3 were not found independent among each other's and that shows all three evaluator's (evaluator#1, 2, 3) values were dependent and related to each other's showing correlation among them(Table 8).

Hair pluck test: 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 to "+1 or +0.5" for Evaluator#1 and 2 indicating that there was a positive correlation between Dermatologist and Evaluator#1 and 2. The value of Pearson correlation and Chi-Square p-value were complementary, giving the value of significance of the correlation between Dermatologist and Evaluator#1 and 2. Hence, it could be considered that two evaluators i.e. evaluator#1& 2 were Dermatologist Trained and Validated Evaluators for performing and reviewing the microscopic slides of the pluck test(Table 9).

Phototrichogram evaluations for Hair Density, Thickness, Hair Growth Rate: All pairs p-values were >0.05 (except pairs 15

and 17) that shows that there was no statistically significant difference in the mean value of evaluator (Tables 10 and11). From pair #1 it was concluded that mean values of Hair Growth for evaluator#1 and evaluator#2 were equal. Similarly for the other two pairs. P-value >0.05 means that there was no statistically significant difference in the two means which showed that they all 3 were performing and taking phototrichogram measurement with minimal variability.

Secondary Outcomes

There were no adverse events or serious adverse events reported during the conduct of the study. In addition to that, none of the subjects experienced erythema, allergic reactions, folliculitis, oiliness, burning, and boils on the scalp during the course of the study.

Conclusion

An accurately performed 60-second hair count is a simple, practical, and reliable tool for the monitoring conditions associated with hair shedding. In addition, clinical scoring, hair pull test, and hair pluck test are standard methods to screen the study population for hair loss stage and activity with regards to inclusion and exclusion criteria of a hair investigation study. The validation method of phototrichogram can be useful for testing new hair loss treatments and quantitatively evaluating hair growth in the same spot over the long term. Our study established the standards to perform test methods and evaluations with the least minimal variability for Phototrichogram and statistically significant correlations between the Dermatologist's Trained evaluator for the Pull Test, and statistically significant correlation between the dermatologist and Dermatologist's Trained Evaluators' to confirm that the designated Evaluator is found Dermatologist Trained and Validated to perform the methods efficacy claims substantiation for Hair Growth products. From this In-House standardization & validation study, we set a new benchmark for Hair Care – Hair Growth Products Safety and Efficacy studies which can produce consistent, compatible, accurate, qualitative, and reproducible results, controlled testing methods for a successful performance of a clinical hair care trial. Any single method of validation is neither 'ideal' nor feasible therefore here we studied different techniques.

However, when interpreted with caution; these are valuable tools for patient diagnosis and monitoring. This study had limitations including the small sample size. As per future perspectives, our study will assist the postgraduate scholars as well as practitioners in their field of research and dermatology. Furthermore, the study with the larger sample size, longer duration, and wider population range in a comparative study design can add up more scientific value to our clinical study.

Table 1 Demographic and baseline characteristics

Gender		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Female	6	50.0	50.0	50.0
	Male	6	50.0	50.0	100.0
	Total	12	100.0	100.0	

Table 2 Descriptive statistics of subjects

Parameters	Subjects (N)	Minimum	Maximum	Mean	Standard Deviation
Height (Cm)	11	157.00	181.00	167.91	9.18
Weight (Kg)	11	46.00	103.00	62.67	15.96
Age	11	19.00	37.00	25.73	6.72
Valid N (listwise)	11				

Note: In this study, there were 6 Males and 5 Females; age of the subjects ranged from 19 to 37 years with the average being 25 years. N=subjects taken.

Table 3 Assessment schedule

Phase	Screening, Enrolment, Evaluations	Evaluations
Day (visit)	Day 1 (Visit 1)	Day 4 (Visit 2)
Details of the Events		
Pre-Entry Inquiry for COVID-19 Exposure history and signs/symptoms	X	X
Informed Consent	X	
Demography, Medical History	X	
Inclusion/Exclusion	X	
General Appearance of Hair	X	
60-Second Hair Count – Hair Combing Method	X	
Hair Pull Test	X	
Trichogram – Hair Pluck Test	X	
Phototrichogram – (Tattoo Method)	X	
Hair Growth Measurement	X	X
Hair Thickness, Density and Scalp Condition		X
Digital Photographs (As applicable)	X	X
Well-being / Adverse Events	X	X
Concomitant Medication	X	X

Note: X represents the test performed on that day.

Table 4 General Appearance of Hair - Statistics and Correlation

Evaluator	Correlation with Dermatologist - AM (Chi-Square P Value)					
	Hair Volume	Hair Density	Hair Reflexion	Hair Plasticity	Hair Shininess	Hair Smoothness
Evaluator #4	0.0002	0.0030	0.0022	0.0182	0.0002	0.0001
Evaluator #1	0.0016	0.0083	0.0079	0.2000	0.0016	0.0006
Evaluator #2	0.0016	0.0083	0.0079	0.2000	0.0302	0.0890
Evaluator #3	0.0004	0.0083	0.1667	0.2000	0.0302	0.0317

Table 5 Scoring of Hair and scalp condition

Scalp assessment by evaluator and dermatologist	N	Minimum	Maximum	Mean	Standard Deviation
Evaluator#4 -Scalp Skin Redness	11	0.00	0.00	0.00	0.00
Evaluator#4- Scalp Skin Roughness	11	0.00	1.00	0.18	0.40
Evaluator#4 -Scalp Skin Scaliness	11	0.00	0.00	0.00	0.00
Evaluator#3 -Scalp Skin Redness	10	0.00	0.00	0.00	0.00

Evaluator#3 -Scalp Skin Roughness	10	0.00	1.00	0.20	0.42
Evaluator#3- Scalp Skin Scaliness	10	0.00	0.00	0.00	0.00
Evaluator#1 -Scalp Skin Redness	10	0.00	0.00	0.00	0.00
Evaluator#1 -Scalp Skin Roughness	10	0.00	1.00	0.20	0.42
Evaluator#1 -Scalp Skin Scaliness	10	0.00	0.00	0.00	0.00
Evaluator#2 -Scalp Skin Redness	10	0.00	0.00	0.00	0.00
Evaluator#2-Scalp Skin Roughness	10	0.00	1.00	0.20	0.42
Evaluator#2 - Scalp Skin Scaliness	10	0.00	0.00	0.00	0.00
Dermatologist- Scalp-Skin Redness	11	0.00	0.00	0.00	0.00
Dermatologist- Scalp Skin Roughness	11	0.00	1.00	0.18	0.40
Dermatologist- Scalp Skin Scaliness	11	0.00	0.00	0.00	0.00
Valid N (listwise)	8				

Note: where N represents number of volunteers

Table 6 60 Seconds hair combing method

	N	Minimum	Maximum	Mean	SD
Dermatologist	11	2.00	88.00	37.18	33.70
Hair Fall Count of Evaluator#1	10	2.00	110.00	40.10	42.08
Hair Fall Count of Evaluator#2	10	0.00	82.00	26.00	26.52
Hair Fall Count of Evaluator#3	10	2.00	73.00	28.90	24.63
Valid N (listwise)	8				

Note: where N represents numbers of volunteers, SD-standard deviation

Table 7 hair fall count pearson correlation

Hair Fall Count		
Evaluator	Correlation with Dermatologist	
	Pearson Correlation	P Value
Evaluator#1	0.991	0.001
Evaluator#2	0.901	0.001
Evaluator#3	0.981	0.001

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 between the Dermatologist (AM) and all three evaluator and these correlations are highly significant for all three evaluator.

Table 8 Pull test method

		Mean	Subjects (N)	Standard Deviation	Standard Error Mean
Pair 1	Pull Test of Evaluator#1	3.11	9	2.47	0.82
	Pull Test of Evaluator#2	2.44	9	2.01	0.67
Pair 2	Pull Test of Evaluator#1	3.22	9	2.54	0.85
	Pull Test of Evaluator#3	2.44	9	3.13	1.04
Pair 3	Pull Test of Evaluator#2	2.11	9	1.96	0.65
	Pull Test of Evaluator#3	2.33	9	3.16	1.05

Table 9 Pluck Test Method

	Pearson Correlation	p-Value	Subjects (N)
Dermatologist (AM) (Evaluator#1)Total No Of Hair Count	0.96	0.0000	10
Dermatologist (AM) (Evaluator#1) Total No Of Anagen Hairs	0.94	0.0001	10
Dermatologist (AM) (Evaluator#1) Anagen Hairs With Sheath	0.92	0.0001	10
Dermatologist (AM) (Evaluator#1) Anagen Hairs Without Sheath	1.00	0.0000	10
Dermatologist (AM) (Evaluator#1) Total No Of Telogen Hairs	0.72	0.0196	10
Dermatologist (AM) (Evaluator#1) % Of Anagen Hairs	0.61	0.0606	10
Dermatologist (AM) (Evaluator#1) % Of Telogen Hairs	0.61	0.0620	10
Dermatologist (AM) (Evaluator#1) Ratio (Anagen/Telogen)	0.34	0.5126	6
Dermatologist (AM) (Evaluator#2) Total No Of Hair Count	1.00	0.0000	10
Dermatologist (AM) (Evaluator#2) Total No Of Anagen Hairs	0.96	0.0000	10
Dermatologist (AM) (Evaluator#2) Anagen Hairs With Sheath	0.87	0.0010	10
Dermatologist (AM) (Evaluator#2) Anagen Hairs Without Sheath	0.74	0.0138	10
Dermatologist (AM) (Evaluator#2)Total No Of Telogen Hairs	0.71	0.0211	10
Dermatologist (AM) (Evaluator#2) % Of Anagen Hairs	0.49	0.1462	10
Dermatologist (AM) (Evaluator#2)% Of Telogen Hairs	0.49	0.1462	10
Dermatologist (AM) (Evaluator#2) Ratio (Anagen/Telogen)	0.91	0.0044	7
Dermatologist (AM) (Evaluator#3) Total No Of Hair Count	1.00	0.0000	10
Dermatologist (AM) (Evaluator#3) Total No Of Anagen Hairs	0.98	0.0000	10
Dermatologist (AM) (Evaluator#3) Anagen Hairs With Sheath	0.91	0.0003	10
Dermatologist (AM) (Evaluator#3) Anagen Hairs Without Sheath	0.90	0.0003	10
Dermatologist (AM) (Evaluator#3) Total No Of Telogen Hairs	0.87	0.0012	10
Dermatologist (AM) (Evaluator#3) % Of Anagen Hairs	0.87	0.0012	10
Dermatologist (AM) (Evaluator#3) % Of Telogen Hairs	0.17	0.6438	10
Dermatologist (AM) (Evaluator#3) Ratio (Anagen/Telogen)	-0.36	0.3811	8

Table 10 Phototrichogram test

	Subjects (N)	Minimum	Maximum	Mean	Standard Deviation
Hair Growth length Average (3Hairs) Visit-01 (µm) Evaluator#1	10	468.00	888.00	612.90	126.96
Hair Growth length Average (3Hairs) Visit-01 (µm) Evaluator#2	10	486.00	768.00	594.50	98.54

Hair Growth length Average (3Hairs) Visit-01 (µm) Evaluator#3	10	404.00	914.00	646.50	157.32
Hair Growth Length Average (3Hairs) Visit-02 (µm) Evaluator#1	10	1100.00	2357.00	1575.90	365.61
Hair Growth Length Average (3Hairs) Visit-02 (µm) Evaluator#2	10	1338.00	1958.00	1593.50	231.47
Hair Growth Length Average (3Hairs) Visit-02 (µm) Evaluator#3 (DJ)	10	1395.00	2049.00	1673.10	237.78
3 Days Hair Growth Rate (µm) Evaluator#1	10	527.00	1469.00	963.00	285.75
3 Days Hair Growth Rate (µm) Evaluator#2	10	612.00	1344.00	998.30	236.33
3 Days Hair Growth Rate (µm) Evaluator#3	10	776.00	1261.00	1026.60	150.61
Per Day Hair Growth Rate (µm) Evaluator#1	10	175.67	490.00	321.03	95.31
Per Day Hair Growth Rate (µm) Evaluator#2	10	204.00	448.00	332.77	78.78
Per Day Hair Growth Rate (µm) Evaluator#3	10	258.67	420.33	342.20	50.20
Hair Thickness (Visit-02) (µm) Evaluator#1	10	14.00	23.00	18.50	2.64
Hair Thickness (Visit-02) (µm) Evaluator#2	10	16.00	22.00	19.50	2.07
Hair Thickness (Visit-02) (µm) Evaluator#3	10	12.00	25.00	16.50	3.69
Hair Density (Visit-02) (sqcm) Evaluator#1	10	160.00	226.00	195.20	19.88
Hair Density (Visit-02) (sqcm) Evaluator#2	10	113.00	226.00	180.80	31.85
Hair Density (Visit-02) (sqcm) Evaluator#3	10	141.00	216.00	172.30	22.98
Valid N (listwise)	8				

Table 11 paired samples test for hair growth measurement

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Standard Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
Pair 1	Hair Growth length Average (3Hairs) Visit-01 (µm) Evaluator#1 - Hair Growth length Average (3Hairs) Visit-01 (µm) Evaluator#2	-12.11	53.90	17.97	-53.54	29.32	-0.674	8	0.519
Pair 2	Hair Growth length Average (3Hairs) Visit-01 (µm) Evaluator#1 - Hair Growth length Average (3Hairs) Visit-01 (µm) Evaluator#3	-48.11	118.40	39.47	-139.12	42.90	-1.219	8	0.258
Pair 3	Hair Growth length Average (3Hairs) Visit-01 (µm) Evaluator#2 - Hair Growth length Average (3Hairs) Visit-01 (µm) Evaluator#3	-41.56	134.09	44.70	-144.63	61.51	-0.930	8	0.380
Pair 4	Hair Growth Length Average (3Hairs) Visit-02 (µm) Evaluator#1 - Hair Growth Length Average (3Hairs) Visit-02 (µm) Evaluator#2	-66.00	238.98	79.66	-249.70	117.70	-0.829	8	0.431
Pair 5	Hair Growth Length Average (3Hairs) Visit-02 (µm) Evaluator#1 - Hair Growth Length Average (3Hairs) Visit-02 (µm) Evaluator#3	-120.22	239.31	79.77	-304.17	63.73	-1.507	8	0.170
Pair 6	Hair Growth Length Average (3Hairs) Visit-02 (µm) Evaluator#2 - Hair Growth Length Average (3Hairs) Visit-02 (µm) Evaluator#3	-41.33	268.47	89.49	-247.70	165.03	-0.462	8	0.656
Pair 7	3 Days Hair Growth Rate (µm) Evaluator#1 - 3 Days Hair Growth Rate (µm) Evaluator#2	-53.11	239.14	79.71	-236.93	130.71	-0.666	8	0.524
Pair 8	3 Days Hair Growth Rate (µm) Evaluator#1 - 3 Days Hair Growth Rate (µm) Evaluator#3	-72.11	204.65	68.22	-229.42	85.20	-1.057	8	0.321
Pair 9	3 Days Hair Growth Rate (µm) Evaluator#2 - 3 Days Hair Growth Rate (µm) Evaluator#3	-0.56	179.95	59.98	-138.88	137.77	-0.009	8	0.993
Pair 10	Per Day Hair Growth Rate (µm) Evaluator#1 - Per Day Hair Growth Rate (µm) Evaluator#2	-17.70	79.71	26.57	-78.98	43.57	-0.666	8	0.524

Pair 11	Per Day Hair Growth Rate (µm) Evaluator#1 - Per Day Hair Growth Rate (µm) Evaluator#3	-24.00	68.30	22.77	-76.50	28.50	-1.054	8	0.323
Pair 12	Per Day Hair Growth Rate (µm) Evaluator#2 - Per Day Hair Growth Rate (µm) Evaluator#3	-0.19	59.98	19.99	-46.29	45.92	-0.009	8	0.993
Pair 13	Hair Thickness (Visit-02) (µm) Evaluator#1 - Hair Thickness (Visit-02) (µm) Evaluator#2	-1.44	2.07	0.69	-3.03	0.15	-2.095	8	0.069
Pair 14	Hair Thickness (Visit-02) (µm) Evaluator#1 - Hair Thickness (Visit-02) (µm) Evaluator#3	1.56	2.88	0.96	-0.66	3.77	1.622	8	0.143
Pair 15	Hair Thickness (Visit-02) (µm) Evaluator#2 - Hair Thickness (Visit-02) (µm) Evaluator#3	3.00	3.28	1.09	0.48	5.52	2.745	8	0.025
Pair 16	Hair Density (Visit-02) (sqcm) Evaluator#1 - Hair Density (Visit-02) (sqcm) Evaluator#2	6.67	17.06	5.69	-6.45	19.78	1.172	8	0.275
Pair 17	Hair Density (Visit-02) (sqcm) Evaluator#1 - Hair Density (Visit-02) (sqcm) Evaluator#3	16.00	18.82	6.27	1.53	30.47	2.550	8	0.034
Pair 18	Hair Density (Visit-02) (sqcm) Evaluator#2 - Hair Density (Visit-02) (sqcm) Evaluator#3	3.22	16.81	5.60	-9.70	16.14	.575	8	0.581

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 between the Dermatologist (AM) and all three evaluator and these correlations are highly significant for all three evaluator.

Figure: 1 Hair counting 60s combing method

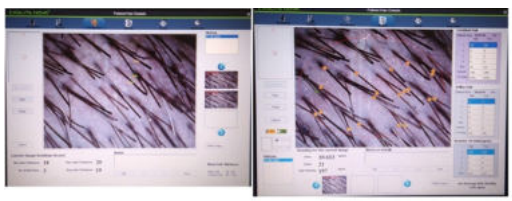
Figure 2 Hair pluck test

Figure 3. trichogram

Figure 4. Mid vertex area in men and woman

Figure 5. Hair growth measurement by phototrichogram

Figure 6. Measurement of hair thickness and hair density



REFERENCES

1. Price VH, et al. Treatment of hair loss. N Eng J Med 1999; 341 (13):964–973.
2. Courtois M, Loussouarn G, Hourseau S, Grollier JF, et al. Periodicity in the growth and shedding of hair. Br J Dermatol. 1996; 134:47–54.
3. Ross EK, Vincenzi C, Tosti A, et al. Videodermoscopy in the evaluation of hair and scalp disorders. J Am Acad Dermatol 2006; 55: 799–806.
4. Wasko CA, Mackley CL, Sperling LC, Mauger D, Miller JJ, et al. standardizing the 60-second hair count. Arch Dermatol 2008; 144:759–62.
5. Shapiro J. Assessment of the patient with alopecia. In: Hair Loss, editor. Principles of Diagnosis and Management of alopecia. 1st ed. London: Martin Dunitz Ltd; 2002.
6. Guarrera M, Semino MT, Rebora A, et al. Quantitating hair loss in women: a critical approach. Dermatology. 1997; 194(1):12-16.
7. Saitoh M, Uzuka M, Sakamoto M, et al. Human hair cycle. J Invest Dermatol 1970; 54:65-81.