

# Hair Care Objectivation

Hair in Beauty represents a wide field of investigation for both devices manufacturers and testing laboratories to serve the ingredients and personal care industry. The hair care global market, more and more inclusive is regularly recording an annual growth of 3% and now exceed the \$ 100 billion. While Beauty may be a largely female-focused industry, the men may be particularly attached to their hair look. Hair is a big part of men or women identity worldwide and, as a culture, they are giving some importance on the look and style of their hair.

**The health treatment of the scalp and the hair** forms an integral part of the daily Beauty routine. The 3 objectives of this regimen are mainly:

- Maintain the good condition of this unique scalp-hair ecosystem considering the internal triggers (stress, diet...): Exfoliation, Moisture, sebum, sweat, microbiota (Malassezia), acidic pH
- Cure and protect it from external aggressions such as UV, heat, and various pollution...
- Make the hair more attractive and change its style and appearance.

Consumers are **seeking new ideas for bettering their hair grooming rituals**, in the form of products that are at once **natural**, **ethical**, **and sustainable** but maintain their cleansing and caring benefits.

Shampoos, conditioners, colouration, permanents, styling gels, offer various ways to follow the hairstyle trends. The ingenuity of the cosmeticians makes possible so many organoleptic designs: shampoo, soap, serum, oil, mask, powder...

A multitude of benefits whatever is the type and ethnicity of hair: Care, Volume, Anti-Frizz, Smoothing, Flat Hair, Repair, Gloss, Shine, Colour, Fine Hair, Curly Hair, straightening effect, Restore, Anti-Sebum, Anti-Dandruff, UV Protection, Anti-Breakage, Moisturising... Hair strength is considered as one of the primary indicators of its health.

**Clinical, use tests and biometrological studies** play a key role in R&D for hair and scalp grooming to evaluate their efficacy and safety and improve hair care beauty products or scalp treatments to market. Finding the

relevant method and the experienced testing partner, and designing the reliable protocol is the ordinary challenge of main testing managers and formulators.

The various biometrological protocols dedicated to fibres and hair testing are focusing different targets: the scalp, the cuticle, the cortex, the hair structure and mechanisms or the growth and can be implemented under controlled conditions of temperature, hygrometry and humidity (Bossa Nova Humidity Chamber) ...

- 1. Fibre physical aspects and swelling Analysis: hair cross-section by dimensional Analysis
- 2. Mechanical analysis for suppleness, flexibility, break resistance, smoothness: with tensile, Stress Relaxation, Fibre Bending, Torsional Analysis, Combing Analysis (Wet or Dry), Friction, Curl Compression, Three Point Bend, Global hairstyling, Anti-Frizz, Volume, Curls, Bending Force, Remain Hold, Flexible Hold, Combability, Shine: Image Analysis, Hair tune...
- 3. Combing and abrasion of the hair: Friba.one, Sirtaki (Bossa Nova)
- 4. Hair volume: 3D reconstruction of a Bolero (Bossa Nova)
- 5. Straightness: hair orientation with the Rumba (Bossa Nova)
- Colour and shine and permeation, Colour Fade, bleaching test using: Goniolux (Orion), GlossyMeter (C+K), SkinColorCatch & SkinGlossMeter (Delfin), Samba Hair, Mambo, Salsa, (Bossa Nova), ColorFace & SpectraCam (Newtone Technologies), C-Cube (Pixience),
- **7.** Hair structure and permeability: infra-red microscopy, Confocal microscopy, Scanning electron microscopy, Optical multiphoton tomography, Atomic Force Microscopy ...
- 8. Specific claims:
  - **Moisturizing:** MoistureMap & Corneometer(*C+K*), Dermalab Hydration, Aquaflux & Epsilon (*Biox*), MoistureMeter SC, MoistureMeterEpi, MoistureMeterD, (*Delfin*),
  - Barrier Function: Vapomerter (Delfin), Aquaflux (Biox), ...
  - Soothing: Tivi 700 & Tivi 8000 (Wheesbridge), Thermographie infrar-red, Neurometer ...
  - Anti-sebum: QuantiSeb, DermaLab Sebum, Sebumeter (C+K), SebumScale including sebum analysis: Shotgun mass spectrometry, Metabolimic MS/MS-16srDNA-PCR (Phylogene), Raman microscopy...
  - Anti-Dandruff by gravimetry and imaging, DandruffMeter (C+K), ...
  - **Scalp surface:** VisioScan (*C+K*),, Antera 3D (*Miravex*), C-Cube (*Pixience*), Skin Damage Vizualizer and all dermoscopes...
  - Hair loss and hair growth by phototrichogramm.

As a complementary point of view to the biometrological evaluation, other protocols including the scorage by dermatologists or hairdressers, the insight of consumers via questionnaire of lifestyles, the sensory analysis and emotions studies are highly recommended.

Moreover, the today inescapable **anti-pollution claims** which is also a big request for hair protection can easily be added to this list. Hair must resist to the environment for four to six, seven and even eight years, compared to skin, which is usually renewed in 28 days. There is some evidence to consider the impact of the environment like cigarette smoke, gases, water, and sun on the hair.

The anti-pollution on human objectivation can be summarised as in situ in polluted metropolis or under standardised pollution conditions (smoke, particles...) with the quantification of the protection effect by:

- Heavy metal Analysis: HPLC
- Particles adhesion visualization: Dermoscopes
- **Lipidic peroxidtion** & Proteins oxidation: biochemical analysis, Carbonyl test Squalene, Monohydroperoxide (SQQOH), Malondildehyde (MDA)
- Omics analysis: MS/MS-16srDNA-PCR genomics, proteomics, transcriptomics, metabolomics.

The new digital era influences undoubtedly the beauty lifestyle **and the personalisation** of the hair care experience. It is propelling hair salons into the digital age using hair diagnostics and augmented reality solutions such as L'Oreal's StyleMyHair, Wella's Colour DJ (Coty) or Schwarzkopf's SalonLab Apps, Choicify (Henkel). These new uses are in the heart of the relationship between the brands, the personal care products, and the consumers. It gives the opportunity, in a 3 in 1 way, to **analyse** the fibre and scalp, make a **diagnosis** and **personalise** hair care treatment on-site based on a client's hair data. The development of the app uses also helps the brands to collect data and insights of both hairdressers and consumers. It strengthens the trade marketing management with the hairdresser and enables to improve the safety of clients and professionals and the efficacy of these products.

The major issue of this beauty category is to answer the needs to combat both intrinsic and external damages (mechanical, thermal, or chemical treatments) and protect from the environmental conditions. To substantiate all claims there are numerous and various protocols analysing at both microscopic and global scales the hair and the scalp. **The preliminary discussions with each CRO's to design the protocol to answer the aim** of the study is crucial to select the best method adapted to the claim substantiation. No need to split hairs anymore!

# Easily find the methods and testing labs to substantiate the Hair Care

- For the *in-vitro* efficacy tests: "Preclinical Testing Platform"
  - For the clinical tests: <u>"Clinical Testing Platform"</u>

Warning Connection: You need to subscribe twice for both platforms

# Our Partners have the floor

We are glad to introduce the several topics presented by our partners

- Expertise of Hair Care Products Evaluation by <u>SP Equation</u>
- <u>Eurofins Cosmetics & Personal Care</u>, your key partner to design complementary strategies to evaluate performance and safety for hair care products
- Innovative Solution to Evaluate Color and Erythema on Scalp with C-Cube by <u>Pixience</u>
- Shampoo bars: clinical studies on tolerance and effectiveness with Intertek
- Characterize Microbiota Metabolism on Scalp with Phylogene
- Hair care products: which method suits your claim by <u>PhDTrials</u>
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- <u>CIDP</u> offers a Wide Range of Hair Care Tests in Ex Vivo And In Vivo Models
- Is hair care the new skin care? Use of "classic" biophysical methods for hair & scalp claims by <u>Courage & Khazaka</u>
- Haircare products: from ex vivo to clinical testing by Complife Group
- Hair Water Retention and Water Content Measurement by <u>Biox</u>
- Claim Substantiation of Hair and Scalp products by Princeton Consumer Research
- Human hair follicle and scalp skin organ culture: highly clinically relevant models for testing your hair care product by <u>Monasterium Laboratory</u>



# Expertise of Hair Care Products Evaluation by SP Equation

Pascal STERLE - CEO

**SP Equation**, **a specialist in in vitro instrumental evaluation in the field of hair**, offers its customers personalized tests that match with their needs and claims, especially for Hair Care products. Maintenance of the **shine** provided by dyeing products, **protection of hair** from the harmful effects **of ultraviolet**, **repair of degradations** provided by dyeing products, .... are all claims that SP Equation can help its customers support.

With more than **25 years of experience in the field of hair and instrumental evaluation**, **SP Equation** supports its customers in highlighting their actives or formulas, selecting the type of hair best suited to the tests to be performed, and evaluating the performance of products using modern techniques, easily exploitable and recognized:

#### **Brightness & Color Evaluation**

- Evaluation of **the brightness** of hair-on-hair strands thanks to the **Samba Hair** which uses polarized light to obtain two images of the wick: a specular image and a diffuse image, and thus make it possible to calculate a **brightness index** (called Luster BNT).
- Protection of the color to successive washes provided by Hair Care products (shampoos / conditioners / masks or coupling of products). This evaluation is carried out by measuring the color of colored strands before and after successive treatments thanks to a spectrocolorimeter adapted to hair strands.
- **UV color** protection. This evaluation is implmented by measuring the color of colored strands before and after irradiation thanks to an Alpha **Xenotest.** A system allowing to alternate insolation and water spraying can also allow the **protection of the color against weather** (sun / rain).

#### **Objectivation of the Mechanical Properties of Hair**

- Straightening effect or volume input, anti-frizz effect of hair products with the help of **Bolero.** This equipment allows, thanks to a camera, to take pictures of a strand of hair that rotates on itself in front of a light background, then to convert them into grayscale. It analyzes them by thresholding to differentiate the body of the strands and frizz
- Evaluation of the mechanical properties of hair (Young's modulus, Extension to breakage, Stress to break) thanks to the Micro-Tensile Tester MTT675 extensometer coupled to the FDAS (Fibre Dimensional Analysis System). This technique evaluates the impact of hair treatments on the mechanical characteristics of the treated fibers. It makes it possible to evaluate the thermal protection provided by hair products or equipment such as straightening irons.
- **Resistance to bending and abrasion** of hair treated with different hair products and after an overlay of treatments. This characterization is done using the **Fibrestress**, equipment intended for the textile industry, whose technology has been adapted to the hair.
- **Protection of fibers from the harmful effects of UV.** This technique enables the characterization of the photoprotection of hair provided by a hair product using the Alpha **Xenotest** for hair insolation. The **Micro-Tensile Tester MTT675 extensometer** evaluates the evolution of the Young's module, characteristic of the degradations observed.

#### **Combing Quantification**

- **Combing breakage test.** This evaluation technique determines the number of **broken hair** after a significant number of combing thanks to **the Sirtaki.** It allows to characterize the ability of a treatment to limit hair breakage to combing
- The evaluation of the ease of combing (or combingability) provided by hair treatments is done on wet hair, it characterizes the "combing force" of strands of hair before and after treatment. This technique uses the Micro-Tensile Tester MTT175 extensometer.

SP Equation, concerned about the satisfaction of its customers, adapts all these protocols to the specific needs of its customers and continues its research to offer ever more innovative tests!

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Discover SP Equation profile and instrumentation on the Clinical Platform: SP Equation



# **Personal Care**

# **Eurofins Cosmetics & Personal Care**, your key partner to design complementary strategies to evaluate performance and safety for hair care products

Dr. Clotilde FOURAULT - Medical Investigator & Geraldine Provost - R&D Project Manager

Healthy-looking hair is an essential part of an overall healthy appearance, influenced by daily routine. Complementary strategies are required to evaluate the performance and safety of haircare products.

# Hair locks testing

Testing hair tresses allows an objective and repeatable evaluation of the haircare product's efficacy on hair fibre.

The use of standardised hair tresses adapting to different types of hair (Caucasian, Asian, Brazilian, African hair, straight hair, wavy or curly, etc.) allows claims to be objectified according to the product's target market. Moreover, treatments can be performed before the product application to weaken hair in order to evaluate its efficacy on sensitised fibre.

Otherwise, technicians' teams are trained on how to ensure repeatability. During analyses, tresses are conditioned in a controlled temperature and hygrometry environment. The different treatments are successively performed, which reduces the time requirement, in comparison to a study on volunteers. These methods, for which the repeatability and the reproducibility are validated during the R&D phase, are performed on a sufficient number of tresses in order to be able to perform robust statistical analyses which contribute to discriminating products, in comparison to a target or a reference, and support claims.

Working with standardised tresses also allows protocols to be adapted in order to better answer to market trends such as water-saving products. For instance, our laboratories developed a protocol to evaluate the reduction of water quantity during product rinsing. This method is applicable to both liquid and solid products, more and more popular among consumers.

# **Clinical testing**

In terms of tolerance, the in-use test is performed under dermatological control, alongside a cosmetic quality questionnaire, on the targeted population (oily hair, dry hair, weakened hair, dandruff, etc.).

In terms of efficacy, good practices for haircare product assessments are based on:

- A panel adapted to the product claims, depending on whether these concerns the scalp (oily, dry, sensitive or even "irritated", with dandruff, alopecia, etc.) or the hair, and in particular the roots, lengths or tips (dry, "tired", thinned, brittle, damaged, split ends, coloured or highlighted, permed, curled, straightened, etc.)
- A protocol specifying the product conditions of application, i.e. on the whole head, half the head under the control of a hairdresser, or on shaved mini-zones of the scalp. The evaluation methods are then specified: clinical scoring of targeted items (dandruff, hair mass, hair colour, shininess, softness, hydration, etc.), self-evaluation of suitable items (scalp itching, stinging, heating, hair detangling, etc.), instrumental measurements (corneometry, trans epidermal water loss, sebumetry, D Squam, phototrichogram, etc.), samples for analysis (hair and scalp, hair for scanning electron microscopy, squames, microbiota, etc.), and statistical analysis. Illustrative pictures and videos of the experiment carried out by the hairdresser complete the study.

The protocols are continuously adapted in order to meet ever-changing trends. Hair loss can significantly impact self-esteem and performant products may help. Their evaluation is based on clinical scoring (hair mass, volume, etc.), self-evaluation by the subjects (hair loss, hair mass, etc.), phototrichogram (photos of shaved minizones, counting of anagen, telogen and total hair density, Tiff Counting method) and illustrative pictures.

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# Innovative Solution to Evaluate Color and Erythema on Scalp with C-Cube by <u>Pixience</u>

#### Romain VIE – Technical Sales Engineer

French leader in digital dermoscopy, Pixience works closely with dermatologists and researchers to develop high quality products for skin and hair imaging and analysis. We strive to bring you innovative instruments that are accurate, reliable and consistent. This is the reason why 50+ dermo-cosmetic laboratories, CROs, and universities around the world have chosen our products for their product efficacy studies.

More than a mere dermoscope, <u>C-Cube Clinical Research</u> – Or C-Cube CR -- is the most versatile instrument for color and surface analysis, specifically designed to be used in dermatology and cosmetic clinical trials. Its patented glossless lighting and exclusive color calibration turn each of its 10 million pixels into an actual color measurement. It also works as a <u>handheld 3D scanner</u> with micrometric resolution, allowing quantitative analysis of the smallest changes in microrelief. With these exclusive features, C-Cube Clinical research can be used to evaluate a variety of product effects: anti-aging, sun protection, moisturizing, pores and seborrhea, blemish and pigmentation, and of course, haircare.

You may use C-Cube CR's color evaluation feature on a strand of hair to get an instant measurement of its color, expressed in the CIE Lab color space. Repeat the procedure over time on a strand of dyed hair, and you can accurately evaluate the **dye resistance**. Similarly, evaluate the **dye's coverage** and **evenness** by studying the color's variance, which C-Cube Clinical Research measures automatically.

You can also evaluate the **scalp's health** by measuring the inflammation or desquamation of the skin: Part the hair to reveal the scalp and quickly capture a few images. Then use our analysis tools to target the skin – while excluding the hair – to quantify the redness, that is characteristic of **erythema or inflammation**. You can also take a sample of dandruffs with a sticky patch and take pictures of the patch with the C-Cube, to then automatically quantify the number of dandruffs, and therefore the **scalp's dryness**.

Are you studying hair growth and hair loss treatments? You can use C-Cube CR for **phototrichogram** protocols and quickly obtain **hair density** and **average growth** measurements. Using <u>Pixience Cloud</u>, our externalized image analysis platform for C-Cube images, you have access to <u>detailed statistical analysis of</u> <u>phototrichograms</u>, performed by our experts.

In summary, C-Cube CR is an incredibly versatile instrument designed to help you with cosmetic product efficacy analysis. It offers relevant use-cases for haircare product evaluation, in addition to its dozens of other applications for clinical evaluation of the skin.

We invented C-Cube Clinical Research to bring you unique observations and convincing illustrations, and to offer an innovative alternative to the many single-application probes and instruments you are used to work with. Soon you may include the C-Cube in most of your studies.

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# Total Quality. Assured.

# Shampoo bars: clinical studies on tolerance and effectiveness with Intertek

Lisa ISENMANN - Operational Marketing Assistant

Shampoo bars are a growing trend for consumers who are looking for alternatives to hair care products which are packaged in plastic bottles and to reduce their impact on the environment.

Intertek, through its network of clinical experts and professional hairdressers, can assist you in conducting tolerance and efficacy studies for your new shampoo bar products:

- Scalp examination: dandruff, erythema, dryness, greasy scalp
- Functional signs: tightness, itching, tingling
- Shine of the hair and hydration of the scalp
- Rinseability and more

#### For all types of hair products, we can assist you in your clinical studies:

#### Anti-hair loss effect:

Our technicians study hair growth uses a **phototrichogram technique to support Anti-hair loss effect claims** 

This approach allows us to measure different parameters of hair growth: total density, the ratio of anagen to telogen hair, percentage of telogen hair, speed of growth, etc. The phototrichogram is performed on a fixed area of the scalp, where the hair is shaved before a first macrophotograph. A second macrophotograph made 2 days later allows to differentiate anagen hair from telogen hair.

To evaluate the effectiveness of an anti-hair loss product, we propose 2 methods:

- **The classic method:** taking photographs with the Canfield Epiflash followed by hair counting by our technicians,
- **The automated method:** taking photographs with the Fotofinder Dermascope followed by hair counting with the Trichoscale software.

#### Anti-dandruff effectiveness

To test the effectiveness of your products, our technicians evaluate the amount of dandruff of volunteers before and after using your products. This evaluation is visual (scoring), achieved using scales.

The effectiveness of an anti-dandruff product is demonstrated when the dandruff score decreases significantly at the end of the study, compared to the score before use. Samples from the dandruff areas of volunteers (Dsquams, swabs...) can also be taken for analysis in our laboratory.

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# Characterize Microbiota Metabolism on Scalp with Phylogene

Gilbert SKORSKI - CEO

Romuald ARNAUD - Business Development Manager

Skin including the scalp surface is the largest organ to repel attacks from external agents and functioning as both a physical and immunological barrier, performing a wide range of innate and adaptive immune functions. Its glandular nature and bacteria rich composition are making it prone to inflammation and infections. Dandruff is a recurrent chronic scalp disorder, affecting a large majority of the population worldwide (1). Several scalp microbiome studies from different populations have revealed the association of dandruff with bacterial and fungal dysbiosis (1, 2). Propionibacterium acnes and Staphylococcus epidermidis emerged as the core bacterial species, where the former was associated with a healthy scalp and the latter with dandruff scalp. Along with the commonly occurring Malassezia species (M. restricta and M. globosa) on the scalp, a strikingly high association of dandruff with yet uncharacterized Malassezia species was observed in the core mycobiome (2).

Eventhough the usage of high throughput next generation sequencing with associated bioinformatics has paved the way for better understanding of scalp-related disorders, the functional role of scalp microbiota in scalp disorders and health remains poorly explored and documented. Metagenomic analysis such as 16s ribosomal gene sequencing provided so far, tremendous insights into taxa's ecosystem. Metaproteomic analysis could unravel functional interactions between microbiota and hosts, paving the way for understanding the mechanisms of action underlying the pathophysiology of scalp-related disorders.

Omics with associated bioinformatics are the latest technologies to fill the gap in host / microbiome studies.

With PHYLOGENE it is now possible to characterize and understand microbiota metabolism on skin and scalp, starting just from our swabs.

High-resolution nano LC-MS/MS quantitative proteomics and HolXplore<sup>™</sup> data processing: <u>The efficient</u> tool for discovery. Bacterial 16s rDNA and fungal ITS sequencing with OTU based estimation to investigate microbial communities' diversity and determine taxonomic composition.

1- Longitudinal study of the scalp microbiome suggests coconut oil to enrich healthy scalp commensals, Rituja Saxena et al, Nature Portfolio 2021

2- Comparison of Healthy and Dandruff Scalp Microbiome Reveals the Role of Commensals in Scalp Health, Rituja Saxena et al, Front. Cell. Infect. Microbiol., 04 October 2018

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# Hair care products: which method suits your claim by **PhDTrials**

Pedro CONTREIRAS PINTO - PhD, MSc

Among the cosmetic market, the search and use of cosmetics for hair care have been consistently increasing and becoming a theme of great interest, which raises the question: how to evaluate and assess the effect of hair care products?

Several methods are available to support the efficacy evaluation of hair care products and is very important to understand which method is more suitable for a claim, from clinical testing to hair locks.

A wide range of methodologies, available in **PhD Trials**® Hair Dynamical Lab., such as imaging and dynamics analysis, like confocal microscopy or trichoscan enables structure analysis and hair loss and hair density assessment.

Moreover, biophysical, and mechanical techniques allow to assess several properties of hair like resistance to breakage, volume (anti-frizz properties), conditioning properties or shinning.

On the other hand, the hair protection became the new trend on hair care, therefore methodologies to assess hair protection against temperature (thermal resistance), radiation, chemicals or anti-pollution assessment turn out to be key points on hair evaluation.

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# Innovative solutions for haircare product evaluation by <u>Newtone</u>

Matthieu JOMIER – Chief Operating Officer

Nowadays, the evaluation of hair products is increasingly important to understand the effects of active ingredients on the hair itself but also on the scalp.

To address these issues, Newtone has developed innovative solutions based on image processing and analysis to evaluate a set of relevant parameters.

While all measurements, such as color, shine, volume, frizz effect, particles deposition, can be done with different technical approaches (polarized camera, optical microscopy, or SEM) in-vitro on strands, it is now possible to perform these assessments in-vivo on the entire hair or scalp directly on the subjects.

It is then possible, from a set of acquired images of the hair to analyze the following parameters:

- Color and homogeneity
- Hair fibers orientation
- Hair messiness
- White/Gray hair evaluation
- Hair growth
- Itching of the scalp

Using a multi-camera system, it is then possible to analyze the volume of a hair in-vivo to evaluate for example the volumizing effect of a product. It is also possible to assess hair messiness by analyzing the orientation of each hair fiber.

Newtone has also developed a macroscopic camera (SkinCam) that allows a high-resolution image capture on a surface of 20x20mm on the scalp and hair. Initially developed for skincare evaluation this solution has been extended and validated for hair and scalp measurements.

Thanks to proprietary algorithms, measurement of the scalp color (L\*a\*b\* values) over time is now possible from the acquired images to evaluate itching of the scalp as well as detection of the dandruff number and size. Using the same acquisition system, it is also possible to evaluate the hair growth or hair density on different sites of the scalp.

Another innovative technique consists in using a specific ATR-FTIR spectroscopy probe to measure several sites on the scalp. With a single acquisition, several parameters can be measured such as:

- Sebum level
- Scalp hydration
- Product/particles deposition

Based on calculated values at specific wavelengths from the acquired infrared spectrum, color maps can be generated on a 3D model of the scalp to show the differences between measurement sites. You can then visualize the real effect of your product directly on the scalp with specific color scales.

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Discover Newtone profile and instrumentation on the Clinical Platform: Newtone



# <u>CIDP</u> offers a Wide Range of Hair Care Tests in Ex Vivo And In Vivo Models

Florence FOURNIER – Client Relationship

As an international CRO with over 17 years of experience in hair care tests, CIDP, has developed different methodologies to substantiate innovative claims for hair care products. With a highly qualified and GCP and GLP trained team of biologists, dermatologists, and hairdressers, CIDP proposes various *ex vivo* and *in vivo* tests on hair care. In addition, owing to its strategic location in cities like Rio de Janeiro and New Delhi, CIDP has access to the biggest cosmetics and hair care consumer markets in the world thereby easing the recruitment of volunteers of skin phototype II to V with different types of hair and particular lifestyles and habits.

Amongst the *Ex vivo* models, CIDP proposes various assays on natural hair of different ethnic origins (Asian, African, Indian and Caucasian) to substantiate claims such as fibre resistance, brushing efficacy, shine, color lasting, hair growth or fall. Furthermore, the hair strands can be exposed to external stressors such as UV, air, and water pollution and assessing different biomarkers such as Malondialdehyde (MDA), free fatty acids, the melanin and protein content, and Tryptophan degradation.

In terms of *In vivo* or clinical evaluation CIDP proposes various medical scales (dandruff, hair fall, hair breakage, hair shine), experts grading (elasticity, resistance, color efficacy, damage, repair, shine, straightening efficacy) and consumer's evaluation including quality of life questionnaires. High-definition imaging with standard repositioning components is another tool that hair care specialist routinely incorporates in the study designs for substantiating clinical claims and providing images that can be used for marketing purposes.

CIDP has also set-up new Innovative involving real life sun exposure, real-life sea exposure, swimmingpool exposure, or even real-life sports activities to better support pioneering cosmetic claims. It allows offers the possibility for clients to combine both the *ex vivo* and *in vivo* techniques in a single study to derive the best results for their active ingredients or end products.

There are various testing services offered by CIDP for hair and cosmetic products and all the tests are tailored as per the client's needs to focus on specific claims of products and/or product kits such as antipollution, damaged hair from chemical aggression, color protection, hair shine and volume, breaking and hair fall, straightening procedures but also focus on upcoming claims requested by consumers such as "hair faint" (great straightening efficacy).

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# Is hair care the new skin care? - Use of "classic" biophysical methods for hair & scalp claims by <u>Courage & Khazaka</u>

Diana KHAZAKA – General Manager

Hair diversity (style, shape, growth pattern or color) is one of the most important features to define us physically.

Therefore, it is no surprise that the market of hair care products with a value of 93.5 billion US \$<sup>1</sup> (Statistica, September 2020) is one of the most important sectors in the complete area of cosmetic products.

Hair care products are developed with a vast amount of imaginable claims. A variety of easy-to-use analysis instruments will assist you in supporting all kind of hair claims.

# Testing hair and scalp in vivo

For the hairless skin, objective testing methods have been known for decades. Some of these "classical methods" have also been used on hair and scalp for some time.

When **sebum** is produced in a high quantity, it leads to greasy hair and scalp problems that cause increased microbial activity and oily dandruff. Fine pollution particles cling to the oil, making the hair look dull and degrading the hair quality. To remove the oil and pollutants, frequent washing is required which will most notably dry out longer hair. Also, this might just trigger the production of more oil to compensate for the frequent removal – leading to a vicious circle. Therefore, the aim would be to balance the oil production to a moderate level or develop products with lipid-restoring properties. The **Sebumeter**<sup>®</sup> is not only the worldwide most used instrument to determine the sebum content of the skin but already in the 1980ies and the early 90ies, work has been presented about the usefulness of this device on hair and scalp.<sup>2,3</sup>

To reach the hairy scalp, a special adapter can be used together with the Sebumeter<sup>®</sup> foil and evaluate it photometrically.

**Hydration** on hair is more difficult to measure than on skin. A promising approach has been tested using capacitance imaging<sup>4</sup>. The **MoistureMap** device using this method could become an interesting tool for such tests.

**Hair colour** and **gloss** are "evergreens" among the claims around hair care. Easy and quick to use **Colorimeter** and **Glossymeter** are suitable to support the booming claims like brightening, luminosity, shine, colour intensity/lasting for hair.

The **pH-Meter** on hair and scalp plays an important role to support claims like "pH-neutral" or "sensitive".

The **barrier function** of the scalp is the key parameter in the safety of products applied on the head. It can be assessed by measuring the transepidermal waterloss (TEWL), the amount of water evaporating through the skin into the environment. The **Tewameter® Nano** (only 2 mm Ø), based on the worldwide most popular open "chamber method" has especially been developed for TEWL measurements on small and difficult to reach surfaces. TEWL measurements are also used as indirect measurements of the water content on the scalp.

Measuring the **firmness of the scalp** with the **Indentometer** is an interesting parameter in the research of hair loss.

#### **Imaging methods:**

The evaluation of the hair length and thickness in mm with the **Visioscan**<sup>®</sup> can be used for different claims in care, shaving and food (-supplements). In hair care and food/food supplements tests, hair length assessment can substantiate hair growth claims. Also, for assessing the quality of shaving, this measurement will be useful.

The DandruffMeter classifies dandruff in number and size.

The **Visiopor**<sup>®</sup> is a handy and efficient camera emitting special polarized UV-light to monitor occurring fluorescence (activity of acne bacteria). The amount, area and intensity of these fluorescent spots is automatically calculated and can be compared over the treatment time.

Is skin care the new hair care? Claims known in skin care for quite some time such as anti-aging, pollution defense, exfoliating scrub, vitamin-infused, collagen boosting become increasingly common in the hair care market as it continues to diversify beyond the standard shampoo, conditioner and styling aids categories.<sup>5</sup>

Or is hair care the new skin care?<sup>6</sup> Our hair is as individual as we are. The skin of the scalp is more permeable than the skin of our face. Personalized trends already known for a long time in skin care, are also gaining ground in hair care.

The same measurement methods used in the development and efficacy testing of hair care can be used in simplified forms at the various points of sale to lead the consumer through the jungle of products and find the individual suitable hair care range.

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# Haircare products: from ex vivo to clinical testing by **<u>Complife Group</u>**

Dr Vincenzo NOBILE - R&D Manager

The haircare products testing is a specialized testing involving the knowledge of both scalp and hair physiology and structure. Like all the studies in the cosmetic field, the hair care studies need experts, know-how and state-of-the-art techniques to be successful. In our laboratories, we have implemented standard and tailor-made *ex vivo* and clinical study protocols to assess the local tolerance and to give real proof of the product effects.

**Safety first!** This is true for all the cosmetic products, but this is even more true for hair care products. From simple epicutaneous patch testing to more complicate safety study protocols, our dermatologists are experienced in evaluating the safety of each hair care product taking in mind its peculiarities. Safety at Complife is not only scoring of the local tolerance reactions, but it is also instrumental measurement of the subclinical skin alterations. Among the others, we can also support the "no tears" claim.

When it comes to the **efficacy** assessment, the *ex vivo* and the testing on human volunteers' approach can be used to give proof of the products efficacy. Both of them can be designed to have instrumental or sensory parameters in relation to the purpose of the study, as follows: instrumental parameters are implemented when it is necessary to have a numeric parameter or a percentage variation before and after product use; while the sensory parameters are implemented when it is necessary to test the Consumer perception of the product efficacy or when it is necessary to fine tune the product cosmetic acceptability with the Consumer habits.

The *ex vivo* testing approach - on human hair locks - is recommended to demonstrate the products effects on the hair shaft for products affecting hair colour, shape, structure, and its physical-mechanical properties. The main advantage of testing on hair locks is related to the wide choice of specific hair characteristics (e.g. colour, texture, etc.) and also to the simulation of different hair stressing or damaging conditions (e.g. bleaching, repetitive washing, straightening). Moving a step forward in the research of new trichological parameters, we have developed - in recent years - innovative biochemical assays to evaluate the hair protein content and amino acids composition, the hair total antioxidant capacity, and the hair oxidative damage (protein carbonylation) due to UV exposure.

The testing **on human volunteers'** approach can be divided in two types: the consumer test and the clinical trial. The Consumer testing allows companies to get a feedback on the product quality and acceptability to "real" consumers. This feedback is helpful to the industry to decide whether a product needs further development, or it is ready to go to the market. However, this approach sometimes is considered as a weak evidence when it is used to substantiate particular claims (especially claims related to product efficacy). To overcome this limitation, the clinical trial approach plays a key role in demonstrating the efficacy of products in protecting the scalp and/or the efficacy of products improving the hair conditions with a mechanism of action different from the physical action on the hair shaft. Even if, during the development of a product the R&D can rely on the results of surrogated *in vitro* assays, the

clinical trial remains the golden standard for claiming the product efficacy with robust data. Collaborating with international brands and key players in trichological products (both cosmetics and food supplements) we have developed a huge and comprehensive knowledge in **trichological clinical testing**. From antidandruff products to products claiming efficacy on telogen effluvium or on androgenetic alopecia (cosmetic grade) we have authored manuscripts in peer-reviewed international journals as well as we have presented data of clinical trials in the congress of dermatology societies.

At **COMPLIFE** the clinical trial on hair products can rely on a large panel of volunteers with different scalp and hair conditions (e.g. hair loss, oily scalp/hair, dandruff), a technical equipment pool always calibrated and maintained and different specialists to cover 360 degrees of the Customer requirements. Along with the techniques normally used in the trichology practice, we have extended our portfolio to scalp/hair microbiome testing by means of 16S rRNA analysis. Start to test your hair care product by asking our experts a trichological testing strategy!

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# Hair Water Retention and Water Content Measurement by **Biox**

Sara BAHMAN - Business Development Manager

It may be said that the cosmetic importance of human hair is equalled only by that of human skin. Although nutrition and pH value affect the healthy appearance and feel of hair, as with skin, water content also plays a key role. Using the **AquaFlux**, a patented *condenser-chamber* evaporimeter, ordinarily used in research studies and claims support involving trans-epidermal water loss (TEWL), we have developed a novel, relatively inexpensive and convenient method for characterizing the ability of hair to retain water.

Healthy hair always contains a certain concentration of water, which is lost to the environment naturally through evaporation at a rate determined by its **water holding capability**. We recorded these hair waterdesorption dynamics by continuously measuring water vapour flux density from small (<10 mm length) hair bundles placed within with the thimble-size AquaFlux measurement chamber. The AquaFlux is the only available evaporimeter designed from the outset for both *in vitro*, and *in vivo* applications. Moreover, it is particularly suited to such studies because its isolated measurement microclimate is unperturbed by external conditions throughout the typically prolonged 1- to 2-hour hair desorption measurement times. With help from **London South Bank University's Bioengineering Research Centre**, mathematical models were developed to describe the hair desorption process. By fitting normalized hair desorption data with the models, we could then find the water diffusion coefficient of each hair sample and relate that information to its water holding capability.

Figure 1 shows normalized hair desorption curves of four volunteers, denoted by letters W, P, M and B, measured with the AquaFlux. Volunteers P and B are male adults, W is a female adult, and M is a female child. The curves show that the child volunteer has the slowest desorption rate, i.e., the best water holding capability, whilst the male adult volunteer P has the least water holding capability.

The above curves show that after preconditioning hair samples in the same relative humidity environment, contrasting moisture desorption rates, and therefore water holding capabilities, are clearly discernible within a few minutes.

The **Epsilon** is a capacitance-based contact **imaging** instrument deploying an array sensor, originally designed for biometric applications. Snapshot and video image data from each of its 76,800 pixels are linearised and calibrated. Readouts from the 13 x 15 mm sensor record both the average dielectric permittivity and sample heterogeneity, with a depth sensitivity of approximately 5 microns.

Additionally, a permittivity filter software feature can exclude non-contacting areas or surface water due to sweat, for example, thus enhancing measurement sensitivity and accuracy. Another software function aligns corresponding regions of interest between images or video frames of the same sample, maximising reproducibility when analysing measurements made over days, weeks or years.

Our previous studies have demonstrated the Epsilon's capabilities for skin hydration imaging, skin surface analysis, 3D skin surface profiles and skin micro-relief, as well as solvent penetration and diffusion dynamics. In a new study, we have found it is also capable of measuring human **hair water content** *in* **vivo**. From measured values of dielectric permittivity, we can infer the near-surface water content in skin, hair and nail. Recorded images show no significant association between hair water-concentration and a subject's age, however, water distribution in the hair of younger volunteers was more uniform. The results also show that surface-water concentration is slightly higher in nail than in hair, with skin being the highest.

This new study suggests that capacitive contact imaging is a potentially useful technique for *in vivo* and *in vitro* hair water content measurement. Compared to other techniques, the Epsilon has advantages of rapid visualisation/inspection, high sensitivity, accuracy, and reproducibility.

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# Claim Substantiation of Hair and Scalp products by Princeton Consumer Research

Nalini KAUL – Vice President Technical

Consumers are constantly searching for new products and improved ways to achieve optimal hair health. They canvas drugstores and salons for products that will help create beautiful, healthy, shiny, and strong hair. Particularly in late summer and fall, when there is an increase in hair shedding, consumers turn to anti-hair loss products which promote hair growth and thickness. Every store shelf is like a congaline of products claiming to help with everyday hair issues. The timeless question returns: which one should they pick?

Experts have consistently recognized that a healthy scalp is the key to maintaining healthy hair. Most scalp and hair products are bought as a result of professional recommendation or claims made on packaging. Substantiation of these claims is critical to safeguarding your brand's integrity and to ensure you're ready when a possible litigation lands at your doorstep. The hair experts at PCR have one of the largest teams of haircare professionals in the industry that have worked on providing claim support for over 20 years. With four full services hair salons located across the world, we are best placed to help you with your claims. We have a database of subjects with diverse ethnicity that reaches every type of hair possible.

Hair evaluations are routinely offered for assessment of hair density, thickness, moisture, frizz control, static, hair manageability, combing forces, hair shine, hair volume, hair breakage, hair softness, hair suppleness, hair color, curl effect, curl retention, gloss, hair anti-loss/growth and hair photo protection. Scalp evaluations offered include dryness/flaking, hydration, irritation, inflammation, erythema, pruritus, oiliness, fatty acids, cytokine levels, overall scalp analyses with in vivo digital photography/microscopy.

PCR is an expert in designing study specific protocols that meet the need of the client and the claim. Our staff work with our hair stylists and scientists employing best methods and our recruitment team finds the right hair types.

Hair loss, alopecia, is caused by an imbalance of the hair growth cycle resulting from various factors, including metabolism, genetics, stress, nutritional, medical and environmental.

These factors lead to a reduced number of growing (anagen) hairs combined with an increased number of degenerating (telogen) hairs. Hair loss affects both women and men, albeit differently. Women experience diffuse hair loss and lose hair from the top of the head, while men hair's loss can involve a substantial area, affecting the top of the head and the temporal area.

A clinical study to test an anti-hair loss or hair-growth product is designed for 3-6 months on volunteers with mild to moderate hair loss. Methodologies for evaluation loss include non-invasive techniques (hair counts, pull tests, pluck tests, standardized wash test, photographs, dermoscopy, hair weight, phototrichogram, trichoscan, electron microscopy and invasive techniques, such as a scalp biopsy. Effects studied include decrease of the telogen hair density, increase of the anagen hair density, increase of the growth coefficient (A/T) ratio at baseline, midpoint and at end of treatment phase. For hair replenishment products, trained evaluators assess the subjects' hair for attributes of shine, volume, thickness, softness, and general appearance/condition at baseline and after 8 to 12 weeks approximately. The Brush Friction Count Method (BFCM) is employed, and intact and broken hair are each counted. Macro photographs are taken of an area on the head with thinning hair and assessed for hair growth and count at baseline and/or at midpoint and at end of treatment period. Also, self-evaluation by subjects for any perceived improvements of specific criteria can provide useful information about the product.

Dandruff and Scalp Seborrheic Dermatitis (SD) are scalp conditions both caused by a yeast called *Malassezia* and result in flaking, erythema, itching and inflammation. The study design involves visual grading for erythema and flaking, bio-instrumentation like squamometry, digital photos along with selfperception questionnaires. Recently, clients are asking for inclusion of scalp microbe because of fits influence on scalp health, and involvement in the pathophysiology of scalp-related disorders. As the scalp surface provides a distinct microenvironment studying pH, sebum level, hydration, transepidermal water loss (TEWL), dandruff severity, as well as the dysbiosis in the cutaneous microbiome assumes great importance. Thus, a systematic measurement of scalp clinical and physiological parameters along with the scalp microbiome and associated functional pathways can help reveal important information.

Lately PCR has had a surge in requests for testing efficacy of hair care regimens that include shampoo, conditioner, scalp & hair serums, and dietary supplements. Clinical testing at PCR can aid in the substantiation of claims including "stimulates hair growth", "extends hair life cycle", "restores hair vitality", "for denser hair." As the demand and options for hair and scalp grooming products is growing, PCR is constantly expanding knowledge and utilizing our experience to keep up with the evolving trends to help our customers with rational and well thought out strategies for testing their products.

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# Human hair follicle and scalp skin organ culture: highly clinically relevant models for testing your hair care product by <u>Monasterium Laboratory</u>

Dr. Marta BERTOLINI - Principal Scientist and Deputy General Manager

Monasterium Laboratory Skin & Hair Research Solutions GmbH provides state-of-the-art pre-clinical human skin models for cosmeceutical application, including hair follicle and scalp skin *ex vivo* models as tools to explore the effects of hair care products.

Since the discovery that microdissected human hair follicles continue to grow *ex vivo* and to produce the hair shaft at similar rate to what can be seen *in vivo*, the human hair follicle organ culture has become the model of choice for testing the impact of cosmeceuticals on hair follicle physiology. This assay is based on the spontaneous regression of anagen VI HFs into catagen during the organ culture within the timeframe of 7-10 days.

Therefore, the traditional so called "Philpott model", in which organ-cultured hair follicles are microdissected below the bulge, is mainly used to investigate the impact of test agents on hair growth and on key signaling pathways involved in the control of anagen-catagen transition. Furthermore, given that hair follicles continue also to produce melanin as long as they remain in anagen IV, this assay can be used also to test the effect of cosmeceuticals on hair follicle pigmentation. Using different microdissection techniques optimized at Monasterium Laboratory, it is now possible also to culture full-length hair follicles, so to investigate the effect on hair follicle stem cells located into the bulge, including melanocyte stem cells, and their progeny.

Advances have been made at Monasterium Laboratory in isolating and culturing telogen HFs, inviting new studies on the telogen-to-anagen transition. However, the major shortcoming of the hair follicle organ culture is that the actives need to be systemically delivered into the aqueous culture medium, therefore challenges are encountered for highly hydrophobic assets. In this regard, scalp skin organ culture can become very useful, as test substances can be delivered not only into the medium, mimicking systemic application, but also applied topically.

The scalp skin organ culture is the closest *ex vivo* model that recapitulates *in vivo* situation and additionally allows the study of interactions of HFs with their surrounding environment, alongside the impact on hair growth, pigmentation, and hair follicle stem cells activities, etc. The main advantages of the HFs or scalp skin organ culture model is that these experiments are performed using serum-free medium, thereby being animal free, a fundamental requirement for cosmetic testing. These assays have been significantly optimized at Monasterium Laboratory for a wide range of applications allowing a variety of instructive functional and mechanistic studies, and to mimic pathological conditions of the HF, e.g. HF immune

privilege collapse, and epithelial-mesenchymal transition in the bulge. In addition, Monasterium Laboratory has access to ethically-sourced patient scalp tissue, and it is now possible also to culture microdissected HFs, follicular unit extraction, or small punches from affected scalp of patients suffering from hair loss.

Therefore, these assays are ideal testing tools not only for developing claims for seasonal or aging-related hair loss, but also for hair growth disorders, such as androgenetic alopecia, alopecia areata, scarring alopecia and hirsutism, which could benefit from adjuvant cosmetic treatments. Using gene silencing techniques, it is also possible to knock-down the expression of genes in microdissected HFs or scalp skin organ culture, in order to identify the mechanism of action of hair care product-technology and develop storytelling for consumer communication. Given the low number of HFs or scalp skin available for these experiments from cosmetic surgeries (i.e. face-lifts or hair transplants), the preferred method of analysis is typically histochemistry or immuno-staining, associated with quantitative (immuno-) histomorphometry.

However, at Monasterium Laboratory, we have further optimized techniques to combine in situ analysis with targeted (qRT-PCR/nanostring), or whole transcriptome, analysis (RNAseq) using the entire tissue, microdissected material, or laser-dissected cell populations from HFs or scalp skin. Given the increased interest in improving hair shaft quality with cosmetic and nutraceutical treatments, Monasterium Laboratory has teamed up with TRI Princeton to optimize the hair follicle organ culture model to allow the simultaneous testing on the "living" hair follicle and "dead" hair shafts.

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