

## FOCUS #10 Testing expert discussion around the evaluation of acne-prone & blemishes skin

### They contribute to this expert panel



## Our partners have the floor.

We are glad to introduce the several topics presented by testing experts.

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## Introduction

Despite its robustness, the skin is susceptible to various disorders, among which acne is one of the most prevalent and psychologically distressing conditions. Acne affects approximately 9.4% of the global population, making it the eighth most common disease worldwide. The pathophysiology of acne is complex and multifactorial, involving increased sebum production, follicular hyperkeratinisation, microbial colonization of the hair follicles by *Cutibacterium acnes* (formerly *Propionibacterium acnes*), and inflammation. The formation of comedones, papules, pustules, nodules and cysts is the result of an obstruction and inflammation of the pilo-sebaceous unit.

Skin imperfections cover a wide range of visible skin alterations with a variety of causes depending on age, skin type, ethnicity and associated disorders, lifestyle, stress, hormonal balance, heredity or exposome. The list is long and includes acne, blackheads or comedons, scars caused by these acne phenomena, pore size, hyperpigmentation, senescence stains, white spots, dartres, eczema, rosacea...

These imperfections depending on their severity can have a significant impact on the wellbeing of men and women, altering their image and interfering in the relationship with others.

Innovative breakthroughs in microbiology, such as microbiome transfers, probiotics or bacteriophages, are being explored to treat acne, mainly through topical application. Nevertheless, an individualized approach that considers psychological aspects remains essential, highlighting the importance of comprehensive care for the affected person.

## Not so easy to classify all claims related to skin imperfections

These claims can use the term "anti" or terms related to restoration, improvement. We can distinguish between different categories of claims that can be studied clinically and causally related to different skin signs:



1. Acne-prone skin

 Related to skin pores: Anti-Bacterial, Anti-Black spots, Unclog pores, Comedolytic, Keratolytic, Non-Comedogenic
Seborrheic status: Anti-Seborrheic, Mattifying,

Purifying, rebalancing, Seboregulation

• **Inflammatory acne**: Anti-Inflammatory, Soothing, Repairing Effect, Healing, Anti-Maskne, Antioxidant, Antipollution, respect the barrier function...

• Respect the skin pH

Pollution plays a significant role in inflammatory acne by exposing the skin to harmful particles and toxins. These pollutants can clog pores, increase sebum production, and trigger inflammatory responses, leading to the formation of acne lesions. Prolonged exposure can worsen acne severity and make skin more prone to breakouts.

- 2. Hyperpigmentation and colour changes
  - Hyperpigmentation, such as melasma: Anti-stains,
  - Redness: Anti-Rosacea, Anti-Redness, covering effect
- 3. Surface irregularities
  - Relief & topography issues: roughness, uneven skin surface: Anti-drying, smoothing, keratolytic
  - Scarring: exfoliating, help the wound healing process, regenerating
  - Skin Hydration: moisturizing
  - Barrier function

#### How to evaluate in vivo acne-prone and blemishes skin

The development of acne-prone skin products involves a meticulous process of preclinical and clinical testing to establish their safety, efficacy, and mechanism of action. In the absence of a legislative demand for clinical proof—proof that is required for the claims of sun protection, for instance -the skin care industry often neglects to search for a real treatment.

The field of claims of these complex products is often mixed with those of products intended for sensitive skin, dark skin, young skin... and very often require a **multi-criterion evaluation** to demonstrate these eclectic performances. Sometimes it seems that the results demonstrated by the **before-after photos are the must-have**. However, these illustrated results are often consolidated by **quantitative measures**, **dermatological assessments and self-evaluation by the subjects** that allow for a comprehensive approach to product activity. The **feeling and self-esteem**, strongly concerned in the well-being provided by an effective anti-imperfection care routine, will also be able to be further evidence well understood by the consumer.

On the **Skinobs Clinical Testing Platform**, you can find different claims regarding acneprone skin and blemishes: anti-black heads, anti-blemishes, anti-seborrheic, astringent, seboregulator...You can find in total **95 methods and 127 providers from 34 countries**.Just connect for free, skinobs.com.

On the **Preclinical Testing Platform**, you can find in **total 98 assays and 55 providers in 14 countries**, for claims related to acne, sebum, and skin imperfections.

Among the scores by experts' protocol, the auto-evaluation by the subjects and the neurosensory studies, here are the methods to evaluate these claims.

#### 1. Sebum and lipids quantification

- Image analysis method: Sebufix and sebumeter (Courage+Khazaka), DermaLab Sebum (Cortex), Quantiseb (Monaderm).
- Gravimetric measurement method: SebumScale
- 2. Skin surface evaluation and image acquisition and analysis: C-Cube Clinical Research, DermaTOP-HE-S, evaSurf, Aeva-HE (Eotech), SpectraCam, NomadCam and SkinCam (newtone), Antera 3D (Miravex), VEOS (Canfield), Clarity (BTBP), Siascope, Visioscan (Courage+Khazaka), Dermalab Videoscope (Cortex)...and others dermoscopes.

#### 3. Lipids composition and quantification in the skin

- Optoacoustic analysis: RSOM Explorer C50
- Shotgun mass spectrometry
- Lipbarvis-HPTLC and Lipbarvis-Microscopy [TEM] (Microscopy services Daenhardt)
- **4. Face: overall analysis:** global image of the face with image acquisition and analysis system: ColorFace (Newtone), EvaFACE-S5, HeadScan Dynamics III, HeadScan VO5 R&D (Orion), VISIA-CR, Visioface (Courage+Khazaka).

While evaluating acne-prone skin and imperfection is important, the overall skin health determine the global aspect of the face including the hypo-seborrheic zones (cheek) and the hyper seborrheic ones (nose, forehead, chin).

#### 5. Microbiota quantification

- Lipbarvis GEN-SSCP analysis, RT-qPCR assays.
- Genome sequencing
- Mass spectrometry
- Profiling and metagenomic sequencing: DNA microarray, Rt-qPCR, 16S-rRNA

Personal care and toiletries are now designed to make the skin healthy. The healthy skin care maintains the skin in good state, protects it from the external aggression and helps to regulate the bad influences of the internal stresses. This is a question of aspect and perceived comfort; it generates a globally positive impression of good health.

It's crucial for investigators to **collaborate closely with Contract Research Organizations** (CROs) to meticulously design protocols, define inclusion criteria, establish measurement

timelines, treatment conditions, and select optimal devices. Investing time in briefing these essential elements is never wasted; it ensures the integrity and reliability of the study outcomes.

The various high-tech biometrological measurements give the opportunity to connect the technology with the new digital use of personalization from the shop to the bathroom. This connection between objectivation and the digital way of choosing and buying may bring the cosmeticians closer to the reality of marketing. Now the **imaging of the skin from the centimetre to the nanoscale** is more and more crucial. Whether for no-comedolytic, radiance, anti-dryness, or seborrheic, the several techniques look for **higher resolution**, **larger measurement area, non-invasive, no-contact and direct methods.** The algorithms and the statistics are the principal future contribution of the success of these new technologies.

The era of connected devices for scientific skin diagnosis or microbiome analysis combined with the personalization treatment sounds great for skin care evaluation. These digital tools enable the development of new products to answer the new request of consumers.

# What are the solutions to validate in-vitro the seboregulation and the inflammation mechanisms in the skin.

The anti-seborrhoeic effect of an active ingredient or cosmetic product can be assessed on human cells, reconstructed skin models or ex-vivo. These approaches not only make it possible to **measure sebum production, but also to analyse the mechanisms of action of the substances**. To complete these tests, the anti-inflammatory effect can be studied.



Cell tests are carried out on sebocyte lines (SZ95) or primary cultures. Once cell viability has been measured (MTT test), lipid production is quantified by gas chromatography-mass spectrometry (GC-MS) or by analysis (Rt-qPCR) of the expression of target genes coding for lipid biosynthesis.

Tests on reconstructed skin models or ex-vivo models of sebaceous glands make it possible to measure the quantity of sebum on the surface of

the skin either by direct measurement or by extraction of lipids and quantification by gas chromatography. Immunohistochemical or protein analyses are carried out to **quantify markers of lipid biosynthesis in acne-prone skin**: Insulin growth factor-1 [IGF1], SREBP-1, diacylglycerol - DGAT acyltransferase, K7 Cytokeratin 7, Keratinocyte growth factor [KGF], Cutibacterium Acnes [ex-Propionibacterium acnes], Squalene MDA (Malondialdehyde) assay. In addition, the morphology of sebaceous glands can be assessed by histology using specific stains (Haematoxylin and Eosin) or by scanning microscopy.

For the associated anti-inflammatory effect, quantification by protein analysis or gene expression of markers such as : Beta defensin 2-4 [BD-2-4], CD1a, CD209 DC-SIGN, CD44, Cytokeratins 17 [K17], Cytokeratins 6 & 16 [K6, K16], Decorin [DCN], Elafin / Skin-derived anti-leukoproteinase [SKALP], Transforming growth factor beta [TGF $\beta$ ], Tumour necrosis factor alpha [TNF $\alpha$ ], Granulocyte macrophage colony stimulating factor [GM-CSF], Filaggrin [FLG], Interferon gamma [IFN $\gamma$ ], Interleukin 22 [IL-22], 31 [IL-31], 6 [IL-6], 8 [IL-8][CXCL8], 1 alpha and 1 beta [IL 1 $\alpha$ -1 $\beta$ ], 17 and 23 [IL 17-23], 4 and 13 [IL 4-13], Kallikrein 5 - 7 [KLK-5-7], Langerin [CD207], Thymic stromal lymphopoietin [TSLP], Matrix metalloproteinases [MMP], Oncostatin M [OSM], Prostaglandin E2 [PGE2], Cathelicidin antimicrobial protein [CAP18], Ribonuclease 7 [RNASE7], Sirtuin 1 [SIRT1]. In addition, the measurement of Reactive Oxygen Species (ROS) is a good indicator of oxidative stress linked to inflammation.

In conclusion, in-vitro assessment of the anti-seborrheic effect on cells requires a combination of quantification techniques to identify and characterize the active compounds.

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## Navigating the clinical evaluation of blemishes and oily skin by Validated Claim Support

Jane Tervooren - Vice President

Beyond the surface frustration of acne, blemishes, and oily skin lies a realm of clinical evaluations that not only pinpoint underlying issues but also help substantiate product efficacy. Acne and blemishes encompass a wide range of imperfections, from pimples and dark spots to scars, hyperpigmentation, and uneven texture.

Validated Claim Support offers a multifaceted approach to clinical evaluation, including bio-instrumentation assessments, expert visual before and after imaging, and self-perception grading, questionnaires. It is important that your clinical evaluations are carried out at an FDA Registered lab in a temperature and humiditycontrolled environment.



Bio-instrumentation assessments utilize objective techniques to provide quantitative data on oily skin, often a root cause of blemishes and enlarged pores. Instruments such as the Sebumeter are employed to measure sebum production, offering insights into the level of oiliness of the skin. Additionally, the Chromameter may be used to measure changes in lesion color, providing valuable objective data on the progression of blemishes. These bioinstrumentation assessments serve as foundational elements in quantifying the changes of acne, blemishes and oily skin, laying the groundwork for supporting claims.

Microbiome collections and analysis adds another layer of understanding to the clinical evaluation of blemishes and oily skin. The skin microbiome, composed of diverse communities of bacteria, fungi, and other microorganisms, plays a crucial role in maintaining skin health and homeostasis. Disruptions in the skin microbiome have been implicated in various dermatological conditions, including acne and blemishes.

Expert visual clinical grading complements bio-instrumentation assessments by providing a qualitative evaluation of acne and blemishes. Trained clinicians assess various characteristics, including size, color, texture, and distribution, to determine the severity of the condition. Validated methods for counting acne lesions, such as comedones, papules, pustules, and nodules, allow for accurate quantification of acne severity and monitoring of progress over time. This expert visual evaluation offers valuable data assessing product efficacy.

**Before and after imaging techniques**, such as the Canfield Visia or VCS Validated Clinical Photography, provide visual evidence of treatment efficacy in a consumer-friendly manner. Controlled studio techniques ensure standardized and high-quality images, allowing for precise assessment of improvements over time. Images are captured in a highly controlled environment, with a neutral background and consistent lighting, to accurately depict changes in blemishes and oily skin. These before and after images serve as powerful tools in demonstrating treatment outcomes giving your intended audience and stakeholders a "reason to believe".



**Self-perception questionnaires** offer the allimportant subjective input from individuals participating in the study, capturing their perceptions of product effectiveness and overall satisfaction. These questionnaires complement objective clinical data by providing insights into the real-world impact of products on subjects' lives. By soliciting feedback directly from participants, you will be able to make claims like "X% of subjects saw an improvement in their troubled skin in only X

weeks". Self-perception questionnaires also serve as valuable tools for assessing subject adherence and satisfaction, informing future marketing strategies.

The clinical evaluation of blemishes and oily skin involves a multifaceted approach that encompasses bio-instrumentation assessments, expert visual grading, before and after imaging, and self-perception questionnaires. VCS integrates these various assessment methods to help substantiate your claims and give your customers a Reason to Believe.





## The power of PCi-SEB for high-throughput screening of antiacne compounds by Phenocell

#### Hélène Leménager - R&D project manager

Acne is the most prevalent dermatological condition before atopic dermatitis. It affects about 80% of the population, with peak incidence between 14 and 19 years depending on the patient gender<sup>1</sup>. Acne development results from several concurrent events, such as increased sebum excretion by sebocytes, alteration of inflammation around the sebaceous unit, colonization of skin by exogenous bacteria and follicular hyperkeratinization. Current human *in-vitro* cell models to study acne include **primary sebocytes<sup>2</sup> and immortalized cell lines<sup>3-6</sup>**. Obtaining primary sebocytes is technically challenging, low throughput and generate donor-dependent results<sup>7</sup>. The most characterized cell line SZ95 harbors major chromosomal abnormalities<sup>3</sup>. Here we present an alternative that allows high-throughput studies on sebocytes, offers a great relevance to the human physiopathology and facilitates analysis in a variety of ethnicities and in both genders.

**Human induced Pluripotent Stem Cells** (iPSC) are obtained after reprogramming of peripheral blood mononuclear cells or fibroblasts obtained from donors under written informed consents. Reprogrammed with non-integrative, episomal vectors, iPSC exhibit a normal karyotype without chromosomal alterations. Their differentiation into sebocytes (PCi-SEB) leads to the production of batches of billions of cells with purity >90% and very low batch-to-batch variability. This allows high throughput studies to screen compound libraries or long-lasting studies that require a similar biological source from experiment to experiment.

PCi-SEB respond to all inducers of sebogenesis known to be related to acne development, e.g. testosterone, dihydrotestosterone, arachidonic (AA) and oleic (OA) acids, inflammatory agents and *C. acnes*, by significantly increasing their lipid content and maturation. As a result, the main pathological processes involved in acne can be thoroughly analysed at molecular levels and the effects of potential acne-reducing agents clearly explained. The availability of sebocytes from Caucasian, Asian and African sources and from the two genders adds more opportunities to the definition of proper leads to counteract skin dysregulations and to develop ethnic-specific care.

**To mimic the bacterial colonization observed in acne**<sup>1</sup>, PCi-SEB were treated with culture medium conditioned by *C. acnes. C. acnes* is a gram-positive anaerobic bacterium that

resides in pilosebaceous follicles as a member of the resident bacteria. Abnormal colonization by *C. acnes* may be trigger by the induction of inflammatory mediators. Fortyeight hours of treatment were sufficient to significantly increase lipid production by sebocytes (Fig. 1A). Concomitantly, the number of cells dropped by 35% compared to the basal control (Fig.1B). An inflammatory reaction was also triggered by *C. acnes*-conditioned medium, with higher levels of secreted pro-inflammatory cytokines (Fig. 1D, 1E). The reduction of cell number illustrates terminal maturation of sebocytes, which will die from critical lipid overload and release their content and cell debris into the extracellular space to produce sebum.



**Figure 1.** *C. acnes* supernatant effects of PCi-SEB cells compared to arachidonic acid (AA)-induced effects and their reduction by a sebostatic agent (AA + inhibitor). **A.** Increase in lipid contents. **B.** Number of cells. **C-D.** Quantification of IL-6 (C) and IL-8 (D) in the culture supernatant. Data are normalized to cell count. Error bars represent the standard deviation. Unpaired *t*-test compared to the basal control with \*p<0.05 \*\*p<0.01 \*\*\*p<0.001\*\*\*\*p<0.0001 or compared to AA with \$p<0.05 \$\$\$

The enzymes that metabolize the free-fatty acid arachidonic acid (AA) are overexpressed in acne-affected skin<sup>8</sup>. Leucotrienes, prostaglandins and 15-HETE, all potent proinflammatory mediators and neutrophil attractants, are synthetized from AA by lipoxygenase. In our set-up, the response of PCi-SEB to AA displayed a similar profile to the response to *C. acnes*, although with higher intensity. AA induced the exacerbation of lipid contents with reduction of cell number and the inflammatory reaction.

**The PCi-SEB model** allows the investigation of key events that lead to the exacerbation of acne. Being amenable to high-throughput screening and analysis, it may lead to quick and reliable identification of anti-acne compounds and allow in-depth characterization of their mechanism of action. Besides acne, the model can be applied to other of inflammatory skin diseases, such as atopic dermatitis and psoriasis.

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## Acne and blemish evaluation with the C-Cube 3 by Pixience

#### Sébastien Mangeruca - CEO

Acne is a **multifactorial** skin disease affecting mainly the pilosebaceous follicles, it represents a common but complex dermatological condition.

Acne can be linked to **hormonal imbalances** but also to **genetic** and **environmental** factors. It affects a wide range of the population, mainly adolescents and young adults, but can also persist into adulthood, thus constituting a major challenge for dermocosmetics.

Given the complexity of this pathology, an accurate evaluation of acne and its effects on the skin requires state-of-the-art tools and analysis methods. In this context, a technology such as the C-Cube represents a significant advance in the clinical world.

The C-Cube 3 has been specifically designed to observe and quantify the effects of your cosmetics ranges. With its **CIE L\*a\*b\*measurements**, it allows you to image and measure the **degree of effectiveness** of your active ingredients on the skin and scalp. With the data contained in each pixel and the placement of ROI in the image, you ensure control and accuracy in your studies.

You can also make 3D acquisitions of the surface of the skin. Using a technique called stereo photometric, the C-Cube system analyzes multiple images under different lighting conditions to estimate surface normals. Combined with geometric calibration, the software produces **elevation information** for each pixel.

By combining rigorous scientific approaches with cutting-edge tools, it becomes possible to better understand the mechanisms of acne, to evaluate the effectiveness of products and thus to optimize its management.

#### The C-Cube is the ideal tool for measuring all acne characteristics.

#### **Pimple Elevation**

Pimples are one of the most common symptoms of acne, manifesting as papules, pustules, or nodules on the skin, resulting from pores and inflammation obstruction. The elevation of the buttons can be reduced by some products.

Thanks to its unique 3D parameter measurement capability, it is possible to measure button elevation with peak height (Sp) and total amplitude (Sz).

An effect of the tested product can be proven if the elevation of the peak decreases over time.



Image source

Resulting data

#### Roughness

The C-Cube is undeniably the best option for assessing even the **most subtle changes.** Beyond measuring pimple elevation, it is capable of observing very small signs of acne before they worsen with inflammation. This is akin to measuring skin texture, invisible to the eye.

This can provide information about **future cysts that** may become inflamed in the following days.



Texture rendering

Elevation map

#### Sebum

Acne-prone skin tends to produce a higher amount of sebum than normal. Many products are intended to **regulate sebum production.** 

Thanks to the C-Cube and its sampling reader, it is possible to collect sebum on the skin and analyze it to study the sebum-regulating effects of your ranges.

The colour of the sampling area is grey and uniform, so that dark sebum spots can be clearly distinguished. The samples taken from these patches are analyzed with the Pixience sample reader and the C-Cube camera in a standardized and systematic way. The analysis of the intensity levels in the sample area makes it possible to determine a threshold between the "background" (the gray area of the sample) and the intensity of the light "form" (the black area, i.e. sebum).

This makes it easy to distinguish them and measure the relative surface area of sebum in relation to the total area of the spot (expressed as a percentage of the total area).



Automatic sebum quantification on Sebutape® patches

#### Blackheads

Blackheads are small heaps of sebum produced by the sebaceous glands and by keratin deposits that clog pores. With the increase in production on acne-prone skin, it is therefore common to be confronted with the appearance of blackheads.

The C-Cube can measure the number of blackheads and the average diameter. Its field of view is constant and calibrated, which means that the surface of the photo is always the same (1.92cm<sup>2</sup>). The density is therefore obtained by dividing the number of pores by the surface area of 1.92 cm<sup>2</sup>.



Image source

Resulting data

Excessive sebum production can affect pore size. These can expand.

Thanks to its reliable dimensional measurements and metric calibration, the C-Cube can detect the number of pores and measure them, in both 2D and 3D.

In 2D, this consists of color analysis, followed by shape identification to eliminate other objects and preserve only the pores.

The results consist of black-and-white processed images and a spreadsheet with the middle area, median diameter, and pore density of each image.



Image source

Resulting data

3D analysis provides an additional dimension of pore depth. The 3D approach improves the sensitivity of pore detection, allowing for the assessment of pore depth and possible pore filling.



The c-cube is a versatile system for evaluating anti-acne products and prove claims.

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## NEWTONE

A QIMA Life Sciences Company

### Prove your product's clinical efficacy through digital imaging analysis by Newtone Technologies

Alix Danoy - Clinical Study Manager

Acne is among the 5 most frequent reasons for dermatological consultations, which is partly explained by the fact that it affects individuals of all demographics, albeit in varying degrees. This well-known skin condition causes lesions that appear with flare-ups. Non-inflammatory lesions include whiteheads (closed comedones) and blackheads (open comedones), while inflammatory lesions include nodules, papules, and pustules. Often, following an inflammatory episode, patients may also experience post-inflammatory lesions, which typically manifest as erythema, hyperpigmentation, or scarring.

#### Dermocosmetic products for acne treatment

Dermocosmetic products designed to treat acne aim to reduce the frequency and severity of both non-inflammatory and inflammatory lesions, minimize the appearance of postinflammatory marks, and even prevent acne onset by targeting its underlying causes, like hyperseborrhea. Thus, **the claims associated with these products cover a wide spectrum**, including "seboregulatory," "mattifying," "astringent," "oil control," "non-comedogenic," "soothing," "anti-imperfections," "spot correcting," "irritation-reducing," "anti-redness," and "scar-reducing."

Demonstrating the clinical efficacy of dermocosmetic products can be challenging, particularly due to the inherent subjectivity of face-to-face volunteer evaluation in clinical settings. At Newtone Technologies (part of the QIMA Life Sciences group), our mission is to assist you in obtaining more precise, sensitive, and reliable data concerning your products' performance, and we do so by providing **advanced digital imaging analysis solutions.** Moreover, we also help you boost your marketing efforts thanks to our **smart illustrations**.

#### Image analysis services

As previously mentioned, hyperseborrhea, or **increased sebum production**, while not solely responsible, is a necessary condition for the development of acne, making it a key target for anti-acne products. Clinically, hyperseborrhea gives the skin a shiny or "glossy" appearance.

The decrease in the gloss parameter can be monitored using our imaging acquisition devices and algorithms to prove seboregulatory effects.

Regarding **acne lesions**, both inflammatory and non-inflammatory, two main approaches exist for their assessment. The first approach involves the monitoring of individual lesions over time with smaller, specialized devices designed for direct analysis of specific areas including the Region of Interest (ROI). The second approach, more holistic, requires a full-face acquisition device to monitor all facial lesions

collectively. In both cases, algorithms are tailored to detect the specific lesion type, which enhances the precision and reliability of the evaluation.

Due to the existing link between an **imbalanced skin microbiota** (dysbiosis) and the onset of acne, we have also developed solutions that allow for the quantification of specific bacterial species on acne-prone skin. Notably, we can study the modulation of the levels of C. acnes —a key acne-contributing bacterium— on the surface of the skin by analyzing porphyrins (one of its metabolites), employing UV acquisition technology.

Moreover, our offer also encompasses the analysis of all types of post-inflammatory lesions. We offer advanced evaluation of **erythema and redness** through the evaluation of blood vessel dilation. Thanks to specially designed image analysis treatments we can generate hemoglobin maps based on cross-polarized image acquisition. We also provide detailed evaluation of **hyperpigmentation**, assessing multiple factors such as color, surface area, and roughness. Additionally, our offerings extend to the evaluation of the severity of lesions using well-established **dermatological scales**, like the PAHPI grading system. These assessments are conducted by our expert in-house dermatologists, ensuring accuracy and reliability.



#### Illustration services

Because clear and impactful communication of your results is just as crucial as proving efficacy, we offer **illustration services to showcase before-and-aftereffects** and thus demonstrate your product's clinical efficacy. We do this by using an average face of the study as a "canvas" where the real effects observed on the skin are displayed and highlighted. Simply choose a volunteer (either the mean or best case), determine the specific time points you want to showcase, and we will project their skin onto the avatar image!

If you have collected biophysical data (like readings from a sebumeter) showing how effective your product is, we are here to help you share those outcomes too. What we will do is craft a digital

continuous color map from your biophysical measurements (either the mean case, best case, or the average study result), and make your results stand out.



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## Approach for a better appearance of acne-prone skin by Zurko Research

Lorena Bellas

#### Approach for a better appearance of acne-prone skin

Impure, acne-prone skin is common among both males and females during puberty and can sometimes persist into adulthood. This condition arises due to increased sebum production influenced by male sex hormones, abnormal follicular keratinization, and subsequent proliferation of skin microflora and inflammatory responses. The skin often appears greasy and shiny, rough with enlarged pores, and has a higher tendency to develop comedones, pimples, and pustules. Many individuals find this skin condition unpleasant and consider it a significant cosmetic issue, highlighting the importance of controlling impure, acne-prone skin.

Currently, there are numerous topical formulations designed for cleansing and caring for this type of skin. These products claim to normalize sebum production, remove excess oil, reduce pore size, and clear skin imperfections. Cosmetic products for impure skin focus on cleaning and caring for the skin. Their claims often include cleansing, degreasing, controlling shine, restoring the balance of oily skin, cleaning pores, removing excess oil and unwanted microorganisms, as well as clarifying, reducing blemishes, and improving the overall health of acne-prone skin.

A balanced approach to evaluating the effectiveness of these treatments involves combining endpoints such as ordinal global assessment scales, acne lesion counts, and instrumental assessments of relevant skin parameters.

Next, we will focus on clinical and instrumental evaluations for this type of skin.

#### Study approachings

#### Sebum and shiny appearance

One of the main concerns is sebum control and, related to it, managing the shiny appearance of the skin due to the presence of oil. In other words, we aim to achieve a more mattified skin appearance by reducing the presence of sebum.

For this, a combined approach can be taken by measuring sebum concentration with the Sebumeter<sup>®</sup> SM 815 and assessing skin shine with the Skin-Glossymeter GL 200.

These effects can be observed in the medium to long term with cleansing or treatment products, but it is also very interesting to see a more immediate effect related to makeup products, such as translucent powders or mattifying primers.

#### Pores appearance

When we seek to improve the appearance of acne-prone skin, evaluating the way we perceive pores is also crucial. On skin that is dirty or appears very oily, pores are more noticeable. Through a visual assessment, we will study whether they are less prominent or perceptible.

#### Prevention

Regarding a preventive aspect, we could conduct a study of the porphyrins present in the skin, as they can indicate potential acne lesions. The accumulation of C. acnes and the production of porphyrins can contribute to the inflammation of the pilosebaceous follicle. The reactive oxygen species generated by the activation of porphyrins can damage surrounding cells and increase the inflammatory response, potentially exacerbating acne lesions. We can visualize them using the UV light filter of the VISIA Skin Analysis.

#### Recovery

Lastly, in order to eliminate the traces of acne lesions that remain on the skin, we can evaluate the intensity of post-acne blemishes: post-inflammatory hyperpigmentation (PIH) and post-inflammatory erythema (PIE). We can conduct this evaluation through clinical assessment and photographic support, but also by studying the hemoglobin and melanin content using the Mexameter® MX 18. In this case, a good selection of panelists will be crucial, as the development of these two types of post-inflammatory lesions is directly related to the skin tone of the panelist.

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#### **Conclusion**

We emphasize the importance of proper care for this type of skin, focusing on prevention, maintaining a healthy appearance, and avoiding negative impacts, which will directly affect the quality of life for individuals with acne-prone skin. Adequate skincare routines not only help in managing current skin conditions but also in preventing future outbreaks and complications. Furthermore, understanding the specific needs and challenges of acne-prone skin can lead to more effective treatments and skincare products, ultimately contributing to better skin health and overall well-being. Continuous research and innovation in this field are essential to provide the best care and improve the lives of those affected by acne.

#### CONTACT

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## Revolutionizing mattifying cosmetic testing with U-Skin™ by Microfactory

Fabrice MONTI - CEO

#### Introduction

In the cosmetics industry, the quest for products with effective anti-acne, anti-seborrheic, and mattifying properties is relentless. Both cosmetic brands and ingredient manufacturers face significant challenges in evaluating these effects accurately and efficiently.

Traditional methods, while useful, often suffer from limitations such as variability in human skin types and reproducibility issues. Microfactory addresses these challenges with its groundbreaking U-Skin<sup>™</sup> technology, which leverages an innovative artificial skin model to mimic human sebum and sweat production. For its mattifying performance, Lucas Meyer chose to evaluate a new R&D ingredient using U-Skin<sup>™</sup>.

#### The innovation of U-Skin<sup>™</sup>: development of a sebum-mimetic artificial skin

Microfactory's U-Skin<sup>™</sup> technology stands out as a revolutionary advancement in cosmetic testing. U-Skin<sup>™</sup> combines a sebum-mimetic artificial skin with advanced microfluidic systems to create a standardized, reproducible platform for evaluating cosmetic products. There are many areas of application, from face care to make-up and dry shampoos. Let's delve into how this technology works and the impact it has on the industry.

#### Technological Components and Mechanisms:

- 1. **Synthetic Epidermis and Dermis:** The artificial skin comprises biocompatible polymers that replicate the texture, elasticity, and permeability of natural human skin. These layers are crucial for mimicking the barrier function and mechanical properties of real skin.
- 2. Artificial Sebaceous Glands: Integrated microfluidic systems simulate sebum production. These systems can vary sebum secretion in response to different stimuli,

closely mimicking the dynamic nature of human skin. The artificial sebaceous glands ensure that sebum production and flow are realistically replicated.

3. **Sebum-like Fluids:** The sebum-mimetic fluids used in U-Skin<sup>™</sup> closely resemble the chemical composition of natural sebum, including lipids, fatty acids, and triglycerides. This ensures that the interactions between cosmetic powders and sebum are realistic, providing accurate data on product performance.



## Evaluating mattifying products with U-Skin<sup>™</sup>: the example of powders (a study performed in partnership with Lucas Meyer Cosmetics).

U-Skin<sup>™</sup> technology allows for precise simulation of sebum production through controlled liquid pulses. Each pulse is followed by a waiting period during which the cosmetic product absorbs the liquid.

This process causes a rapid increase in gloss with each pulse, which then decreases during the waiting period.

After several cycles, if the average gloss increases, it indicates low absorption capacity, suggesting that the powder lacks mattifying power. Conversely, if the gloss remains stable or decreases, it confirms the powder's ability to control gloss or even provide a mattifying effect. Notably, three cycles in this simulation are equivalent to 24 hours in real life.

This methodology was applied to test three powder samples: Rice, Maize, and a powder under development. The results demonstrated varying capacities of these powders to control gloss, providing valuable insights into their effectiveness.



#### Benefits for cosmetic ingredient manufacturers

For manufacturers of cosmetic ingredients, U-Skin<sup>™</sup> technology offers several key benefits:

- **Accurate screening:** Enables precise screening of functional ingredients, providing reliable data on their performance.
- **Cost-Efficiency:** Reduces time and costs associated with traditional testing methods.
- **Enhanced reproducibility:** Provides standardized and reproducible results, ensuring consistent data across tests.

#### Advantages for cosmetic brands

Cosmetic brands seeking to reformulate their products with more natural ingredients or to eliminate certain components can significantly benefit from U-Skin<sup>™</sup> technology. It allows them to:

- **Rapidly test new formulations:** Evaluate new formulations efficiently before moving to in vivo testing.
- **Ensure product efficacy:** Guarantee that reformulated products meet high-performance standards for mattifying effects.

#### Conclusion

Microfactory's U-Skin<sup>™</sup> represents a significant advancement in the cosmetic industry, providing a reliable and efficient method for testing the efficacy of mattifying products. By closely mimicking human skin, this technology offers unparalleled insights into product performance, helping manufacturers and brands develop superior cosmetic products.

We would like to thank Lucas Meyer, and especially Audrey MANIERE (<u>Audrey.Maniere@lucasmeyercosmetics.com</u>).

By adopting these innovative testing methods, the cosmetic industry can continue to push the boundaries of product development, ensuring that consumers receive high-quality, effective products.

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## Skin imperfections: which methodologies to adopt? Evaluation of the Efficacy of cosmetic products against skin imperfections by IEC

Jean-Robert CAMPOS - Scientific Director



Skin with imperfections is characterized by irregularities such as enlarged pores, blackheads, pimples and even acne, especially in oily skin or skin with an oily tendency.

These imperfections are caused by several factors: thickening of the epidermis (hyper-keratinization), excessive sebum production (hyper-seborrhea) and the multiplication of a bacterium (Propionibacterium acnes).

To evaluate the effects of a cosmetic product on skin imperfections, and to claim a **decrease of imperfections/anti-imperfections**, it is possible to monitor the evolution of various parameters such as the **number of pimples**, **shine**, **sebum rate/flow**, **number and size of pores**, and **inflammation** caused by bacterial flora.

In addition to the previous skin problems linked to cutaneous imperfections, we also have **pigmentation spots** and **post-inflammatory hyper-pigmentation scars** (PIH).

To support product development or the justification of recommendations for "antiimperfections" claim, IEC propose an In Use Test with, as a first option, a visual assessment by experts which may include a count of retention and inflammatory elements enabling **non-comedogen and/or comedolytic claims** to be attributed, a scoring of shine over the whole face, and the number and size of pores using photographic scales. These assessments can be carried out in the presence of the subject, or on the basis of photographs.These scorings are complemented by subjects'self-assessments and specific consumer studies. The above propositions can be objectified during kinetics and/or In Use Test by instrumental methods to reinforce claims, such as the **seboregulating effect** [Sébutape® / Quantiseb Station Monaderm], or a **matifying effect** by measuring skin shine linked to seborrhea on the whole face [ColorFace® – Newtone Technologies - QIMA Life Sciences] or in macro image [SkinCam®, Newtone Technologies] or monitoring sebum evolution [Sebumeter® or Glossymeter® - Courage & Khazaka].

**An astringent effect** by analyzing pores (surface area, density, apparent depth) using photos in parallel or cross-polarized light *[Colorface®]* or with the Visiopore® PP34 *[Courage & Khazaka]*, which in addition to pore number and size can also measure the intensity of porphyrin produced by P.acnes, a marker of the evolution of **the inflammatory and bacterial state** of the skin. Porphyrin fluorescence intensity can be measured under UV light using ColorFace®.

The **astringent effect** can also be assessed using C-Cube<sup>®</sup> [*Pixience*] or Fringe Projection [*Dermatop*<sup>®</sup> or *AEVA*<sup>®</sup> - *EOTECH*].

**Inflammation** can be assessed using ColorFace<sup>®</sup> whole-face or SkinCam<sup>®</sup> macro-image, by monitoring acne lesion parameters (density, surface, color).

**Inflammation** (cytokines, fatty acids, PGE2) can also be determined from non-invasive epidermal samples *[Synelvia- QIMA Life Sciences]*, which can be combined with **anti-microbial activity**, **seboregulation** and the **qualitative and quantitative composition of sebum**.

Spot imperfections and PIH are assessed by direct instrumental measurements [Chromameter®, Spectrophotometer®, Mexameter® - Minolta, SIAScope - Siametrics <sup>TM</sup>] or by photographic image analysis [Colorface, C-cube®], hyperspectral imaging [SpectraCam® or SpectraFace® - Newtone Technologies] and specific software [FrameScan - Orion Concept].

With its multi-tone panel due to the geographical distribution of its testing centers [Europe, Asia, Africa]: Caucasian [Bulgaria, France: Lyon & Saint-Etienne], Asian [Singapore, Japan, China, Korea], African [Cape Town, South Africa], the IEC Group offers a multitude of methodologies for evaluating the efficacy of cosmetic products against skin imperfections.

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## Acne and skin microbiome in summer: how to avoid the rebound effect! By ByomeLabs

Stéphanie Badel-Berchoux - Laboratory Director and Margaux Jeanmougin Marketing Manager

In summer, the sun shines, and your skin looks radiant, giving the impression that your acne has vanished... Unfortunately, this is an illusion! Come autumn, the pimples will make a strong comeback, which is known as the rebound effect. Where does this phenomenon come from? How can you avoid it while preserving your skin microbiome?

The skin itself is an ecosystem sensitive to external variations in temperature, humidity, and ultraviolet radiation (UVA and UVB), which specifically modulate the composition of its microbiome and its ability to colonize the skin, challenging its entire balance.

Acne, an inflammatory skin condition affecting more than 6 million people in France, is often caused by increased sebum production, hyperkeratinization, inflammation, and the colonization of follicles by the bacteria *C.acnes*.

Beyond its impact on the skin, this condition has significant psychosocial repercussions. Often a source of embarrassment and frustration, acne seems to miraculously disappear with the first rays of the sun, but this is far from the case.

While the sun can temporarily reduce blemishes, it is ultimately detrimental to our skin microbiome as it dries out the skin, leading to increased sebum production to compensate for the loss of hydration.

Research has highlighted the essential role of the skin microbiome in skin health. Composed of microorganisms living on the surface of our epidermis, it plays a key role in protecting against infections by producing antimicrobial peptides, and some of these microorganisms are involved in the production of molecules that maintain hydration.

## Excessive sun exposure, combined with inappropriate use of certain cosmetic products, disrupts this delicate balance.

Exposure to UV rays (UVA and UVB) decreases the Shannon index, indicative of microbial diversity on the skin's surface. Microbial diversity is an indicator of good skin microbiome health. *Lactobacilli* are significantly affected, although they fully contribute to skin balance. Conversely, UV rays increase the number of cyanobacteria producing lipopolysaccharides, which are irritating to the skin.



Inflammation and hyperseborrhea, which clog the skin's pores, are two factors that favor the proliferation of *C.acnes*, thus exacerbating skin problems, including acne flare-ups after sun exposure.

This is where BYOME LABS comes in, a French biotech startup specializing in microbiome skin testing and analysis. With our team of experts, we test the impact of cosmetic products on the skin flora and support cosmetic brands in developing innovative solutions to preserve this balance. BYOME LABS has also created a "Microbiome Friendly" certification, ensuring consumers that their cosmetic products respect their skin microbiome.

To limit the damage caused by the interactions between the sun, skin microbiome, and acne-causing bacteria, it is essential to adapt your beauty routine:

- Cleanse your face daily with gentle cleansers to remove impurities without irritating the skin and further disrupting the skin microbiome.
- Hydrate your skin according to its needs to prevent dryness. Be careful to avoid photosensitizing ingredients that could weaken the skin!
- Use exfoliating products to unclog pores and remove dead skin cells. To preserve your skin flora, opt for chemical exfoliants (fruit acids, AHA, BHA, PHA...) without overusing them.

- Protect your skin from the sun with high SPF to avoid damage from UVB and UVA rays, year-round!

Preventing the rebound effect of acne is not limited to using appropriate products during or after sun exposure. It is also important to take care of your skin throughout the year by using products suited to your skin type, avoiding overly harsh products that could disrupt the skin microbiome, and adopting a regular skincare routine.

By enabling cosmetic brands to scientifically demonstrate their products' activity on the skin microbiome and exploring prebiotic or probiotic activities in their products, BYOME LABS paves the way for preserving our skin's balance, allowing you to fully enjoy the pleasures of summer with peace of mind.

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#### Global evaluation to assess acne skin by PhD Trials

Acne is a common skin alteration that affects people worldwide in different degrees being an important concern for the population, especially for teenagers. It is characterized by presenting inflammatory and non-inflammatory facial lesions such as papules, pustules, nodules and comedones. This has an impact on the skin at different levels:

- 1. Skin texture imperfections and pores.
- 2. Skin tone homogeneity redness and pigmentation.
- 3. Skin oiliness lipidic index and shininess.
- 4. Comfort & wellbeing sensorial methods.

At PhD Trials<sup>®</sup> we can evaluate and quantify the major aspects of acne alterations to globally evaluate the improvement resulted by a cosmetic treatment.

**Facial skin imperfections** lead to an important impact on skin texture. To evaluate the state and evolution of the skin surface can be used the AEVA-HE system, a 3D scanner that produces very accurate data about the skin roughness and smoothness. **Pores** are more visible on this skin condition population. VISIA® image analysis to study and obtain parameters of the number and area of the pores is an appropriate method to study the improvement in this aspect.

**Skin homogeneity** is also affected, dyschromia is visually perceived due to inflammatory redness or post-inflammatory pigmentation resulting in an uneven face skin. To evaluate these aspects can be used colorimetric measurements directly on the spot or quantify in a more global way by the acquisition of VISIA® images and their analysis by a specific software, obtaining parameters such as number of spots and area covered by red or brown spots.

**Skin oiliness** is generally increased in this skin condition showing the skin shinier and oilier. Products that act in the sense of regulating the level of sebum can be objectively evaluated using the well-known Sebumeter<sup>®</sup> system to assess the immediate decrease of lipidic index or the decrease of a sebum rate after a defined period after a single product application or after several weeks of a treatment. Also, it is proposed to evaluate the skin shininess reduction by the acquisition of VISIA® images taken with cross and parallel polarization light and posterior image analysis using specific algorithms.

Related with acne oiliness and using also VISIA® system, there is an interesting parameter to be evaluated that is the porphyrins level, bacterial excretions that can become lodged in pores. In this case, the images acquisition will be performed using UV light.

**Comfort and wellbeing** perception of people experiencing this skin condition can be importantly affected, resulting in low self-esteem and lack of confidence. PhD Trials® offers an advanced method to evaluate how this self-perception can be improved when using an effective treatment. The proposed sensorial method involves several evaluations among them the Galvanic Skin Response (GSR), Facial Expression Analysis, Eye Tracking and Electroencephalography (EEG) to conclude about how strong and what type of feelings and sensations a subject is experiencing.

Clinical studies performed in PhD Trials<sup>®</sup> involving robust and objective quantification of the presented parameters will give you a deep knowledge of the efficacy of your product. Contact PhD Trials<sup>®</sup> to know how we can help you in the development of your products targeting acne skin.







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## Use of a standardized photographic bench in evaluating acne by Eotech

Edouard Macé - CEO



Acne is an aesthetic discomfort that many people face during their lifetime. Not being related to aging and corresponding in most cases to a relatively short period of life, it is often suffered with resignation. However, it can become more permanent in some subjects and be experienced as a real handicap.

Several types of treatments are available today

(topical products, laser, etc.) and their persistane is very variable. The quantification of results is therefore essential to assess the performance of the treatments and their durability. It is mainly based on a visual scoring of patient's photos, hence the importance of being able to take photos in standardized and reproducible conditions.

Most of the existing photography systems allow you to take photos in good conditions but they have several disadvantages:

- Large footprint
- Significant initial operator training required by specific softwares
- Imperfect repositioning resulting in differences in facial angles and skin reflections
- Face holder hindering the full-face photo (chinrest,)

The solution proposed by Eotech, CBright, is based on this observation and capitalizes on the company's experience in positioning benches developed for several decades for 3D imaging and analysis of skin relief. This compact photographic bench offers a standardized environment and recordable positioning variables for each subject.

#### The CBright environment consists of:

- A neutral grey matte background (RAL 7011) with 2 folding side panels
- An illuminating frame consisting of 4 fixed intensity LED strips (4200°K)
- A head holding device attached to a motorized height-adjusting column

The shots are made using a CANON 250D camera combined with a 24mm lens (2.8 focal). Its 24Mp sensor and its very low weight are its main assets. It is controlled from a PC installed near to the CBright thanks to a specially developped software ensuring photos shooting support and managing the studies and the subjects. It integrates a "ghost" function, very useful for the fine repositioning of subjects.

The positioning variables of the subject specific to each shot are:

- Height of headrest
- The nature of polarized light
- The shooting angle (0, ± 30°, ± 45°, ± 60°, ± 90°)



In addition to these features, each shot is associated with its order number, subject ID and measurement time, so that each record is unique.

Apart from these benefits, the CBright exhibits a small footprint, thanks to its one-piece design. After the photo session is complete, the panels can be folded and the arm supporting the camera folded to 90°. The footprint is then one meter wide by 46 cm deep. This point is often

decisive for clinical testing laboratories or dermatological centers.

All these benefits recently decided the CLIPP (Centre Laser International de la Peau Paris) to select CBright for a study on the effectiveness of CUTERA's new AviclearTM laser in the permanent treatment of acne. Patients' expectations about this new device justify the need for perfectly comparable before/after photos.



Stay tuned!

## Coming in December

## FOCUS #11 Testing expert discussion around **Tolerance and safety evaluation**

If you wish to publish an article, please contact Ilona Salomon at palomon@skinobs.com

