THE EFFECTIVENESS OF EPIDERMAL GROWTH FACTOR SERUM FOR THE IMPROVEMENT OF FACIAL SKIN HYDRATION, ELASTICITY, PIGMENTATION, AND WRINKLES AT THE INSTITUTE OF DERMATOLOGY: A SINGLE-CENTERED PILOT STUDY WITH A ONE-GROUP BEFORE-AND-AFTER DESIGN

NCT05724589,

Research Methodology

1. Population and Samples

1.1 Population

The study will include Thai individuals, both male and female, aged between 40-65 years.

1.2 Sample size

There is no previous study to reference, the researchers have opted for a pilot study with a sample size of 28 participants.

1.3 Inclusion criteria

1) Gender: Male or female

2) Nationality: Thai, capable of reading, writing, and understanding the Thai language

3) Age: Between 40 - 65 years

4) Not using any facial creams or lotions that may impact the study, such as those with anti-aging effects (e.g., containing Vitamin A, fruit acids, or Hydroquinone)

5) Individuals with mild to moderate skin conditions, assessed using the Hyperpigmentation status scale 1-2, and Wrinkle severity grading scale 2-3.

6) Willingness to avoid sunbathing and minimize sun exposure during the study period

7) Availability for scheduled research team meetings

1.4 Exclusion criteria

1) Individuals with allergies to components tested in the serum

2) History of any cancer, especially skin cancer

3) Acute or chronic skin conditions causing inflammation, such as Eczema or Atopic dermatitis

4) Pregnant women (positive pregnancy test), breastfeeding mothers, or those planning pregnancy during the study

5) Individuals who underwent facial procedures (e.g., laser treatments, energy-based devices like focus ultrasound, LED, or Infrared, facial surgeries, filler injections) within the last 3 months before joining the study

6) Skin damage in or near the testing area, including sunburn, tattoos, or scars

7) Consumption of vitamin supplements and/or hormonal supplements

2. Research Format

Pilot study and one group before-after design.

2.1 Research Operation Steps

1) Volunteer Preparation Phase

Selection of volunteers based on inclusion and exclusion criteria.

Schedule a meeting to provide information about the research to the volunteers.

Collect personal and relevant research-related data from volunteers during the meeting.

2) Inter-rater Reliability Assessment

Two dermatologists will review approximately 20 images of the sample group.

Assess inter-rater reliability and calculate the Interclass Correlation Coefficient (ICC).

If ICC is less than 0.75, both assessors will meet to align their evaluations before the actual research begins.

3) Skin Condition Measurement for Volunteers

Volunteers receive appointments for skin condition measurements. Skin measurements occur at the same time each week. Rescheduling is allowed for volunteers unable to make appointments due to non-study-related commitments or COVID-19 concerns.

4) Laboratory Location

The research will be conducted at the Biogenomics Laboratory on the 4th floor of the Queen Sirikit National Institute of Child Health, Monday to Friday.

5) Skin Cleaning Instructions for Volunteers

Volunteers are advised to cleanse their skin lightly with the provided soap before testing. Cleaning conditions are standardized to maintain consistency across all tests.

6) Evaluation Parameters

Five parameters will be measured using various tools to assess experiment results.

Measurement of skin moisture and water loss using Corneometer and Tewameter, respectively.

Measurement of skin elasticity using Cutometer.

Measurement of skin color using Mexameter.

Evaluation of skin wrinkles using Visioscan.

Assessment of facial skin changes using Visia-CR imaging.

These steps are meticulously designed to ensure high-quality and reliable data for the dermatological research.

The measurements for Corneometer, Tewameter, and Cutometer will be taken at four specific positions:

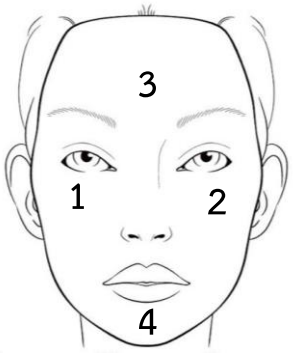
- Right Malar prominence area

- Left Malar prominence area

- Central forehead

- Central chin

Measurements will be performed three times at each position, as illustrated in the diagram below. Each volunteer will calculate the average value for all four areas to represent their data. Assessors will use a marking technique on transparent sheets to ensure consistent measurement points are maintained across all sessions.



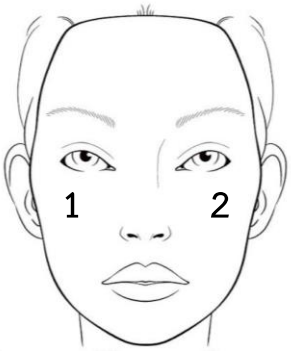
The positions for measurements using Corneometer, Tewameter, and Cutometer

When using the Mexameter to measure the Melanin Index (MI), readings will be taken at two specific positions:

- Right Malar prominence area

- Left Malar prominence area

Measurements will be conducted three times at each position, as depicted in the diagram below. Each volunteer will calculate the average value for both areas to represent their data. Assessors will utilize a marking technique on transparent sheets to ensure consistent measurement points across all sessions.



The positions for measurements using Mexameter

Visioscan is used to assess wrinkles using a 4-grade percentage wrinkle improvement scale as follows:

Grade 1 (0-25%): No change to a slight improvement in wrinkles.

Grade 2 (> 25-50%): Moderate improvement in wrinkles.

Grade 3 (50-75%): Marked improvement in wrinkles.

Grade 4 (>75%): Significant to no wrinkles.

The assessment involves scoring at four positions:

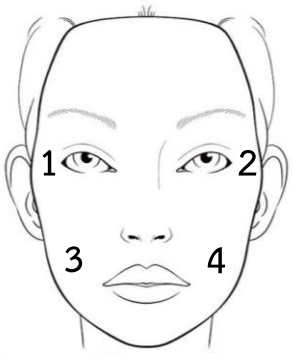
- Right crow feet area

- Left crow feet area

- Right cheek line area

- Left cheek line area

Positions are determined based on Hamilton's classification of contour changes of facial skin, specifically targeting static wrinkles effectively. Volunteers will calculate the average value for both areas to represent their data. The assessment will be performed by two board-certified dermatologists, each evaluating both areas, ensuring a thorough and reliable evaluation of wrinkle improvement.



The positions for measurements using Visioscan

Visia-CR is utilized as a detailed facial imaging system to compare changes before and after product usage. The assessment is done by comparing images using the Subject Global Aesthetic Improvement Scale (SGAIS) as follows:

Score 1: Worse (no improvement)

Score 2: Mild improvement (<25% improvement)

Score 3: Improvement (25-49% improvement)

Score 4: Much improved (50-74% improvement)

Score 5: Very much improved (75% improvement or more)

The evaluation is conducted by two board-certified dermatologists, each specializing in skin health. The images captured by Visia-CR allow for a thorough comparison of facial conditions before and after product usage, providing a detailed understanding of the product's impact on the skin's aesthetic improvement.

The medical technicians will perform measurements of skin moisture, water loss, skin elasticity, skin color, and capture facial images using a specialized imaging device, taking approximately 30 minutes for each

participant. This research will measure these parameters on the first day (d1) before applying EGF serum to establish a baseline.

7) Baseline Measurements:

Participants will undergo measurements on the first day, assessing moisture, water loss, skin elasticity, skin color, and facial images using Corneometer, Tewameter, Cutometer, and imaging devices.

8) Application of EGF Serum:

Participants will apply EGF serum twice daily for 8 weeks, using three drops each time, covering the entire face.

9) Sunscreen Application:

After using EGF serum in the morning, participants will apply Skin Intelligence Sunscreen Cream SPF30 PA+++, using 2 fingertip units (approx. 1 gram) each time.

10) Second Visit (2 Months):

Participants return for a second assessment after 2 months, with measurements conducted similar to the baseline.

11) Subsequent Visits:

Participants stop using the serum and return for measurements in the 3rd and 4th months, following the same procedure.

Note:

If adverse events occur or if there are signs of an allergic reaction, participants will cease product usage and receive standard treatment from the institute. Severe reactions may lead to the participant's exclusion from the study.

3. The materials and equipment

Tested Product:

Objective: Serum containing 75 ppm Epidermal Growth Factor (EGF)

Composition:

0.5% Acrylate Copolymer (Thickener)

0.5% Triethanolamine (TEA) (Neutralizer)

Phenoxyethanol / Chlorphenesin (Preservative)

Deionized Water (Solvent)

EGF 75 ppm

Safety Standards and Packaging:

- The product must adhere to safety standards, be free from contaminants, and not contain harmful substances. The product will undergo Microbiology Test (contamination test) by ALS and Irritation Test by DermScan Asia before testing begins.

- Phototoxicity testing is not required based on research by Henry et al., indicating no phototoxicity in the proteins present in the product (25).

- Cytotoxicity testing of rh-EGF by NSTDA showed minimal contamination below injectable drug standards, and animal testing indicated no cytotoxicity.

- The product will be stored according to the sponsor company's recommendations, at a temperature below 30 degrees Celsius, avoiding sunlight, and has an approximate shelf life of 2 years.

Equipment:

Temperature and Humidity Control Room:

Objective: Maintain conditions at 20±3°C and 50±3% RH.

Tewameter TM 300:

Manufacturer: Courage & Khazaka (C&K) Electronic GmbH, Germany

Purpose: Measures transepidermal water loss (TEWL) from the skin.

Corneometer CM 825:

Manufacturer: Courage & Khazaka (C&K) Electronic GmbH, Germany

Purpose: Measures skin hydration.

Cutometer MPA580:

Manufacturer: Courage & Khazaka (C&K) Electronic GmbH, Germany

Purpose: Measures skin elasticity.

Mexameter MX18:

Manufacturer: Courage & Khazaka (C&K) Electronic GmbH, Germany

Purpose: Measures melanin index (MI) for skin color.

Visioscan VC98:

Manufacturer: Courage & Khazaka (C&K) Electronic GmbH, Germany

Purpose: Measures skin wrinkles.

Visia-CR:

Manufacturer: Canfield Scientific, Inc., US

Purpose: Captures detailed facial images for before-and-after comparisons.

4. Table of activities:

Activities \ Day/wk	d1	2 wk	4 wk	8 wk	12 wk	16 wk
Record data	√					
Apply EGF serum	√	√	√	√		
Apply sunscreen cream	√	√	√	√	√	√
Measuring skin moisture and water loss using Tewameter and Corneometer	√			√	√	√
Measuring skin elasticity using Cutometer	√			√	√	√
Measuring melanin index using Mexameter	√			√	√	√
Measuring wrinkle using Visioscan	√			√	√	√
Capturing facial images using Visia-CR	√			√	√	√
Evaluation by Specialists (Two Board-Certified Dermatologists)				√	√	√
Assessment of Participant Satisfaction				√	√	√
Assessment of adverse effects				√	√	√

Assessment of adverse effects via videocall application line		√	√			

5. Informed Consent Process

Before volunteers participate in the research project, the researcher will explain the research objectives, methodology, potential risks or side effects of the research, treatment, anticipated benefits from the research, and inform the volunteers of their right to voluntarily choose to participate or withdraw from the research at any time without coercion. Volunteers will be assured of medical care accessibility during and after participation. Once the volunteers decide to join, they will sign a consent form, certifying their voluntary participation in the research project.

6. Data Collection

Data collection for all participant types will commence after obtaining approval from the ethics committee at the Institute of Dermatology, and information will be gathered through the following:

Participant Assessment Data:

Pre-research assessment (baseline) and post-treatment assessments at 2, 3, and 4 months.

Research Outcomes:

6.1 Primary Outcome: Measurement of five parameters, including skin moisturization, skin elasticity, skin color, skin wrinkle assessment, and facial imaging using the Visia-CR system. Measurements will be taken at baseline, 2 months, 3 months, and 4 months.

6.2 Secondary Outcomes:

1) Assessment of participant satisfaction post-treatment at 2, 3, and 4 months using a quartile grading system (0 = unsatisfied, 1 = slightly satisfied, 2 = satisfied, 3 = very satisfied).

2) Evaluation of adverse events, including irritation, rash occurrence, new dark spots, and infections post-treatment through videocall applications at 2 weeks and 4 weeks. On-site evaluations will occur at 2 months (after completion), 3 months, and 4 months.

7. Statistical Analysis

7.1 Descriptive Statistics for General Participant Information: Descriptive statistics will be used for continuous quantitative data, presenting mean and standard deviation or median and interquartile range (IQR). For non-continuous quantitative data and qualitative data (ordinal scale, nominal scale), results will be reported as percentages.

7.2 Primary Outcome Analysis: The primary outcome data, comparing values for skin moisturization, water loss from the skin, skin elasticity, skin color measurement, and wrinkle assessment before and after using the serum at 8 weeks, 12 weeks, and 16 weeks, will be analyzed. If the data follow a normal distribution, a repeated-measure ANOVA will be used, with $p < 0.05$ considered statistically significant. In cases where the data distribution is not normal, the Wilcoxon signed-rank test will be employed.

7.3 Descriptive Statistics for Adverse Events: Descriptive statistics, reported as percentages, will be used to analyze adverse events after using the serum with Epidermal Growth Factor.

7.4 Descriptive Statistics for Participant Satisfaction: Descriptive statistics, reported as percentages, will be used to analyze participant satisfaction after using the serum with Epidermal Growth Factor.