CLINICAL STUDY PROTOCOL

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Study Title	A Clinical Study to Evaluate the Interstitial Dermal Fluid, Efficacy and Sun Protection Factor (SPF) of Two Test Materials According to an Adaptation of the Guidelines of the FDA Over-the-Counter Monograph M020: Sunscreen Drug Products for Over-the-Counter Human Use
Sponsor	Good Molecules, LLC 8605 Santa Monica Blvd #21334, West Hollywood, CA 90069
Sponsor Representative	Ivan Souza, R&D Director
Test Materials	• Good Molecules Hybrid SPF 30 DII, code GM23091405 • Benchmark Chemical SPF 30, lot # GM22349110-B • No SPF Placebo, lot # GM22349110-A
Principal Investigator	Alexis Chantal, PA-C Director Clinical Operations, Eurofins CRL Cosmetics LLC
Sub-Investigators	Carrie Cobb, MD FAAD Medical Director, Eurofins CRL Cosmetics LLC Chelcia Mejia Manager, Photobiology Yvette Gonzalez SPF Team Lead

Investigating Laboratory	Eurofins | CRL, Cosmetics LLC 411 W 4th St. Suite 100 Winston-Salem, NC 27101 Phone: (336) 725-6501
Institutional Review Board	Allendale Investigational Review Board 14 Somerset Lane Old Lyme, CT 06371 Telephone:(860) 575-0092
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TABLE OF CONTENTS

1.0 Introduction 5
2.0 OBJECTIVES 5
3.0 STUDY DESIGN 6
4.0 SPF Standard 7
5.0 TEST MATERIALS AND RECORD RETENTION 7
5.1. IDENTIFICATION 7
5.2. STORAGE AND RETENTION 8
5.3. PRODUCT USE INSTRUCTIONS 8
5.4. APPLICATION INSTRUCTIONS FOR TEST MATERIALS 9
6.0 STUDY POPULATION 10
6.1. INCLUSION CRITERIA 11
6.2. EXCLUSION CRITERIA 12
6.3. SUBJECT TERMINATION AND WITHDRAWAL 13
7.0 RANDOMIZATION 14
8.0 BLINDING 14
9.0 STUDY EVALUATIONS 14
9.1. SOLAR SIMULATORS AND RADIOMETER/DETECTOR SYSTEMS 14
9.2. CHROMAMETER 15
9.3. DIGITAL CAMERA 16
9.4. DERMAL EVALUATION 17
9.5. DOSE SERIES INFORMAITON 18
9.6. MEXAMETER MX 18 MDD 4 19
9.7. THERMOMETER READING 19
9.8. COLLECTION OF DERMAL INTERSTITIAL FLUID 20
9.8.1. Dermal Interstitial Fluid Analysis – Eurofins Discovery 21
9.8.1.1. Project Description 21
10.0 TEST METHOD 22
10.1. SUBJECT IDENTIFICATION 22
10.2. DAY 1 22
10.2.1. Subject Enrollment 22
10.2.2. Initial Unprotected MED (MEDuI) Dose Administration 23
10.3. DAY 2 23
10.3.1. MEDuI Determination 23
10.3.2. Application of Test Material(s) and Standard Sunscreen 23
10.4. DAY 3 (FINAL VISIT) 24
10.4.1. Evaluation of Responses to UV Doses for MEDuR, ssMEDp, and tpMEDp 24
11.0 PROTOCOL AMENDMENTS AND DEVIATIONS 24
12.0 DATA TABULATION 25
12.1. SPF COMPUTATION 25
12.2. EFFICACY AND SAFETY PARAMETERS 26
13.0 Invalid /Missing Test Data 27
14.0 ETHICS 28

14.1. Ethical Conduct of the Study 28
14.2. Ethics Committee and Regulatory Approval 28
14.3. Informed Consent 28
14.4. Subject Confidentiality 28
15.0 ADVERSE EVENTS 29
15.1. Definition of an Adverse Event 29
15.2. Definition of a Serious Adverse Event 29
15.3. Documentation and Reporting of Adverse Events 29
15.4. Expected Events 30
15.4.1. UV Expected Events 30
15.4.2. Test Material Expected Events 30
15.4.3. Process for Suspected Allergic Reactions 30
15.5. Micro Needling Expected Reactions 31
16.0 QUALITY ASSURANCE PARAMETERS 32
17.0 CLINICAL STUDY REPORTING 32
18.0 References 32
19.0 PROTOCOL APPROVAL 33
19.1. Sponsor Approval 33
19.2. Principal Investigator Acceptance 33
19.3. Sub-Investigator Acceptance 34
Appendix I – Randomization 35
Appendix II – Collection of Dermal Interstitial Fluid Instructions 38

1.0 INTRODUCTION

This modified FDA Over-the-Counter protocol describes the procedures for determining the static sun protection factor (SPF).

The Static SPF is defined as the ratio of the minimal erythema dose of ultraviolet radiation for sunscreen-protected skin to that for unprotected skin. The minimal erythema dose (MED) is the lowest dose of ultraviolet (UV) radiation that produces the first perceptible unambiguous erythema (sunburn) with clearly defined borders (filling more than 50% of the exposure subsite), 16 to 24 hours after UV exposure administration. UV radiation doses, expressed in J/m^2-eff, are administered using a xenon arc lamp that simulates solar radiation and satisfies the requirements of the FDA Over-the-Counter Monograph M020 [1].

Interstitial dermal fluid serves as an immediate environment for skin cells, facilitating the exchange to the fluid-filled spaces between the tissue cells in the dermis, which is the outer layer of skin. This fluid, also known as interstitial fluid, serves as an immediate environment for all cells in the body, facilitating the exchange of substances necessary for cellular processes.

Eurofins Discovery will conduct an evaluation of 350 human interstitial fluid samples for levels of IL-1α, IL-6, IL-8, and VEGF-A using Luminex methodology. Each sample is to be tested in duplicate at one optimal dilution. Prior to sample analysis, a feasibility study will be conducted to assess matrix interference and identify the optimal sample dilution.

2.0 OBJECTIVES

The first objective of this study is to assess the effects of known UV Radiation dosage on light and dark skin. The second objective is to assess the protective effect of two SPF test materials, placebo product, and standard sunscreen (‘Pad O’) on skin, before and after a known UV Radiation dosage. The third objective of this study is to collect the interstitial dermal fluid from approximately 60 subjects and send to Eurofins Discovery for analysis.

3.0 STUDY DESIGN

Approximately 70 subjects (35 subjects per light and dark group) will be enrolled in this clinical study (to complete with a minimum of 10 subjects per skin type (I-VI) to assess the effects of known UV Radiation dosage on light and dark skin, assess the protective effect of two SPF test materials, placebo product, and standard sunscreen ('Pad O') on skin, before and after a known UV Radiation dosage and collect the interstitial dermal fluid from approximately 60 subjects and send to Eurofins Discovery for analysis. The following study evaluations will be obtained: SPF Determination, Visual Assessment of Erythema, Chromameter and Mexameter measurements, Digital photography, Thermometer readings and Collection of Dermal Interstitial Fluid (IDF).

A study schedule appears below.

Table 1: Study Schedule

Study Procedures	Day 1	Day 2	Day 3
Informed Consent	X		
Determine if Subjects Qualify through Medical History/Eligibility Questionnaire	X		
Subjects will acclimate to room temperature for 10 minutes before evaluations.	X	X	X
Baseline Visual Assessment of Erythema By Trained SPF Technician	X		
Baseline Mexameter Measurements for Melanin and Erythema Index By Trained SPF Technician	X		
Baseline Digital Camera Images of Test Sites	X		
Baseline Chromameter Measurements for L*a*b* and ITA Calculation By Trained SPF Technician	X		
Baseline Thermometer Reading for Skin Temperature	X		
Baseline Collection of Dermal Interstitial Fluid By Trained Technician (Micro Collection Needles)	X		
Administer UV Doses to unprotected site for MED_{uI}	X		
Monitor for Adverse Events	X	X	X
Identify and Document Concomitant Medications	X	X	X
Evaluate UV Responses; Determine MED_{uI}		X	
Apply Standard Sunscreen for SPF (Pad-O)		X	
15-Minute Drying Time		X	
Apply Static and Placebo Test material(s)		X	
15-Minute Drying Time		X	
Administer UV Doses to Protected Site for ssMEDp and tpMEDp, and to Unprotected Site for MEDuR		X	
Post Treatment Visual Assessment of Erythema by Trained SPF Technician (Evaluate UV Responses; Determine ssMEDp, tpMEDp, and Unprotected MEDuR)			X
Post Treatment Mexameter Measurements for Melanin and Erythema Index By Trained SPF Technician			X
Post Treatment Digital Camera Images of Test Sites			X
Post Treatment Chromameter Measurements for L*a*b* and ITA Calculation By Trained SPF Technician			X
Post Treatment Thermometer Reading for Skin Temperature			X
Post Treatment Collection of Dermal Interstitial Fluid By Trained Technician (Micro Collection Needles)			X
Compliance Assessment		X	X

4.0 SPF STANDARD

A standard sunscreen formulation will be tested concurrently with test materials. The standard will be 7% Padimate-O and 3% Oxybenzone ('Pad-O') Certified Formula SPF Standard (SPF 16.3 ± 3.43 or 12.87 to 19.73).

5.0 TEST MATERIALS AND RECORD RETENTION

5.1. IDENTIFICATION

The test materials will be identified by Eurofins | CRL, Cosmetics LLC (ECRL) study numbers, in accordance with distribution. Test material identification is as follows;

Sponsor Identification	Identification Number
Good Molecules Hybrid D II SPF 30, code GM23091405	ECRLNC2026-0016-01
Benchmark Chemical SPF 30, lot # GM22349110-B	ECRLNC2026-0016-02
No SPF Placebo, lot # GM22349110-A	ECRLNC2026-0016-03

The following accessory materials will be provided by ECRL:

Accessory Product Identification
7% Padimate-O and 3% Oxybenzone "Pad-o"

The following accessory materials will be provided by Sponsor:

Accessory Product Identification
Fluid Collection Kits and Ascilion Extraction Units

The Sponsor assumes responsibility for the purity, stability, characterization, and adequate preservation of the test materials. The Sponsor shall provide assurance that the test materials submitted have been determined to be safe for use in humans and have been developed following the guidelines in the FDA Over-the-Counter Monograph M020.

5.0 TEST MATERIALS AND RECORD RETENTION (CONTINUED)

5.2. STORAGE AND RETENTION

Prior to use in the clinical study and for the duration of the study, the test materials will be securely stored at room temperature and humidity and protected from direct sunlight, unless otherwise directed by the Sponsor. One subject's worth of the test materials will be retained by ECRL for a minimum of 6 months per ECRL SOP.

All original forms of this study will be retained by ECRL as specified in ECRL Standard Operating Procedures.

5.3. PRODUCT USE INSTRUCTIONS

Test material, placebo and standard sunscreen SPF ('Pad-O') use instructions:

The technician will use a double-weighing procedure incorporating weighing the syringe/pipette + product + fingercot, prior to and after test material application, to ensure the required quantity of test material is delivered. The technician will apply up to* 100 ± 2.5 mg (2.0mg/cm^2 ± 0.05mg/cm^2) of the test material, evenly using a fingercot, in its randomly designated rectangle.

*Alternatively, 80 ± 2mg (2.0mg/cm^2 ± 0.05mg/cm^2) of each test material will be applied (Section 5.3 and 5.4) if 40 cm^2 test sites are used.

Approximately 20 droplets of the test material will be deposited with a syringe/pipette on the designated test site. Using the fingercot and light pressure, the test material is spread with circular movements followed by vertical and horizontal movements or a similar application method. The application time will be between 20 to 50 seconds, dependent upon the ease of test material application.

5.0 TEST MATERIALS AND RECORD RETENTION (CONTINUED)

5.4. APPLICATION INSTRUCTIONS FOR TEST MATERIALS

Test materials will be thoroughly mixed and/or shaken well prior to application.

Prior to test material application, the testing area(s) may be cleaned using a dry cotton pad or equivalent.

Evenness of application will be verified by observation with a Wood's lamp. If noticeable non-uniformity or streaking of the test material is noted, the test site shall be rejected and may not be used for testing. If another test site is available, reapplication of the test material may be attempted.

The technician will document product formula designations, test site locations, amount of time for test material application, application time of test material, along with the test material applied weight.

Test materials will be applied accordingly such that dry time will be at least 15 minutes, prior to UV exposures, or prior to water immersion, as applicable.

6.0 STUDY POPULATION

Approximately 70 subjects (35 subjects per light group Fitzpatrick skin type I – III, approximately 10 subjects for each Fitzpatrick skin type, and dark group Fitzpatrick skin type IV – VI, approximately 10 subjects for each Fitzpatrick skin type) will be enrolled in this study (to complete with 60 subjects, 30 subjects per light group Fitzpatrick skin type I – III, 10 subjects for each Fitzpatrick skin type, and dark group Fitzpatrick skin type IV – VI, 10 subjects for each Fitzpatrick skin type,).[1]

See Table 2 below for Fitzpatrick Skin Types.

Table 2 Fitzpatrick Skin Types (FST):

ITA Value (Individual Typology Angle)	Skin Color Categories	Fitzpatrick Skin Type	Fitzpatrick Definitions
>55°	Very Light	I	Always burns easily; never tans
>41° to 55°	Light	II	Always burns easily; tans minimally
>28° to 41°	Intermediate	III	Burns moderately; tans gradually
>10° to 28°	Tan	IV	Burns minimally; always tans well
>-30° to 10°	Brown	V	Rarely burns; tans profusely
≤-30°	Black	VI	Never burns; deeply pigmented

Any subjects who are disqualified due to non-compliance or adverse event will be replaced. Subjects whose data do not permit successful computation of SPF values will be replaced, provided the maximum study sample is not exceeded.

[1] As with all clinical studies, it is beyond the control of the test facility to guarantee the number of subjects that will actually complete the study. It is the responsibility of the Sponsor to commission a sufficient number of subjects in order to maximize the probability of ending with the intended target number.

6.0 STUDY POPULATION (CONTINUED)

6.1. INCLUSION CRITERIA

A subject may be eligible for study participation if all of the following criteria are met:

a) Subject is male or female between 18 - 75 years old, providing legally effective, written informed consent in conformance with 45 CFR Parts 160 and 164;
b) Subject has a Fitzpatrick Skin Type of I, II, or III, Light Skin Group or IV, V, or VI Dark Skin Group with an approximately equal distribution of all skin types (approximately 10 of each Fitzpatrick skin type) within each group as determined by ITA Angle;
c) Subject agrees to undergo the micro needling procedure on their back (a total of 5 sites);
d) Subject should not have their back shaved or waxed 3 days prior to the start of the study;
e) Subject is able to read, write, speak, and understand English;
f) Subject is in good general health;
g) Subject agrees to avoid UV exposure to the test area for the duration of the study;
h) Subject is willing to wear loose-fitting clothing that exposes mid to lower back for study visits;
i) Subject is dependable and able to follow directions as outlined in the protocol and anticipates being available for all study visits;
j) Subject is willing to participate in all study evaluations and follow all instructions;
k) Subject has a current Subject Profile Form on file at ECRL;
l) Subject has uniform skin color over the test area; ITA° calculation range ≤10-point difference (example: 35.6°-45.6°);
m) Subject agrees to sign a Photography Release Form, providing consent for the capture of digital images for use in relation to this clinical study;
n) Subject has completed a HIPAA Authorization Form in conformance with 45 CFR Parts 160 and 164;

6.0 STUDY POPULATION (CONTINUED)

6.2. EXCLUSION CRITERIA

A subject is not eligible for study participation if any of the following criteria are met:

a) Female subject is pregnant, nursing, planning a pregnancy, or using inadequate birth control;
b) Subject has a history of abnormal response to sunlight/UV radiation; sensitivity to any ingredient of the test materials, standard or latex; or any known allergies to sunscreen, cosmetic, and/or toiletry products;
c) Subject has a history of or any current significant dermatological condition, such as atopic dermatitis (eczema), psoriasis, skin cancer, melanoma, active dermal lesions, nevi, keloids blemishes, abnormal scarring or moles, lupus, diabetes, or connective tissue disease that would increase the risk associated with participation;
d) Subject has received treatment with sympathomimetics, antihistamines, vasoconstrictors, non-steroidal anti-inflammatory agents, and/or systemic or topical corticosteroids within one week prior to initiation of the study.
e) Subject has used a prohibited medication (per separately provided prohibited medication list) within the specified timeframes prior to initiation of the study;
f) Subject has used/applied any personal care products (e.g. lotions, sunscreens, sunless tanners) and/or topical medications in/on the testing area (the back between the shoulder blades and the waist line) within 24 hours prior to Day 1 or during the study;
g) Subject has any known communicable disease(s) (*e.g.* HIV, STD's, Hepatitis B, Hepatitis C, etc.);
h) Subject has any skeletal protrusions and/or extreme areas of curvature in the test area;
i) Subject with medical treatment/vaccination planned during the study, which would make the subject ineligible, place the subject at undue risk, or confound the outcome of the study, per the discretion of the PI.
j) Subject has undergone any surgical procedure in the last 12 months;
k) Subject has undergone chemical or physical treatment procedures in the test area within the last 12 months;
l) Subject is planning hospitalization during the study;
m) Subject exceeds the weight limit of 300 pounds due to equipment limitations;
n) Subject has any condition that might confound the study results, increase the risk associated with participation, or interfere with study participation;
o) Subject has any visible sunburn or suntan in testing area;
p) Subject has used an artificial tanning lamp or had recent sun exposure to the test area within the past 2 months;
q) Subject has participated in another UV exposure clinical study in the last 2 months, or is currently enrolled in a clinical study utilizing the same test site (i.e., no back patches during a UV exposure study).

6.0 STUDY POPULATION (CONTINUED)

6.3. SUBJECT TERMINATION AND WITHDRAWAL

A subject may be discontinued from study participation at any time if the Principal Investigator, or designated medical staff feels that it is not in the subject's best interest to continue.

The following is a list of possible reasons for study discontinuation other than non-compliance with inclusion and exclusion criteria:

a) Adverse event that, in the opinion of the PI, would be in the best interest of the subject to discontinue study participation.
b) Protocol violation; all deviations must be documented.
c) Sponsor request for early termination of the entire study.

All subjects are free to withdraw from participation at any time, for any reason, specified or unspecified, and without prejudice. Reasonable attempts will be made by the Principal Investigator, or designee to provide a reason for subject withdrawals. The reason for the subject's withdrawal from the study will be specified in the subject's source documents and included in the final report.

7.0 RANDOMIZATION

In this study, test sites will be randomized to ensure blinded evaluations. All qualified subjects will be assigned a randomization scheme on Day 1 of testing based on the study objective and listed in Appendix I. [1]

The sites for where the micro needling procedure will take place are listed in Appendix I.

[1]The assigned randomization may be modified by the technician performing testing to fit the subject's test area avoiding beauty spots, scars, etc. Any randomization modifications will be documented.

8.0 BLINDING

Subject will be blinded to the name of the test material. The investigatory staff will not be blinded, with the exception of the evaluator. Test materials will be labeled with unique ECRL study identification upon test material receipt by ECRL.

For practical reasons, the only site that is known to the evaluator is the MEDuI site. If the evaluator has questions about any discrepancies in test site location or issues that may have occurred during testing, the evaluator may receive assistance from the technician who applied the test materials and administered the UV doses; however, the technician may not influence the evaluator in the grading of UV responses.

9.0 STUDY EVALUATIONS

9.1. SOLAR SIMULATORS AND RADIOMETER/DETECTOR SYSTEMS

Solar simulators used in the study will be multi-port xenon arc lamps (Model 601; Solar Light Co., Philadelphia) equipped with 1 mm WG320 UVC blocking filters, 1 mm UG-11 visible and infrared blocking filters, and heat-rejecting dichroic mirrors, that comply with the FDA Over-the-Counter Monograph M020: Sunscreen Drug Products for Over-the-Counter Human Use [1] (Model 601, Solar Light Co., Philadelphia). The size of the exposure site is $\geq 0.5 cm^2$. The solar simulator system used on subjects will not be interchanged during testing with the exception of a malfunction.

The Model 601 solar simulator will be used with radiometer DCS-2/PMA2100 and detector Erythema PMA2108 showing output in MED/hr (Solar Light Co., Philadelphia).

The technician will document solar simulator, radiometer and detector, UV doses, lamp effective irradiance reading before and after UV doses, Immediate Response Codes after each UV subsite exposure, and the UV dose series completion time.

9.0 STUDY EVALUATIONS (CONTINUED)

9.1 SOLAR SIMULATORS AND RADIOMETER/DETECTOR SYSTEMS (CONTINUED)

Requirements for Irradiation Source

1. Each solar simulator will have a minimum warm up time of 20 minutes prior to capturing the lamp effective irradiance readings.
2. Continuous emission spectrum from 290 to 400 nm
3. Emission spectrum measured at least annually, after replacement of lamp bulb or any change in optical components, using an appropriate spectroradiometer system that is calibrated to a NIST traceable source.
4. Daily radiation intensity monitored before and after each phototest, or at least at the beginning and end of each test day using an erythemally-weighted radiometer with a calibration consistent with the spectroradiometer system.
5. No significant time-related fluctuations in the exposure plane (±20%)
6. Good beam uniformity (±20% from centerline reading)
7. UVAII (320-340 nm): ≥ 20% of Total UV Irradiance
8. UVAI (340-400 nm): ≥ 60% of Total UV Irradiance
9. Total Irradiance from 250 to 1,400 nm ≤1,500 W/m^2
10. Percent erythemal dose contributions as shown in Table 3:

Table 3.:

Wavelength Range	Percent Erythemal Dose Contribution
<290	**<0.1**
290-300	**1.0-8.0**
290-310	**49.0-65.0**
290-320	**85.0-90.0**
290-330	**91.5-95.5**
290-340	**94.0-97.0**
290-400	**99.9-100.0**

9.2. CHROMAMETER

This study will use a reflectance colorimeter, CM-700d (Konica Minolta) or equivalent, a handheld device that measures skin color in the L*a*b* color space. The L*, a*, and b* values will be measured from at least 3 different locations on the subject's back and then used to calculate the ITA°, which can be used to estimate a subject's MED$_u$[2].

Chromameter measurements will be obtained at Day 1 Baseline and Day 3 post treatment for L*a*b* for the following test sites: Untreated area, and for the areas treated with No SPF Placebo, Mineral SPF 30, and Chemical SPF 30 before (Day 1) and after UV Exposure (Day 3).

9.0 STUDY EVALUATIONS (CONTINUED)

9.3. DIGITAL CAMERA

Photographs will be taken of test sites using a Cannon Rebel T7 (or equivalent to be listed on the study specific paperwork). The camera system should be agreed upon with sponsor before study initiation, if requested. Photographs (which do not reveal the subject's identity) may be taken for training purposes, to document unexpected results, to document AEs, for scientific purposes, and possibly for research/scientific publications and may be anonymously used for marketing.

Images will be saved using the following filename:

Sub_1_L1

Images will be saved into a shared drive that will be shared with the Sponsor.

Images will be obtained prior to any microneedling extractions.

One photo per subject will be obtained with each test site marked at Baseline. At the Day 3 timepoint, one photo per test site with each test site marked will be obtained.

9.0 STUDY EVALUATIONS (CONTINUED)

9.4. DERMAL EVALUATION

A technician will determine post UV dose immediate responses using the assessment scale listed below:

Table 4. Immediate Response Codes

N	No Immediate response
D	Immediate Darkening
R	Immediate Reddening
H	Immediate Generalized Heat Response Spreading Beyond the Subsite

A trained evaluator will grade responses of the UV exposed sites, 16-24 hours post UV Dose administration, under warm white fluorescent or tungsten illumination of at least 450 lux, using the grading scale shown below in Table 5.

Table 5. Grading Scale for Erythema Responses to UV Doses Administered to Untreated Sites and Sunscreen Treated Sites

0	No erythema response or inconclusive response
0.5	Ambiguous erythema and/or no clear border and/or not filling more than 50% of the exposure subsite. (Minimally perceptible erythema)
1	Perceptible unambiguous erythema with clearly defined borders (filling more than 50% of the exposure subsite)
2	Moderate to intense erythema*

Grading for subjects with Fitzpatrick skin types 4, 5 and 6 will likely be inconclusive due to their darker skin tone and difficulty to show redness.

For Fitzpatrick skin types 4, 5 and 6 site darkening alignment with the grading scale above may be used if subjects present with no redness.

*If moderate or intense erythema does not reach borders of exposed site, an explanation will be provided in the comments section of evaluation forms.

Table 6. Grading Scale for Erythema on Day 1 and Day 3

Erythema
0 = None
± = Barely Perceptible
1 = Slight
2 = Mild
3 = Moderate
4 = Severe

9.0 STUDY EVALUATIONS (CONTINUED)

9.4 DERMAL EVALUATION (CONTINUED)

The MED will be determined as the first exposure site in the series that produces an erythema grade of at least 1 (Perceptible unambiguous erythema with clearly defined borders (filling more than 50% of the exposure subsite)). The progression of erythema grades must be consistent with the UV doses administered.

The trained evaluator will document grades for UV-exposed test site(s), any comments and time of completion.

Photographs of the test area, which do not reveal the subjects' identities, may be taken for training purposes, to document unexpected results or to document Adverse Events.

9.5. DOSE SERIES INFORMAITON

A series of 5 UV Doses will be used.

Table 7. UV Doses for MEDuI

Expected MEDu				
0.64x	0.80x	1.00x	1.25x	1.56x

Table 8. Over-the-Counter Monograph M020 Standard Dose series for protected sites

Expected SPF	Multiple of Expected SPF and Subject MEDuI				
SPF < 8	0.64x	0.80x	1.00x	1.25x	1.56x
SPF 8 to 15	0.69x	0.83x	1.00x	1.20x	1.44x
SPF >15	0.76x	0.87x	1.00x	1.15x	1.32x

The technician will document the UV Doses administered to each test site.

9.0 STUDY EVALUATIONS (CONTINUED)

9.6. MEXAMETER MX 18 MDD 4

The Mexameter provides a quick, easy and accurate method to measure melanin and erythema in the skin.

The Mexameter is a spectrometer measurement technique, based on light reflection and absorption. The probe emits three wavelengths of light, chosen to correspond to the different absorption rates of melanin and hemoglobin. This light emitted by the probe is reflected by the skin and the receiver in the probe measures this reflected light. It is only the diffuse and scattered light that is measured. The results are shown in 1 second as index numbers between 0 and 999. The probe allows the measurement to be made quickly (1 second). The probe head is spring loaded so that a constant pressure is provided.

This device specifically measures the content of melanin and hemoglobin in the skin. These are the two components mainly responsible for skin color. The measurement is based on the absorption principle. The probe of the MX-18 emits light of three defined wavelengths. A receiver measures the light reflected by the skin. As the quantity of emitted light is defined, the quantity of light absorbed by the skin can be calculated.

Three Mexameter measurements will be obtained from the subjects randomized test sites on the back at each designated time point to assess erythema and melanin**.**

9.7. THERMOMETER READING

A Fisher brand IR Thermometer will be used to obtain the subjects skin temperature from the following test sites: Untreated area, and for the areas treated with No SPF Placebo, Hybrid SPF 30, and Chemical Benchmark SPF 30 before and after UV Exposure.

9.0 STUDY EVALUATIONS (CONTINUED)

9.8. COLLECTION OF DERMAL INTERSTITIAL FLUID

The collection of dermal interstitial fluid will be obtained using the PELSA system which consists of several components that together are used for the sampling of dermal interstitial fluid. The sample can then be used for different types of biomarker analyses. These analyses are not performed using the PELSA system and appears in Appendix II.

A trained staff member will extract a total of 5 samples per subject. Micro-needling will not take place over any area that has irritation or that was recently shaved. There will be no hair in the location of the extraction. At the Baseline timepoint, a sub-site to the top left of the testing area will be used for the micro-needling.

If the first attempt is not successful, a second attempt will be performed after a 90 second wait period.

Samples will be obtained and stored with dry ice and sent fresh to Eurofins Discovery on the day that they are obtained. Once the samples are received by Eurofins Discovery they will be frozen for batch testing until all samples have been received from all subjects at all timepoints.

9.0 STUDY EVALUATIONS (CONTINUED)

9.8.1. Dermal Interstitial Fluid Analysis – Eurofins Discovery

9.8.1.1. Project Description

Client-provided human interstitial fluid samples and commercially-sourced artificial interstitial fluid will be evaluated at multiple dilutions for levels of IL-1α, IL-6, IL-8, and VEGF-A using Luminex methodology (Thermo Fisher ProcartaPlex catalog #PPXT-04). Two (2) client-provided human interstitial fluid samples will be evaluated at 8 dilutions in singlicate.

Artificial dermal interstitial fluid (BioChemazone catalog #BZ428) and simulated interstitial fluid (BioChemazone catalog #BZ254) will each be evaluated at 8 dilutions in triplicate, spiked with high and low levels of the kit-provided standard or vehicle control to evaluate matrix interference at all sample dilutions. Cytokine levels of all samples will be interpolated from a standard curve using 4- or 5-parameter non-linear regression analysis, or as suggested by the kit manufacturer. Percent recovery will be calculated for spike/recovery samples.

350 client-provided human interstitial fluid samples will be assayed at one optimal dilution (determined in Milestone 1), in duplicates*, for the cytokines listed above using a commercially available Luminex kit (Thermo Fisher ProcartaPlex catalog #PPXT-04) according to the manufacturer's protocol. Commercially-sourced artificial or simulated interstitial fluid (chosen based on Milestone 1 results) will be spiked with high and low levels of the kit-provided standard or vehicle control for spike/recovery controls on every assay plate. Levels of the cytokines listed above will be interpolated from standard curves using 4- or 5-parameter non-linear regression analysis, or as suggested by the kit manufacturer.

Note: Any samples with insufficient volume for duplicate evaluation will be evaluated in singlicate or at a higher dilution.

10.0 TEST METHOD

10.1. SUBJECT IDENTIFICATION

All subjects will be initially identified by a permanent ECRL identification number. Once the subject meets qualification criteria, a study subject number will be assigned. This study subject number will be assigned in sequence as subjects are enrolled in the study.

10.2. DAY 1

10.2.1. Subject Enrollment

Prospective subjects will report to the testing laboratory and be given the Informed Consent (IC) and Photo Release Form. Subjects will acclimate to room temperature for approximately 10 minutes before evaluations. Subject will be given time to read the document. If they choose to participate, site staff will review, document, obtain necessary signatures from the informed consent document and complete inclusion/exclusion criteria and medical history interviews.

The back, between the beltline and shoulder blades, will be examined for uneven skin tones and blemishes using a Wood's lamp. If there is visible irritation or redness in the testing area, subject will acclimate to room temperature for 10 minutes. Subjects with persisting irritation or redness after acclimation of 10 minutes on any of the three study days will be discontinued. The study technician will draw, using an indelible marker and template, eight rectangles 40 cm^2 on the subjects' backs between the beltline and shoulder blades, lateral to midline. Using a colorimeter, each subject's ITA° will be determined with at least 3 recorded color measurements per sponsor's request. Up to 3 measurements may be taken and recorded. An ITA° calculation range must be a ≤10-point difference (example: 35.6°-45.6°). Any subject whose ITA° range is a >10-point difference will result in disqualification.

The following study evaluations will be obtained: Mexameter Measurements for Melanin and Erythema Index, Chromameter Measurements for L*a*b* and ITA Calculation, images using the digital camera, Thermometer Reading for Skin Temperature for the following test sites: Untreated area, and for the areas treated with No SPF Placebo, Hybrid SPF 30, and Chemical Benchmark SPF 30 before and after UV Exposure. Collection of Dermal Interstitial Fluid from a sub-site to the top left of the testing area (following the instructions in Appendix II).

10.0 TEST METHOD (CONTINUED)

10.2.2. Initial Unprotected MED (MEDuI) Dose Administration

A series of 5 UV doses, increasing in 25 percent increments (J/m^2-eff), will be administered to an unprotected test site in its randomly designated rectangle, as shown in Table 7 in section 9.5.

Subject will be instructed to avoid UV exposures and prohibited medications, and to return to the testing laboratory 16 to 24 hours after completion of UV doses.

The technician will document as described in Sections 9.1, 9.4, and 9.5.

10.3. DAY 2

10.3.1. MEDuI Determination

Subject will return to the testing laboratory within 16 to 24 hours after completion of MEDuI doses for evaluation of responses and will be questioned to assess compliance, to identify concomitant medications and to monitor for adverse events. If there is visible irritation or redness in the testing area, subject will acclimate to room temperature for approximately 10 minutes prior to the evaluation. Subjects with persisting irritation or redness after acclimation of 10 minutes on any of the three study days will be discontinued.

The trained evaluator will document the determined visual erythema response grades as described in section 9.4 table 5.

Every attempt will be made to have subjects adhere to their scheduled study visits.

10.3.2. Application of Test Material(s) and Standard Sunscreen

The technician will apply the placebo, test material(s) and standard sunscreen (‘Pad-O’) in the designated rectangle(s) as described in sections 5.3 and 5.4 (respectively) and document as described in section 5.5.

10.0 TEST METHOD (CONTINUED)

10.4. DAY 3 (FINAL VISIT)

10.4.1. Evaluation of Responses to UV Doses for MEDuR, ssMEDp, and tpMEDp

Subjects will return to the testing laboratory within 16 to 24 hours after completion of MED doses for evaluation of responses and will be questioned to assess compliance, to identify concomitant medications and to monitor for adverse events. Subjects will acclimate to room temperature for approximately 10 minutes before evaluations.

The trained evaluator will document the determined visual erythema response grades as described in section 9.4 table 5.

The following study evaluations will be obtained from subsite 1.00x: Mexameter Measurements for Melanin and Erythema Index, Chromameter Measurements for L*a*b* and ITA Calculation, images using the digital camera, Thermometer Reading for Skin Temperature (for the following test sites: Untreated area, and for the areas treated with No SPF Placebo, Mineral SPF 30, and Chemical SPF 30, before and after UV Exposure. Collection of Dermal Interstitial Fluid from the target SPF site (following the instructions in Appendix II).

11.0 PROTOCOL AMENDMENTS AND DEVIATIONS

Neither the testing facility nor the Sponsor will modify this protocol without the agreement of the other. In the event that the protocol must be changed, a protocol amendment or administrative change will be documented. A protocol amendment will be submitted when changes in the existing protocol are required that significantly affect safety of subjects, scope of the investigation, or scientific quality of the study. Protocol amendments will be submitted to the Sponsor and IRB for review and signature (sponsor) and will be signed and implemented by the Principal Investigator. Minor changes to the existing protocol that do not significantly affect safety of subjects, scope of the investigation, or scientific quality of the study will be documented as administrative changes to the protocol and will be maintained in the study master file only after approval is received by sponsor. Administrative changes will be approved and signed by the Sponsor. Once all administrative changes are agreed upon, the protocol will then be submitted to the IRB for review and approval. Unplanned or unexpected non-compliances with the protocol will be documented as protocol deviations. Protocol deviations should be avoided whenever possible and will be documented in the associated study file and included in the final report to the Sponsor.

12.0 DATA TABULATION

The per-protocol (PP) population will be the primary population for all statistical analyses. The PP population will include all subjects who received treatment and completed the study in general accordance with the protocol. Only the data of completing subjects will be included in the statistical analysis.

Subjects may be removed from the analysis in the case of an AE, SAE, noncompliance, or Investigator decision. The reason for any subject(s) excluded from an analysis population will be documented in a note to file and included in the study report.

The sample size determination of this study is based on the Sponsor's recommendations.

Demographic data and baseline characteristics will be summarized according to the analysis population. For continuous variables, descriptive statistics including number of subjects (N), mean, median, standard deviation (SD), minimum (MIN) and maximum (MAX) values will be presented. For categorical variables, the frequency and percentage of each category will be provided.

Analyses will be performed separately in two groups, one set for Fitzpatrick Skin Group 1-3 and one set for 4-6 with 10 subjects per Fitzpatrick Skin Type. Data will be separated into two panels based on FST (i.e. Fitzpatrick Skin Types 1 – 3 will have a minimum of 30 subjects in each data set, Fitzpatrick Skin Types 4 – 6 will have a minimum of 30 subjects; for a total of 2 data sets for each evaluation parameter).

All raw data will be shared with Sponsor at the end of the study.

12.1. SPF COMPUTATION

The test material's static SPF, Placebo, and the Standard Sunscreen's ('Pad-O') SPF values will be computed, as applicable, for each of the subjects, as follows:

SPF values for individual subjects (SPFi) will be calculated as:
$SPFi = MEDp/MEDuR$

The mean SPF and standard deviation (SD) will be calculated from valid SPFi values. SE is the standard error of the mean.

The Standard Error (SE) will be calculated as:
$SE = SD/\sqrt{n}$
(n equals the number of subjects who provided valid test results.)

The t value from a one-sided Student's t distribution table corresponding to the upper 5% point with n-1 degrees of freedom will be obtained.

12.0 DATA TABULATION (CONTINUED)

The labeled SPF value will be determined as the largest whole number less than the following calculation:

Labeled SPF = Mean SPF – (t * SE)

At the discretion of the Principal and/or Sub-Principal Investigator, additional statistical analyses may be performed and/or reported.

In order for the SPF determination of the test material to be valid, the SPF value of the standard sunscreen should fall within the acceptable range of the expected SPF. [1] See Table 9 for standard sunscreen acceptance limits.

Table 9. Standard Sunscreen Acceptance Limits

Standard Sunscreen	Mean SPF	Acceptance Limits	
		Lower Limit	Upper Limit
7% Padimate-O and 3% Oxybenzone	16.3	12.87	19.73

12.2. EFFICACY AND SAFETY PARAMETERS

Three Chromameter measurements will be averaged for each treatment group and visit, and the averaged value will be used for statistical analysis.

Dermal Interstitial Fluid samples will be processed and analyzed by Eurofins Discovery.

Paired t test or Wilcoxon signed rank test depending on the normality of data (checked by the Shapiro-Wilks test at 0.01 significance level) will be applied to determine the differences between baseline and each post-treatment interval for Mexameter Measurements for Melanin and Erythema Index, Chromameter Measurements for L*a*b* and ITA Calculation, Thermometer Reading for Skin Temperature.

Paired sample t-test or Wilcoxon Signed Rank test depending on the normality (Shapiro-Wilk test at the threshold of 1%) will be applied to compare the difference in change from baseline at each post treatment visit between the treatment groups (i.e., Untreated site, No SPF placebo, Mineral SPF, and Chemical SPF samples).
for the above-mentioned parameters.

Change from baseline and % of subjects improved will be calculated at each post-treatment interval for the above-mentioned parameters. Statistical significance exists for two-sided p-values ≤ 0.05 at the 95% significant level.

13.0 INVALID /MISSING TEST DATA

SPF test data is deemed invalid and shall be rejected under the following circumstances as per the monograph:

a) Erythema (sun burn) is not present on either the unprotected or protected (test material and/or standard sunscreen) test sites.
b) Erythema (sun burn) is present at all subsites on either the unprotected or protected (test material and/or standard sunscreen) test sites.
c) The responses are inconsistent with the series of UV doses administered.
d) The subject was noncompliant with testing protocol.

A maximum of three subjects may be invalidated for the above reasons. In the event of a fourth subject being invalidated, the whole panel is automatically invalid, testing will be stopped, and no SPF will be calculated. [1]

Other reasons for data rejection and exclusion from SPF calculation other than those listed above per the monograph may include:

a) Subject lost to follow up.
b) Subject discontinued due to Adverse Event.
c) Subject disqualified due to technical failure.

14.0 ETHICS

14.1. ETHICAL CONDUCT OF THE STUDY

ECRL follows established, standardized procedures such as Good Clinical Practices (GCP) and International Council of Harmonization (ICH) for clinical testing designed to ensure the wellbeing of clinical study subjects and the generation of reliable study data. The Study Sponsor is responsible for ensuring the study complies with applicable Drug, Cosmetic or Medical Device regulations, which vary by product.

14.2. ETHICS COMMITTEE AND REGULATORY APPROVAL

The Institutional Review Board (IRB) review is critical to verify the adequacy of informed consent and to ensure that study protocols incorporate appropriate inclusion/exclusion criteria for subject selection (both to protect test subjects and to ensure the accuracy of results). The IRB will review and approve the clinical study protocol and Informed Consent Form prior to study initiation in accordance with Title 21 of the Code of Federal Regulations (CFR), Parts 50 and 56. Individual product ingredient lists may be submitted to the IRB for review per Sponsor's request. Additional study documentation, including advertising, recruitment, and screening materials, may be submitted to the IRB for review and approval at the discretion of the Principal Investigator or at the request of the IRB.

14.3. INFORMED CONSENT

Each subject will be given a copy of the IRB-approved Informed Consent Form (ICF) and Photograph Release Form and have the nature, the purpose, and the risks of the study explained by ECRL personnel. Prior to entry into the study, the subject must give voluntary written consent to participate. The ICF will be signed and dated by the subject and site staff. The Principal Investigator must retain the original signed Informed Consent Form and signed Photograph Release Form in the subject's file and must give a copy of the signed and dated Informed Consent Form and Photograph Release Form to the subject.

14.4. SUBJECT CONFIDENTIALITY

The Principal Investigator must ensure that the research subject's confidentiality is maintained. Subject will be identified by a permanent ECRL identification number. Documents will be kept in strict confidence by the Principal Investigator and designees. Any use of personally identifiable data or private health information must be justified by the Principal Investigator and approved by the IRB.

15.0 ADVERSE EVENTS

15.1. DEFINITION OF AN ADVERSE EVENT

An adverse event is any untoward medical occurrence, whether or not it is considered study related, including death, experienced by a subject. An event may consist of a disease, an exacerbation of a pre-existing illness or condition, an occurrence of an intermittent illness or condition, a set of related symptoms or signs, or a single symptom or sign.

15.2. DEFINITION OF A SERIOUS ADVERSE EVENT

A serious adverse event is any event in which the subject is, in the view of the Principal and/or Sub-Principal Investigator, (A) at immediate risk of death, a life-threatening experience, causes hospitalization/prolongation of hospitalization, a persistent or significant disability or incapacity, a congenital anomaly or birth defect, an infection, or significant disfigurement (including serious and persistent rashes, second- or third-degree burns, significant hair loss, or persistent or significant alteration of appearance), other than as intended under conditions of use that are customary or usual; (B) requires, based on reasonable medical judgment, a medical or surgical intervention to prevent an outcome described in (A) above.

Note that this definition does not include an event that, had it occurred in a more serious form, might have caused death or persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions. Examples of serious adverse events include but are not limited to death due to any cause, whether or not it is felt to be related to the study; events that require subject hospitalization; or events that result in a congenital anomaly or birth defect.

The description of the serious adverse event must be well documented as the Manufacturer will need to provide detailed description of the serious adverse event to the FDA, related or not to the test material.

15.3. DOCUMENTATION AND REPORTING OF ADVERSE EVENTS

All adverse events will be promptly recorded and sufficiently documented by the Principal Investigator, Sub-Principal Investigator, or designated medical staff in the source documentation and case report form, even if the adverse event is assessed as unlikely to be related to the study by the Principal Investigator or designated medical staff. The Principal Investigator or designated medical staff will report the occurrence of any serious adverse event to the Sponsor's representative and the IRB within one business day, regardless of the causal relationship to the study, and follow-up with written documentation within three business days of awareness. All adverse events, serious or not serious, related or not related to the test material, will be summarized and reported in the final report. All adverse events will be followed up until resolved, stabilized, the subject is lost to follow-up, or the event is otherwise explained.

15.0 ADVERSE EVENTS (CONTINUED)

15.4. EXPECTED EVENTS

15.4.1. UV Expected Events

During the course of the study, because of irradiation with the solar simulator, varying degrees of redness will develop on the skin. Multiple areas of skin are involved; however, since the UV exposed sites are about 1/3 inch in diameter, there should be little or no discomfort. The skin response to the UV light may include: erythema (sun burn), mild discomfort, itching, edema (swelling), dryness, peeling, papules, stinging/burning, ulceration and heat. In some cases, the reddened areas will "tan" and pigmentation may persist for months or years. The effects of the solar simulator on the skin will not be considered an AE including the effects of the UV on a test site where the test material did not perform up to expected protection level. However, if the visual evaluation outcome appeared to be highly exacerbated or more than normally associated with the test procedures as determined by the PI or designee, the event will be documented as an AE.

15.4.2. Test Material Expected Events

As any product applied to the skin, there is a possibility of the following skin reactions: redness, dryness, peeling, itching, burning/stinging or an allergic reaction. The reactions associated with the application of the test material on the test area are to be considered an AE as determined by the PI or designee and it will be recorded.

15.4.3. Process for Suspected Allergic Reactions

There is always a possibility that a subject can have irritation or a skin reaction to any product applied to the skin. Examples of possible skincare product reactions are:

- Redness
- Dryness/tightness
- Peeling
- Itching
- Burning/stinging
- Allergic reaction

Any skin reaction side effects experienced by the subjects associated with the application of the test material, to the testing area, are to be considered AEs. The side effect/event will be determined by the PI or designee, and the event will be documented as an AE.

15.0 ADVERSE EVENTS (CONTINUED)

15.5. MICRO NEEDLING EXPECTED REACTIONS

Redness, swelling, bleeding, scarring, and long-term discoloration and pinpoint bleeding of the skin at the sampling site that disappears within 1-3 days may occur.

During the Skin Penetration phase, the Needle Unit microneedle chip penetrates the outer layers of the skin of the subject. The microneedle chip contains a matrix of microneedles sharp enough to penetrate the skin and long enough to reach the IDF in the skin.

16.0 QUALITY ASSURANCE PARAMETERS

This clinical study will be conducted in accordance with the clinical study protocol and ECRL SOPs. The full panel clinical study file will be reviewed for compliance with the clinical study protocol, ECRL SOPs, and applicable guidelines and regulations by the Principal Investigator and the Quality Assurance representative.

17.0 CLINICAL STUDY REPORTING

A final audited topline with all data from all timepoints will be submitted to the Sponsor within approximately 10 business days following study completion. A clinical study report will be issued within approximately 6 weeks of full panel study completion. Study-related documents, including source documents or raw data, will be reviewed by the Principal Investigator and/or designated study staff prior to issuance of the final report. The final signed study report will summarize the study objective(s) and test methodology. At the completion of the study, the sponsor will be provided with a tabulation of subjects' demographics; MEDs; SPF values; descriptive and inferential statistics, instruments/measurements (Mexameter, Chromameter, thermometer measurements and dermal evaluations), images that are obtained using a digital camera, protocol deviations, AEs, conclusions, and lamp calibration data. Data will be separated into individual panels based on Fitzpatrick Skin Group 1-3 and 4-6 (i.e. Fitzpatrick Skin Types 1 – 3 will have a minimum of 30 subjects in each data set, Fitzpatrick Skin Types 4 – 6 will have a minimum of 30 subjects; for a total of 60 subjects in 2 data sets for each evaluation parameter).

A Eurofins Discovery analysis report of the IDF samples will be provided to the sponsor once it has become available from the vendor and will also be included in the final report.

***Please note: ECRL is an independent, third-party testing laboratory. To preserve objectivity and ensure that no bias or preference is introduced, the laboratory does not generate, endorse, or substantiate marketing claims. Our study reports present only the data collected, indicating statistical/clinical significance or measurable improvements in the parameters evaluated under the study design.**

18.0 REFERENCES

1. U. S. Food and Drug Administration. Over-the-Counter Monograph M020: Sunscreen Drug Products for Over-the-Counter Human Use; September 24, 2021.
2. Guideline for the colorimetric determination of skin color typing and prediction of the minimal erythema dose (MED) without UV exposure. Colipa, 2007

19.0 PROTOCOL APPROVAL

19.1. SPONSOR APPROVAL

I have read the foregoing protocol entitled "A Clinical Study to Evaluate the Interstitial Dermal Fluid, Efficacy and Sun Protection Factor (SPF) of Two Test Materials According to an Adaptation of the Guidelines of the FDA Over-the-Counter Monograph M020: Sunscreen Drug Products for Over-the-Counter Human Use" and approve it.

.....

Ivan Souza, Date
R&D Director
Sponsor Representative

19.2. PRINCIPAL INVESTIGATOR ACCEPTANCE

I have read the foregoing protocol entitled "A Clinical Study to Evaluate the Interstitial Dermal Fluid, Efficacy and Sun Protection Factor (SPF) of Two Test Materials According to an Adaptation of the Guidelines of the FDA Over-the-Counter Monograph M020: Sunscreen Drug Products for Over-the-Counter Human Use" and I agree to conduct the study in compliance with the protocol approved by the Sponsor.

.....

Alexis Chantal, PA-C Date
Director Clinical Operations, Eurofins CRL Cosmetics LLC
Principal Investigator

19.0 PROTOCOL APPROVAL (CONTINUED)

19.3. SUB-INVESTIGATOR ACCEPTANCE

I have read the foregoing protocol entitled "A Clinical Study to Evaluate the Interstitial Dermal Fluid, Efficacy and Sun Protection Factor (SPF) of Two Test Materials According to an Adaptation of the Guidelines of the FDA Over-the-Counter Monograph M020: Sunscreen Drug Products for Over-the-Counter Human Use" and I agree to conduct the study in compliance with the protocol approved by the Sponsor.

.....

Carrie Cobb, MD FAAD Date
Medical Director, Eurofins CRL Cosmetics LLC
Sub-Investigator

I have read the foregoing protocol entitled "A Clinical Study to Evaluate the Interstitial Dermal Fluid, Efficacy and Sun Protection Factor (SPF) of Two Test Materials According to an Adaptation of the Guidelines of the FDA Over-the-Counter Monograph M020: Sunscreen Drug Products for Over-the-Counter Human Use" and I agree to conduct the study in compliance with the protocol approved by the Sponsor.

.....

Chelcia Mejia Date
Manager, Photobiology
Sub-Investigator

I have read the foregoing protocol entitled "A Clinical Study to Evaluate the Interstitial Dermal Fluid, Efficacy and Sun Protection Factor (SPF) of Two Test Materials According to an Adaptation of the Guidelines of the FDA Over-the-Counter Monograph M020: Sunscreen Drug Products for Over-the-Counter Human Use" and I agree to conduct the study in compliance with the protocol approved by the Sponsor.

.....

Yvette Gonzalez Date
SPF Team Lead
Sub-Investigator

Appendix I – Randomization

This randomization code may be modified if the Principal Investigator determines that the test site is not appropriate due to scars, tattoos, or other aesthetic concerns and recruitment needs.

Subject Number	MEDuI	MEDuR	Standard ('Pad-O')	Untreated Site	Placebo	TP1	TP2
01	L2	R4	L1	L3	L4	R3	R2
02	L2	L3	L4	R4	R2	R1	L1
03	R4	L2	L3	L1	R1	R3	R2
04	R1	R3	L3	L1	L4	R2	L2
05	R4	L3	R1	L4	L1	L2	R2
06	L1	R4	L4	R1	L2	R3	L3
07	R2	L3	L4	L1	R1	R4	L2
08	R1	R2	L4	L2	L3	R3	R4
09	L4	R4	L1	L2	R3	R2	R1
10	L4	R2	L2	R1	L3	R4	R3
11	R3	R4	L4	L2	L1	L3	R2
12	L1	R4	L3	R3	R2	L2	R1
13	L2	R2	R1	R3	L3	L1	R4
14	R3	R2	L3	L1	R4	L4	R1
15	R2	R1	L1	L3	L4	R4	R3
16	R4	R3	L2	R1	L1	L4	L3
17	R2	R1	L2	R4	L3	R3	L4
18	R3	R4	R1	L1	L4	L2	R2
19	L3	R2	L2	R4	L1	R1	L4
20	R1	R3	R4	R2	L2	L3	L4
21	L2	R3	L1	R4	R2	R1	L3
22	R2	L4	R4	R3	R1	L1	L2
23	R3	R1	L1	R2	L2	L4	R4
24	L4	L1	R2	R4	R3	L3	L2
25	R3	R4	L1	L2	L3	R2	R1
26	L2	R4	L1	L3	L4	R3	R2
27	L2	L3	L4	R4	R2	R1	L1
28	R4	L2	L3	L1	R1	R3	R2
29	R1	R3	L3	L1	L4	R2	L2
30	R4	L3	R1	L4	L1	L2	R2
31	L1	R4	L4	R1	L2	R3	L3
32	R2	L3	L4	L1	R1	R4	L2
33	R1	R2	L4	L2	L3	R3	R4
34	L4	R4	L1	L2	R3	R2	R1
35	L4	R2	L2	R1	L3	R4	R3

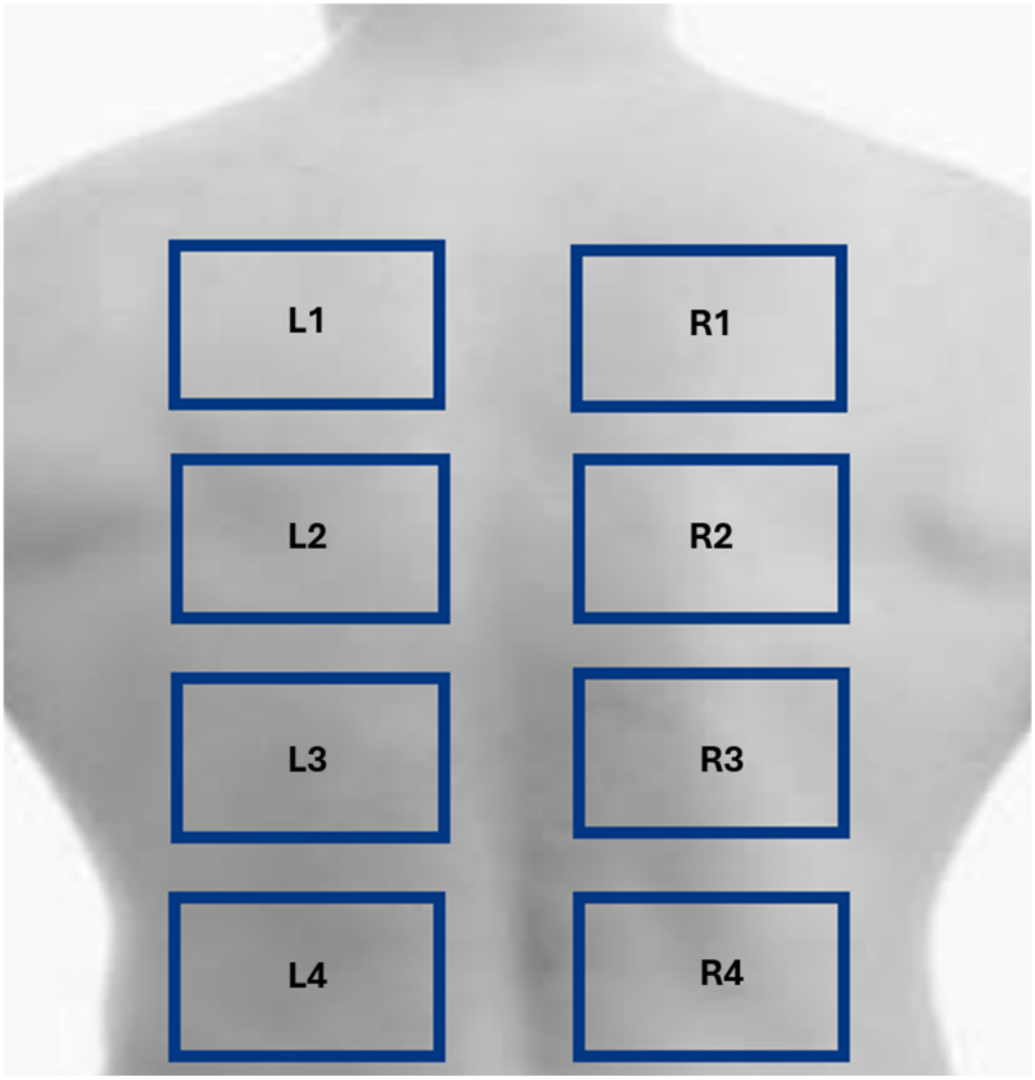
The site locator is listed on page 37.

Appendix I – Randomization (Continued)

Subject Number	MEDuI	MEDuR	Standard ('Pad-O')	Untreated Site	Placebo	TP1	TP2
36	R3	R4	L4	L2	L1	L3	R2
37	L1	R4	L3	R3	R2	L2	R1
38	L2	R2	R1	R3	L3	L1	R4
39	R3	R2	L3	L1	R4	L4	R1
40	R2	R1	L1	L3	L4	R4	R3
41	R4	R3	L2	R1	L1	L4	L3
42	R2	R1	L2	R4	L3	R3	L4
43	R3	R4	R1	L1	L4	L2	R2
44	L3	R2	L2	R4	L1	R1	L4
45	R1	R3	R4	R2	L2	L3	L4
46	L2	R3	L1	R4	R2	R1	L3
47	R2	L4	R4	R3	R1	L1	L2
48	R3	R1	L1	R2	L2	L4	R4
49	L4	L1	R2	R4	R3	L3	L2
50	R3	R4	L1	L2	L3	R2	R1
51	L1	R4	L4	R1	L2	R3	L3
52	R2	L3	L4	L1	R1	R4	L2
53	R1	R2	L4	L2	L3	R3	R4
54	L4	R4	L1	L2	R3	R2	R1
55	L4	R2	L2	R1	L3	R4	R3
56	R3	R4	L4	L2	L1	L3	R2
57	L1	R4	L3	R3	R2	L2	R1
58	L2	R2	R1	R3	L3	L1	R4
59	R3	R2	L3	L1	R4	L4	R1
60	R2	R1	L1	L3	L4	R4	R3
61	R4	R3	L2	R1	L1	L4	L3
62	R2	R1	L2	R4	L3	R3	L4
63	R3	R4	R1	L1	L4	L2	R2
64	L3	R2	L2	R4	L1	R1	L4
65	R1	R3	R4	R2	L2	L3	L4
66	L2	R3	L1	R4	R2	R1	L3
67	R2	L4	R4	R3	R1	L1	L2
68	R3	R1	L1	R2	L2	L4	R4
69	L4	L1	R2	R4	R3	L3	L2
70	R3	R4	L1	L2	L3	R2	R1

The site locator is listed on page 37.

Appendix I – Randomization (Continued)



Appendix II – Collection of Dermal Interstitial Fluid Instructions

Please see the instructions for the Collection of Dermal Interstitial Fluid below.

ascilion

PELSA SYSTEM

INSTRUCTIONS FOR USE

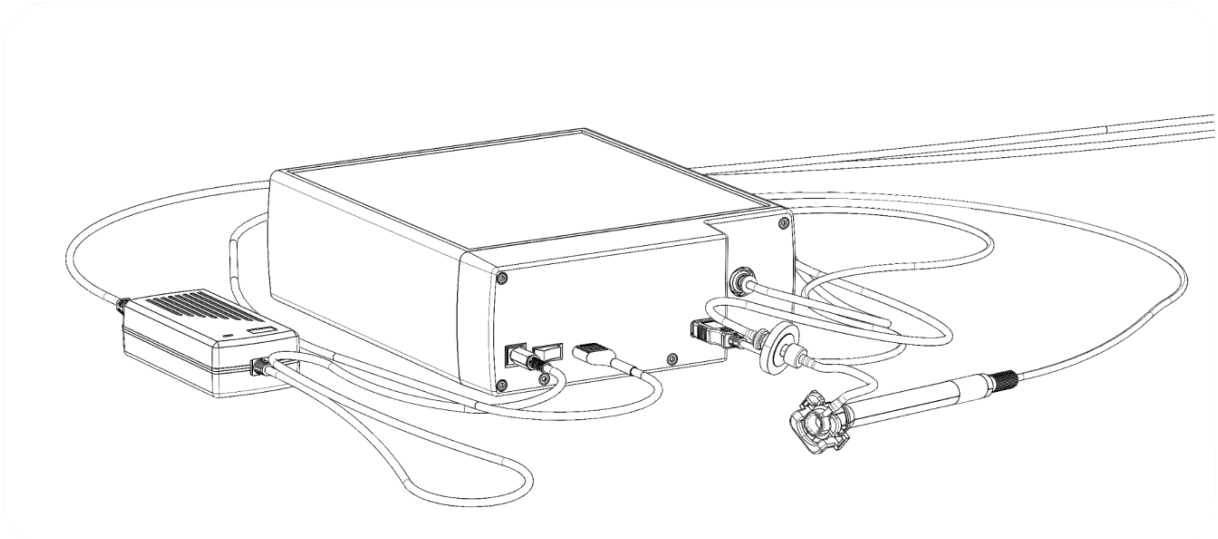


Table of content

1 INTENDED PURPOSE 3

2 TERMS, ABBREVIATIONS AND SYMBOLS 4

3 SYSTEM OVERVIEW 5

4 DETAILED DESCRIPTION OF PELSA SYSTEM PARTS 6

5 SYSTEM ASSEMBLY 9

6 SAMPLING PROCEDURE 10

7 MAINTENANCE AND CLEANING 18

8 STORAGE CONDITIONS 18

9 TROUBLESHOOTING 19

10 TECHNICAL SPECIFICATIONS 22

11 ELECTROMAGNETIC COMPATIBILITY INFORMATION 23

12 WARNINGS, CAUTIONS AND RESIDUAL RISKS 24

13 APPENDIX 1. EMC INFORMATION 25

1 Intended purpose

The PELSA system is intended for sampling and collection of dermal interstitial fluid from the skin of adult humans.

The PELSA system shall be used in medical facilities.

1.1 Intended user

The PELSA system is intended to be used by healthcare professionals or scientists with knowledge in sampling of biological material from subjects. The user shall be trained in the PELSA system by Ascilion representative before usage.

1.2 Contraindications

The PELSA system shall not be used:

- On persons under 18 years of age.
- On persons determined by a trained professional in interviews or by examination to be vulnerable (including pregnancy).
- On persons with an open wound in or near the sampling area.
- On persons with any type of tattoo or piercing anywhere in or near the sampling area.
- On acral skin.

1.3 Performance characteristics

The device is exclusively for clinical investigations and the device does therefore not have any performance characteristics claims yet.

2 Terms, abbreviations and symbols

2.1 Terms and abbreviations

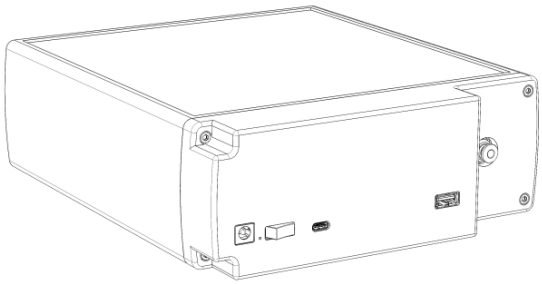
Term / abbreviation	Description
ISF	Interstitial Fluid
dISF	Dermal Interstitial Fluid

2.2 Symbols

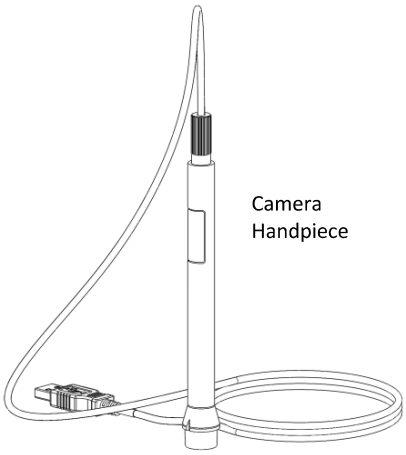
	Refer to Instructions for Use		Batch code
	Sterilized using irradiation		Serial number
	Do not use if package is broken		Catalogue number
	Do not re-use		Temperature limit
	Single sterile barrier with protective packaging inside		Humidity limitation
	Use-by date		Atmospheric pressure limitation
	Country and date of manufacture		Do not re-sterilize
	Manufacturer		Medical Device
	Electrical equipment, discard separately		

3 System overview

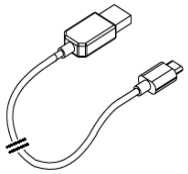
The PELSA system consists of several components that together are used for the sampling of dermal interstitial fluid (dISF). The sampled dISF can then be used for different types of biomarker analyses. These analyses are not performed using the PELSA system.



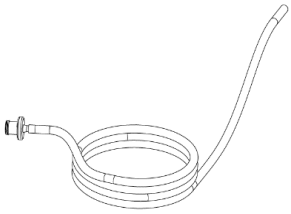
Control Unit



Camera Handpiece



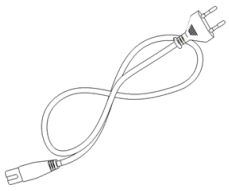
USB Cable



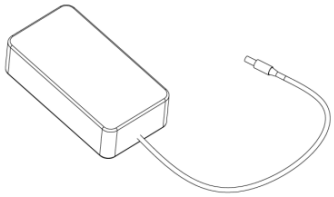
Extension tube



USB Flash drive with software



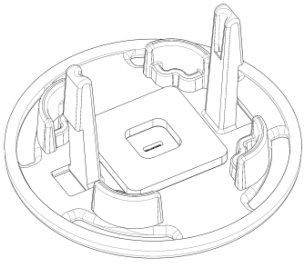
Power Cord



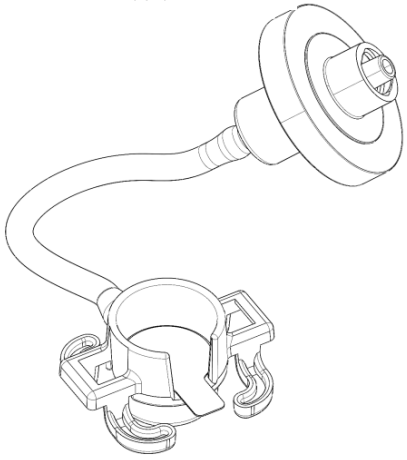
Power supply 24V/36W



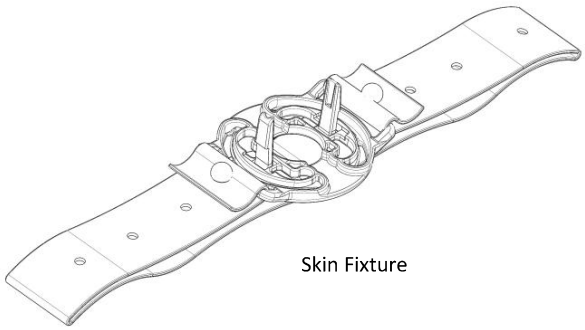
Instructions for Use



Needle Unit



Chamber Unit



Skin Fixture

The following parts are included in the PELSA system. The Needle Unit (including the microneedles), the Chamber Unit, and the Skin Fixture are all single-use disposables. The PELSA Control Apparatus consists of all re-usable parts, i.e. Control Unit, Camera Handpiece, Extension tube, USB Cable, Power Supply, Power cord and USB Flash drive (with software).

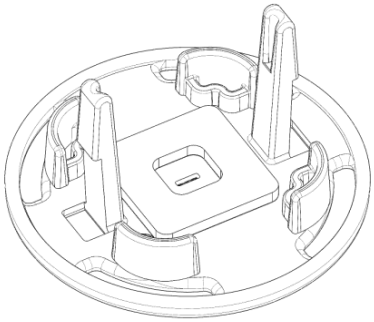
4 Detailed description of PELSA system parts

4.1 Needle Unit

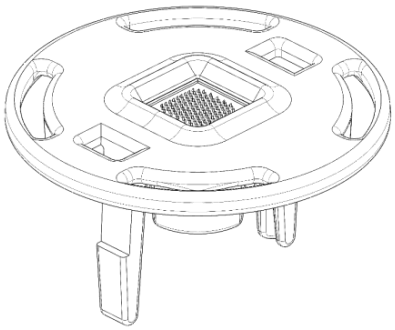
The Sampling procedure consists of two phases – the Skin Penetration phase and the dISF Extraction phase. During the Skin Penetration phase, the Needle Unit microneedle chip penetrates the outer layers of the skin of the subject. The microneedle chip contains a matrix of microneedles sharp enough to penetrate the skin and long enough to reach the dISF in the skin.

Quick-release clips or snaps are used to connect and release the Needle Unit from the Chamber Unit. The Needle Unit is a single use device and is discarded after use, see chapter 6.6 Needle unit – Safe removal and disposal.

The Needle Unit is supplied sterile. It has been sterilized using irradiation. The microstructures in the Needle Unit cannot be cleaned after use. Do not attempt to re-sterilize the Needle Unit.



Needle Unit – Top view



Needle Unit – Underside view showing microneedle chip

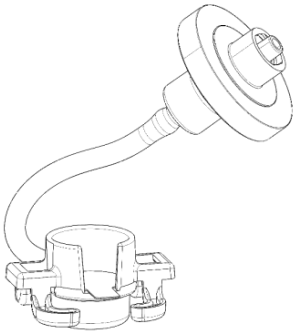
4.2 Chamber Unit

The Chamber Unit attaches to the Camera Handpiece and the Needle Unit is then attached to the Chamber Unit for use during the Skin Penetration phase. The Chamber Unit is also connected to the Control Unit via the Extension tube (included). The Chamber Unit is a single use device and is discarded after the dISF Extraction phase, see chapter 6.7 Chamber Unit – Safe removal and disposal of the Chamber Unit.

At the completion of the Skin Penetration phase the Needle Unit is removed and the Chamber Unit is positioned on the skin for the dISF Extraction phase. The Chamber Unit can be held in position on the skin manually by the Operator or by connecting to a correctly positioned Skin Fixture. During the dISF Extraction phase negative pressure is used to stretch the skin so that dISF droplets form and merge on the surface of the skin.

At the completion of the dISF Extraction phase, , the dISF on the skin surface must be collected using a pipette. To access the dISF, either remove the plastic 'Tape lid' part of the Chamber Unit or remove the entire Chamber Unit (leaving the Skin Fixture in position). Please note that this plastic lid is essential to maintain a negative pressure chamber and so should not be removed until after the dISF Extraction step is completed. It is not possible to reattach the lid. See chapter 6.5 dISF collection and transport.

Each Chamber Unit includes a short tube and filter that filters the air pumped from the Chamber Unit when applying pressures (see below). This tube and filter are single use and should not be reused.

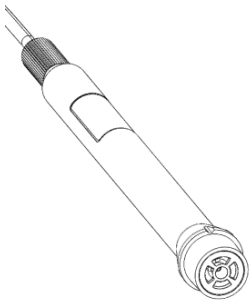


Chamber Unit

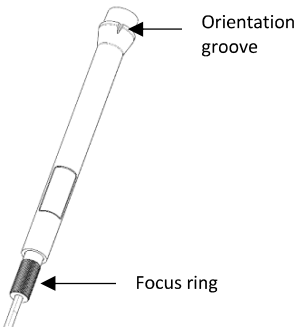
4.3 Camera Handpiece

Sampled volumes are in the microliter range and a high magnification camera is used for real-time visualization of the sampled fluid, to support assessment of volume and quality of the sample through detection of blood contamination. The Camera Handpiece is comprised of a USB camera with a holder/housing that is used to connect to the Chamber Unit. The groove on the Camera housing indicates the orientation of the top of the image that appears in the PELSA GUI software.

The camera USB cable connects to the Control Unit. The image from the camera is displayed in the PELSA GUI Software. Image quality may be adjusted via a focus ring at the top of the housing. LED intensity may be adjusted via an adjustment wheel on the cable.



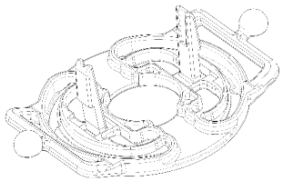
Camera Handpiece



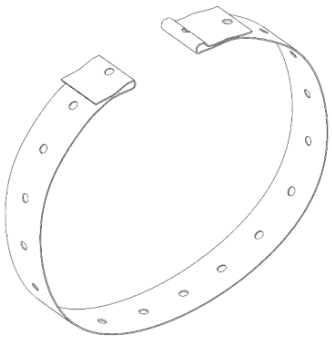
Camera Handpiece

4.4 Skin Fixture

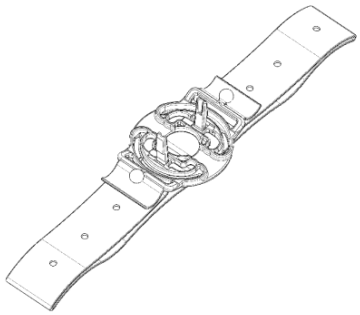
The Skin Fixture is used to hold the Camera Handpiece and Chamber Unit in position during the dISF Extraction phase. It is comprised of two parts, a buckle and an armband.



Buckle



Armband



Skin Fixture

4.5 Control Unit

The Control Unit allows for precise application and control of the negative pressures used in sampling. The Control Unit is controlled from a Windows PC or tablet using the PELSA GUI software.

The primary purpose of the Control Unit is to apply and control negative pressures. The unit should be connected to an electrical outlet (110 or 230 VAC, 50-60 Hz) using the supplied power supply (XP Power AKM36US24C2) and to a PC or tablet (Windows 11) via the supplied USB cable for communication. Pneumatic connection to the Chamber Unit is via the Extension tube's female Luer lock connection. Applied pressures are displayed in the PELSA GUI software.

4.5.1 PELSA GUI Software

The PELSA GUI software is used to control and display the pressures from the Control Unit. The software also displays the image from the Camera Handpiece.

The version number of the installed PELSA GUI software can be found by selecting 'About' in the PELSA GUI display.

4.6 Auxiliary equipment

- A computer or tablet running Windows 11 is required. See 5.2.1 for minimum requirements.
- Protective gloves shall be used when handling the PELSA sampling system.
- Alcohol wipes shall be used to wipe off the skin before penetration of microneedles and during the blood management protocol.
- A pipette with volume range of 1-10 µl and associated pipette tips are needed for collection and further handling of the sampled fluid. Gilson Pipetman P10 pipette and D10ST pipette tips, or Witeg Witopet Mini 10µl with appropriate tips are recommended.
- A collection vessel or medium such as an Eppendorf tube

5 System assembly

5.1 Preparation

Familiarize yourself with the different system components, packages and pouches. Do not open the sterile Needle Unit pouch until it is time for it to be used. Familiarize yourself with the nomenclature and intended purpose of each part of the system and the information on the labels.

5.2 Software installation

5.2.1 PC Requirements

To install and run the PELSA GUI software, a Windows PC or Tablet (Windows 11) with one available USB port, a minimum of 1GB free hard disk space, a minimum of 8GB of RAM and a screen resolution of 1920x1080 or more shall be used.

The installation of the PELSA system requires administrative privileges on the computer, but the usage of the software after installation does not. The user organization shall determine which level of protection against unauthorized access is required and implement the necessary protection measures. The PELSA GUI does not require any personal data to be collected or stored for the intended purpose of the PELSA system.

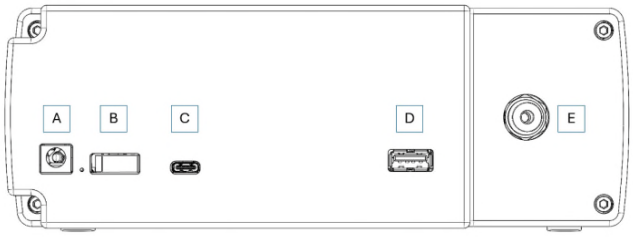
The PELSA GUI does not require any network connection to function. Connection to an IT-network that includes other equipment could result in previously unidentified risks to patients, operators or third parties. The user organization should identify, analyze, evaluate and control these risks. Subsequent changes to the IT-network could introduce new risks and require additional analysis. Changes to the IT-network include changes to the IT-network configuration, connection of or disconnecting additional items to the IT-network, and update or upgrade of equipment connected to the IT-network.

5.2.2 Installation

Run the provided Setup file located on the USB flash drive and follow the on-screen installation guide.

5.3 Control Unit setup

1. Unpack the Control Unit and place on a flat, dry and stable surface.
2. If power switch (B) is set to ON (I), set the switch to OFF (O)
3. Connect the power cable and power supply to the power port (A) of the Control Unit.
4. Connect the Camera Handpiece to the USB-A connector (D) of the Control Unit
5. Remove any other connected USB cables from the PC.
6. Using the USB cable (USB-A/C to USB-C), connect a USB-A/C port on the Windows PC or Tablet to the USB-C connector (C) of the Control Unit.
7. If present, remove the protective part from the pressure tube (E) connector of the Control Unit by pressing in the blue ring and pulling the protective part straight out. Save the protective part if the device will be stored for longer periods of time or transported.
8. Connect the Extension tube by inserting the tube all the way into the pressure port (E).
9. Turn the device on using the ON/OFF switch (B). A LED indicates when the device is on.



Control Unit

6 Sampling procedure

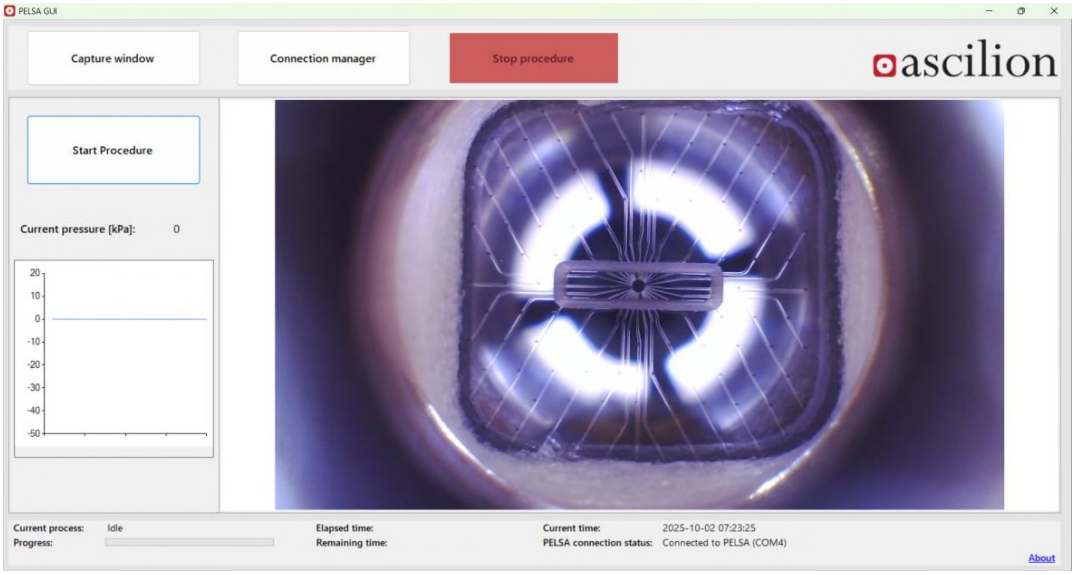
6.1 Software setup

Start the PELSA GUI software. The Control Unit and the Camera connect automatically to the PELSA GUI software when powered on.

Connection and disconnection of the Control Unit and the Camera can also be manually performed from the Connection manager dialog. Click the “Connection manager” button to open the dialog. Camera resolution can also be changed here.

If connection problems occur, consult the Troubleshooting section.

The small grove in the Camera housing is oriented to the top of the image shown in the software. The Camera might require rotation to align correctly. If needed, adjust the Camera illumination level using the adjustment wheel on the Camera cable.



PELSA GUI with Chamber Unit and Needle Unit connected to Camera Handpiece.

6.2 Sampling site skin preparation

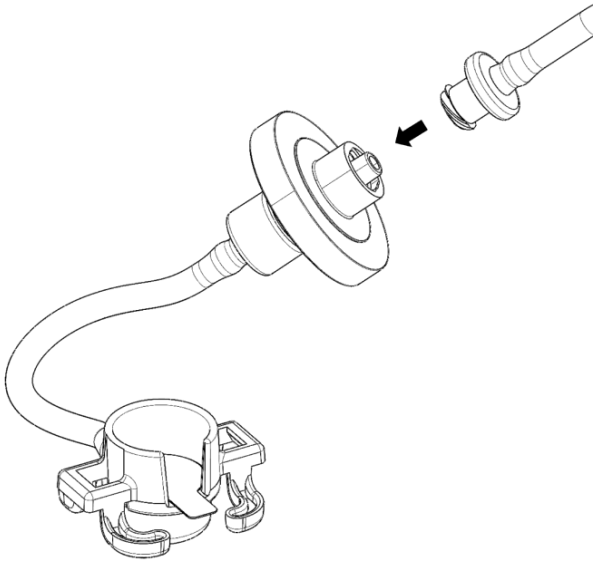
The site preparation steps are as follows:

1. Select a suitable site for sampling on the skin. Avoid uneven or bony skin areas.
2. The skin should be free from hair and contaminants for an effective dISF sampling.
3. If not already shaved, shave the sampling area using water only (avoid soap products) to remove any hair and let dry.

6.3 Prepare Disposables

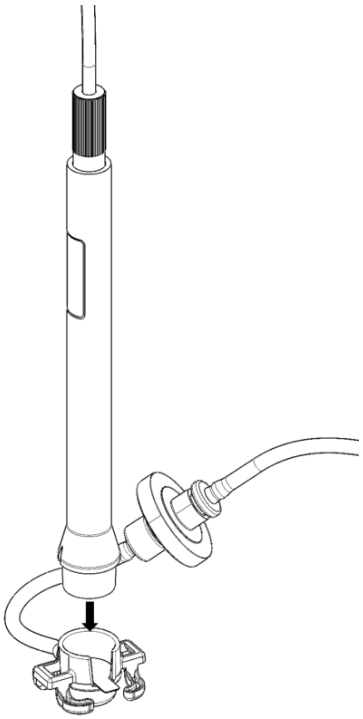
The Chamber Unit, Needle Unit, and Skin Fixture are single-use disposables that shall be replaced after each use. Protective gloves shall be used during assembly and sampling. The procedure to prepare the disposables for a sampling is as follows:

1. Inspect the packaging of the Needle Unit and Chamber Unit. Do not use if packaging is broken.
2. Remove the Chamber Unit from its pouch and place it on a clean and flat surface.
3. Connect the Chamber Unit filter to the Extension tube using the Luer lock connector.

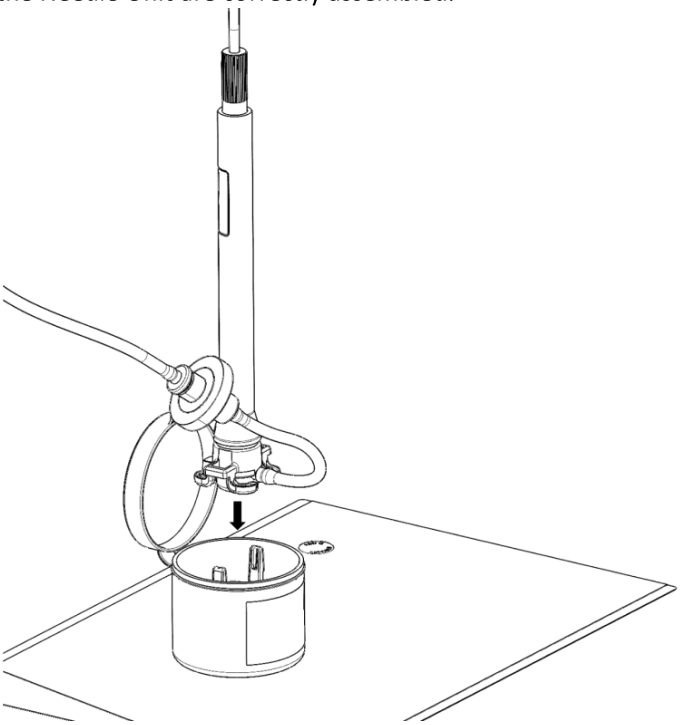


Note: The Luer lock connector should only be rotated ‘finger tight’. Excessive force may lead to difficult disassembly.

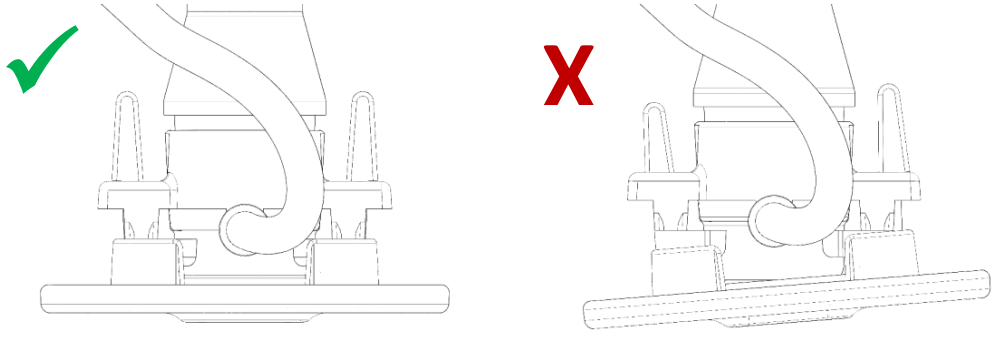
4. Connect the Chamber Unit by gently pressing the Camera Handpiece fully into the cavity in the Chamber Unit.



5. Open the Needle Unit pouch and remove the plastic container from the pouch.
6. Open the plastic container, but do not remove the Needle Unit from the container.
7. Holding the Camera Handpiece, align the Chamber Unit to the snaps of the Needle Unit. Note that the snaps are two different sizes and that the larger snap of the Chamber Unit must be aligned to the larger snap of the Needle Unit.
8. Press the Chamber Unit assembly vertically into the Needle Unit. A click sound is heard when the Chamber Unit and the Needle Unit are correctly assembled.

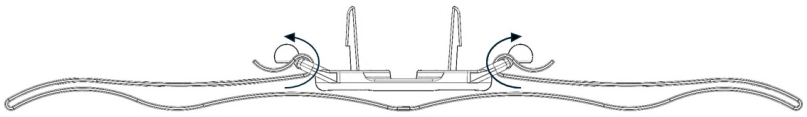


9. Lift the assembled parts out of the plastic container.
10. Inspect the alignment between the Chamber Unit and Needle Unit. If the connection is misaligned, press in the snaps to detach the Needle Unit from the Chamber Unit, replace the Needle Unit in the container and repeat steps 7-10 until the two parts are correctly attached according to image below.



Correct (left) and incorrect (right) assembly of the Needle Unit and Chamber Unit. In case of incorrect assembly, disassemble and re-assemble the two parts.

11. Prepare the Skin Fixture. This should be pre-assembled, but if not see below how to attach the band. Insert the end of the band from below the buckle through the slit and then up and over the hook on each side of the buckle.



6.4 Sampling Procedure

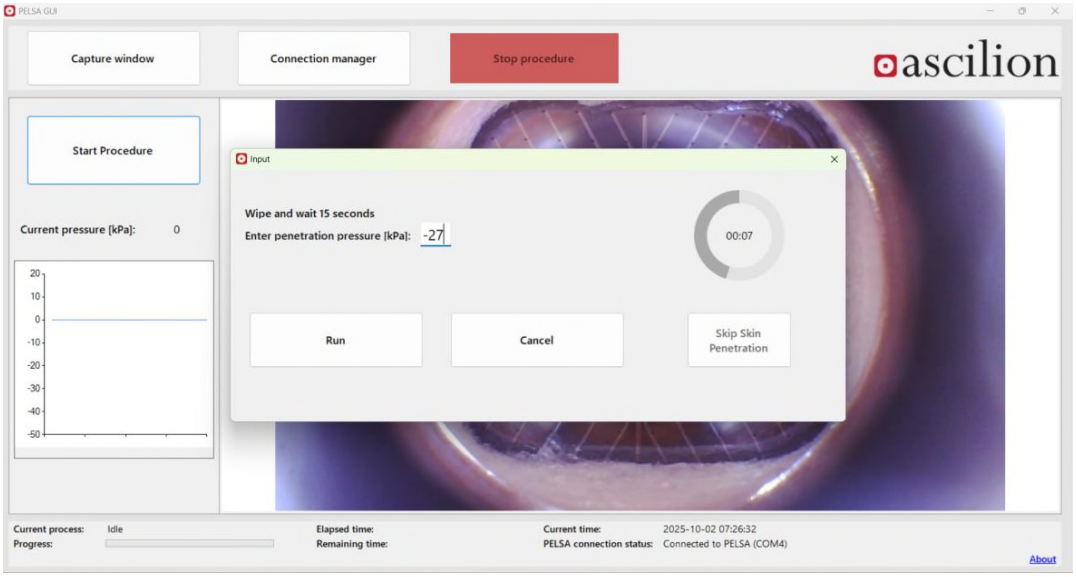
The Sampling procedure consists of two phases – the Skin Penetration phase and the dISF Extraction phase.

Note: A Sampling procedure can be aborted at any time by clicking the red "Stop procedure" button. However, it is also always possible to easily remove the sampling disposables from the patient's skin even with negative pressure applied.

6.4.1 Skin Penetration Phase

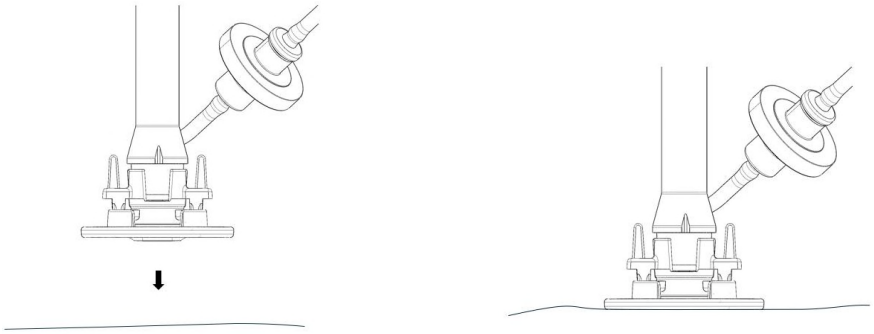
To perform the Skin Penetration phase:

1. In the PELSA GUI software click the button labeled "Start Procedure".



Start Procedure dialog in PELSA GUI

2. Wipe the skin sampling site area four times with a 70% IPA alcohol wipe and wait 15 seconds (a 15 second timer is displayed). Note that the 15 second timer may be reset by clicking on it.
3. Position the Needle Unit against the skin as shown in the figure below.
4. Click "Run" to start Skin Penetration.



5. The Skin Penetration phase will run for 1-2 minutes and involves 10 cycles of skin penetration by the microneedles. Completion is indicated by the message “Skin penetration completed”.
6. After the Skin Penetration phase is completed, remove the Needle Unit and discard according to 6.8 Needle unit – Safe removal and disposal.

Note: The Skin Penetration phase can be aborted at any time by clicking “Stop Skin penetration”.

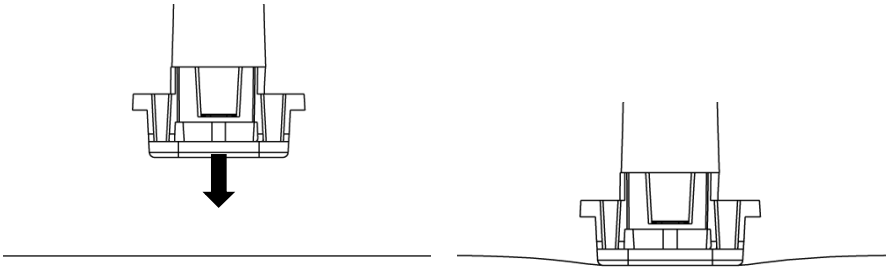
Note: For Advanced Users only - the Skin Penetration phase can be skipped completely by selecting ‘Skip Skin Penetration’

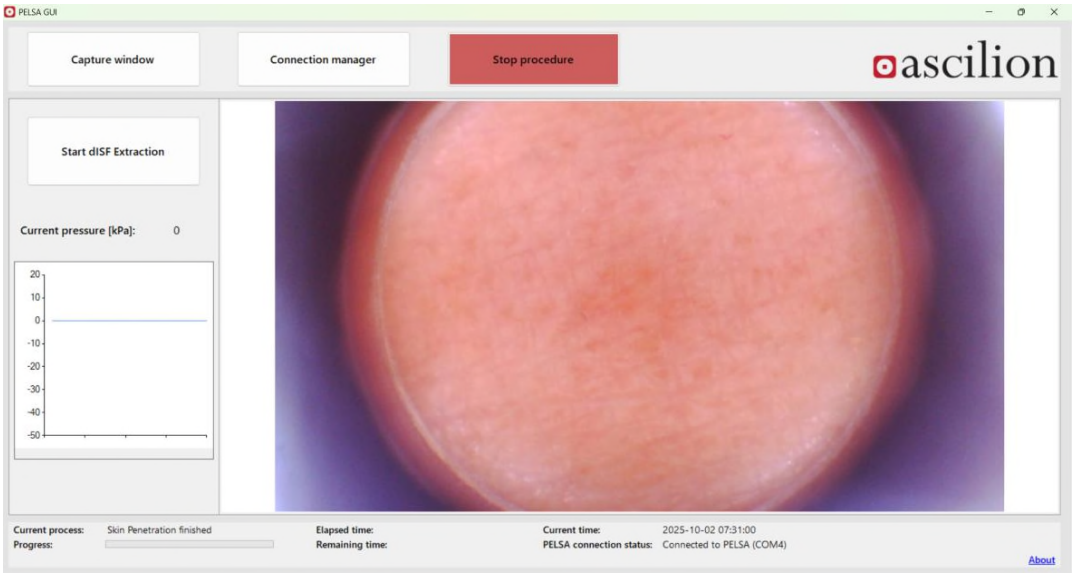
6.4.2 dISF Extraction Phase

1. Position the aperture of the Skin Fixture (if used) so that the area of microneedle penetration is in the center of the Skin Fixture aperture and tighten the band.

Note: To position, it is recommended to carefully lift the Skin fixture off the surface of the skin, position the aperture so that it is centered on the imprint from the microneedle penetration and then lower back onto the skin. This will ensure that the imprint is not compromised.

2. Next, align the Chamber Unit and connect it to the Skin Fixture (you will hear a ‘click’ from each side of the connector).
3. The dISF extraction Phase may be performed without the Skin Fixture. In this case align the Chamber Unit above the area of microneedle penetration and manually press and hold the Chamber Unit against the skin for the entire dISF Extraction phase.





View of the skin surface after removing the Needle Unit and placing Chamber Unit on the skin. The needle imprint can be seen before starting the next step.

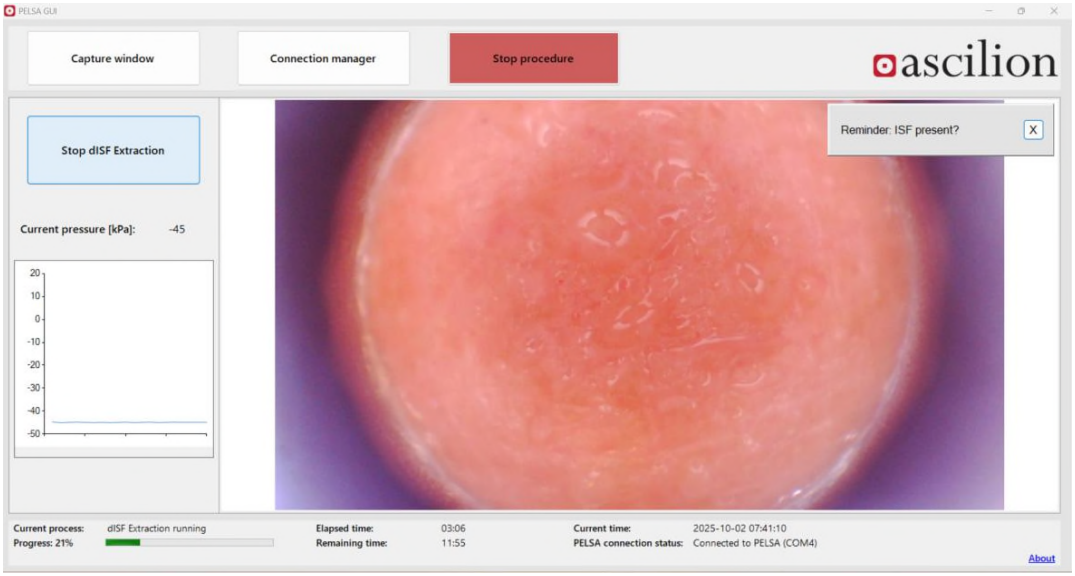
4. Click the software button labeled “Start dISF Extraction”.
5. The negative pressure slowly decreases until the maximum negative pressure is reached. The dISF Extraction phase will run for 15 minutes. A progress bar illustrates elapsed time and remaining time. After completion, the software returns the pressure to zero.
6. Reminder messages are displayed after 90 seconds (Check for blood!) and 3 minutes (Check dISF)



Check for blood reminder during the dISF Extraction phase. A few blood spot are clearly visible.

a. Wait for the 'Check for blood!' reminder to appear (after approx. 90 seconds). If blood spots are visible at this point, follow these steps:
   i. Click "Stop dISF Extraction"
   ii. Remove Chamber unit from the skin. Do not remove Skin fixture if used.
   iii. Wipe the blood and any dISF away with an alcohol wipe
   iv. Wait for 2 minutes (a two-minute timer will be displayed)
   v. Repeat from Step 2 in 6.4.2 dISF Extraction Phase.

b. If blood is again seen on the second attempt within 90 seconds of starting dISF Extraction, follow the steps:
   i. Repeat from 6.2 Sampling Skin Preparation, Step 1 using a new Needle Unit and select a new skin penetration site.
   ii. Enter a lower penetration pressure of -24 kPa.



ISF Present? reminder during the dISF Extraction phase. Droplets of dISF have formed on the skin surface.

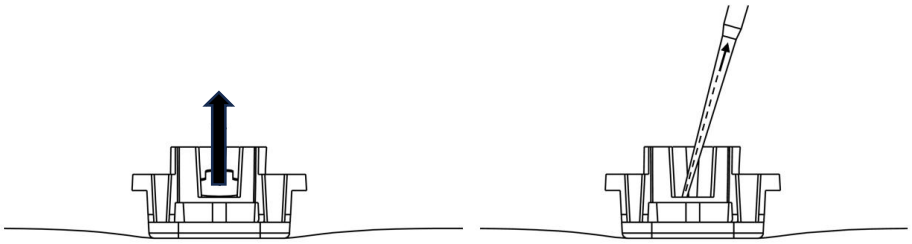
c. If no dISF is visible after the 'ISF present?' reminder is displayed (after approx. 3 minutes), follow these steps:
   i. Click "Stop procedure"
   ii. Repeat from 6.2 Sampling Skin Preparation Step 1 including attachment of a new Needle Unit and select a new skin penetration site.
   iii. Enter a penetration pressure of -30 kPa.

7. After 15 minutes the dISF Extraction phase is complete and the pressure returns to zero.
8. The dISF sample can now be collected, see the next section.

6.5 dISF collection and transport

1. Prepare the collection vessel/media and pipette by attaching the pipette tip, see 4.6 Auxiliary equipment. dISF evaporates quickly so it is important to prepare the pipette etc. so that the dISF can be collected *immediately* when the dISF Extraction phase is complete.
2. Remove the Camera Handpiece from the Chamber Unit by twisting and pulling while holding the Chamber Unit firmly to the skin.

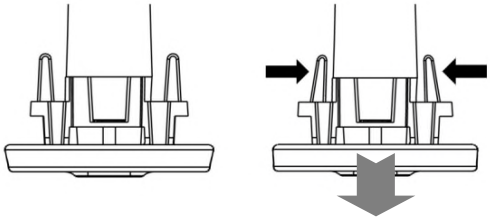
3. Remove the Tape lid from the Chamber Unit by pulling up on the Tape lid tab while holding the Chamber Unit firmly to the skin. Alternatively, remove the Chamber unit and collect directly from the skin.
4. As soon as possible after removing the Tape Lid, insert the tip of the pipette into the Chamber Unit opening and collect the fluid from the skin surface inside the reservoir then deposit the dISF in an appropriate medium (Eppendorf tube, paper or similar). Repeat as necessary. Work as quickly as possible to prevent evaporation of the dISF.
5. Finally, remove the Chamber Unit from the skin and collect the remaining dISF with the pipette directly from the skin. Always check the inside of the Chamber Unit as dISF may be present there.



6.6 Needle unit – Safe removal and disposal

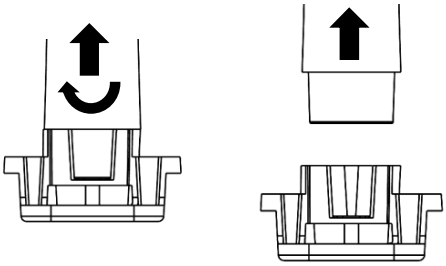
Position the Needle Unit *above* the Needle Unit plastic container and detach the Needle Unit from the Chamber Unit by pressing the two snaps inwards - so that the Needle unit detaches into the plastic container. This is easier than trying to detach the Needle Unit while it is placed inside the plastic container.

The Needle Unit contains sharps and should be disposed of as biological waste containing sharps.



6.7 Chamber Unit - Safe removal and disposal

Remove the Chamber Unit from the Camera Handpiece by twisting and pulling the handle while firmly holding the Chamber Unit. Disconnect the Chamber unit from the extension tube. The Chamber unit shall be disposed of as biological waste.



6.8 Auxillary equipment – Safe removal and disposal

Remove the pipette tip from the pipette and dispose the tip as biological waste. Also dispose of the tape lid, Skin Fixture and used alcohol wipes as biological waste.

7 Maintenance and cleaning

No calibration or preventive maintenance is required other than cleaning. All parts in contact with the patient and/or bodily liquids are single use devices that shall be discarded after each use.

Use a dry cloth and wipe the Control Unit, Camera Handpiece, cables and extension tube as needed. In case of spillage of sampled fluid, carefully disinfect contaminated parts and avoid ingress into the electrical devices.

Note: There are no serviceable parts of the PELSA system. Do not attempt to open any enclosure of the PELSA system.

If any re-usable part shows signs of material degradation, contact Ascilion to replace the part.

8 Storage conditions

All parts of the PELSA system shall be stored at 10-35°C, 30-85% RH, 90-106kPa.

If the Control Unit is stored for longer periods of time or is to be moved/transported, remove the Extension Tube and insert protective part delivered with the Control Unit into the pressure port ‘E’ to protect the pressure port from dust.

9 Troubleshooting

Problem	Possible reason	Solution
General		
No dISF visible or insufficient dISF volume produced	Insufficient skin penetration during Skin Penetration phase Hair or skin surface irregularities in sampling area causing pressure leakage during dISF Extraction phase	Repeat 6.2 Sampling Skin Preparation Step 1 including attachment of a new Needle Unit using penetration pressure of -30 kPa on a new skin site. Alternatively, shave the current skin site and repeat. Alternatively choose a more appropriate site that is without hair or surface irregularities that could cause leakage.
Washed out or excessively yellow image from Camera Low quality/'grainy' image that is difficult to focus	The Camera LED illumination level is too low for the Camera to acquire an acceptable image.	Increase the LED illumination level on the camera (via the adjustment wheel on the cable) to maximum, then decrease slowly until an acceptable image is obtained. This will cause the camera to automatically re-calibrate the image. Note that it may be necessary to manually adjust the Camera focus.

Problem	Possible reason	Solution
Air bubbles or foam appear in the collected dISF.	Leakage of air into the Chamber Unit from the interface between the Chamber Unit and the surface of the skin – potentially due to hair or dry /scaly skin.	If the bubbling is restricted to the edges of the Chamber Unit continue the dISF Extraction as normal. In most cases, the sampling can continue even with bubbling. Collect the dISF with a pipette from the skin surface below the bubbles/foam. If bubbles have filled most or all of the chamber it may be necessary to stop dISF extraction, collect the dISF that is present, wipe, reposition the Chamber Unit, and repeat part or all of the diSF Extraction phase. If significant bubbling still occurs, stop and restart penetration on a new site.
Hardware		
Insufficient or unstable negative pressure (e.g. increased or continuous pump activity)	Needle Unit and Chamber Unit not assembled correctly.	Remove and reattach the Chamber Unit to the Needle Unit and ensure that both snaps are fully engaged.
	Leakage between tubing and Chamber unit	Check all tube connections and ensure that all are correctly assembled.
	Leakage between Tape Lid and Chamber Unit	Seal the Tape Lid by pressing the lid firmly onto the Chamber Unit
	Leakage between tubing and Control Unit	Detach and fully re-insert the Extension tube into the pressure port of the Control Unit.
	Leakage between the skin surface and the Needle or Chamber Unit.	Shave any hair. Choose a more appropriate site that is flatter and without hair or surface irregularities that could cause leakage.
Software		
Full PELSA GUI window cannot be seen	Screen resolution too low	Set screen resolution to at least 1920x1080
	Screen resolution scaling too high	Set screen scaling factor to 150% or less

Problem	Possible reason	Solution
Connection to USB camera not possible	Camera not connected correctly	Remove and re-insert camera cable to USB-A port of Control Unit
	Windows OS version older than 11	Use a PC with Windows OS version 11
	Power cable not connected correctly	Remove and re-insert power cable to Control Unit
	Damaged camera	Contact Ascilion for replacement
	Damaged power supply or power cable	Contact Ascilion for replacement
	Damaged USB-C to USB-A cable	Contact Ascilion for replacement
Connection to COM port not possible	USB cable not connected correctly	Remove and re-insert USB cable (both to Control Unit to PC)
	Windows OS version older than 11	Use a PC with Windows OS version 11
	Power cable not connected correctly	Remove and re-insert power cable to Control Unit
	Damaged power supply or power cable	Contact Ascilion for replacement
	Damaged USB cable	Contact Ascilion for replacement
	Windows lacks Virtual COM port driver	Contact Ascilion for guidance
PELSA GUI cannot be opened	Windows OS version older than 11	Use a PC with Windows OS version 11
	Unsuccessful installation of PELSA GUI	Consult the Software installation Section or contact Ascilion
Unresponsive or slow GUI	Computer specification not in accordance with recommendations or excessive load on computer from other applications	Close other applications on computer Adjust the camera resolution(s) until GUI responds as desired

Contact Ascilion in case problem remains for further troubleshooting assistance.

10 Technical specifications

Type	Value
Maximum vacuum/Negative pressure	-45 kPa
Maximum pressure	45 kPa
Vacuum/pressure resolution	1 kPa
Applied part	Needle unit, Chamber unit, Skin fixture
Input voltage range	100-240 VAC
Frequency range	50 – 60 Hz
Power rating	25 W
Separation from mains supply	Disconnect the power cord from mains to separate the PELSA system from mains. Do not position the equipment so that it is difficult to disconnect the power cord from mains.
Usage conditions	10-35°C, 30-85% RH, 90-106kPa
Storage conditions	10-35°C, 30-85% RH, 90-106kPa
Transporting conditions	-10-70°C, 10-85% RH, 75-106kPa
Dimensions	Control Unit: 242 x 210 x 76 mm Camera Handpiece: 160 x Ø18 mm
Weight	Control Unit: 3.5 kg Camera Handpiece: 60 g Needle unit: 5 g Chamber unit: 7 g Skin fixture: 9 g
USB cable length	2 m
Power cable length	1.8 m
Camera Handpiece cable length	1.5 m
Extension tube length	1.2 m

10.1 Disposal information

At the end of the PELSA system lifetime, each re-usable part shall be disposed of according to local requirements. The following table can be used as guidance.

Part	Disposal information
Control Unit	Contains electronic circuit boards, metallic and plastic casing
Camera Handpiece	Contains electronic circuit boards, metallic and plastic casing
Power supply	Contains electronic circuit boards
Power cable	Contains electrical wires and insulation
USB Flash drive	Contains electronic circuit boards
Extension tube	Plastic, primarily DEHP-Free PVC
Chamber unit	Disposed of as biological waste
Needle unit	Disposed of as sharps, biological waste
Skin fixture	Disposed of as biological waste

11 Electromagnetic compatibility information

Note: The emissions characteristics of this equipment make it suitable for use in industrial areas and hospitals (CISPR 11 class A). If it is used in a residential environment (for which CISPR 11 class B in normally required) this equipment might not offer adequate protection to radio-frequency communication services. The user might need to take mitigation measures, such as relocating or re-orienting the equipment.

Note: The PELSA System has no essential performance as defined by IEC 60601-1 and IEC 60601-1-2.

Warning: Use of this equipment adjacent to or stacked with other equipment should be avoided because it could result in improper operation. If such use is necessary, this equipment and the other equipment should be observed to verify that they are operating normally.

Warning: Use of accessories, transducers and cables other that those specified or provided by the manufacturer of this equipment could result in increased electromagnetic emissions or decreased electromagnetic immunity of this equipment and result in improper operation.

Warning: Portable RF communications equipment (including peripherals such as antenna cables and antennas) should be used no closer than 30 cm (12 inches) to any part of the PELSA system, including cables specified by the manufacturer. Otherwise, degradation of the performance of this equipment could result.

12 Warnings, cautions and residual risks

Note: The PELSA system is currently not approved as a medical device. It shall only be used for research and clinical investigations where the use of the device has been approved by the relevant authorities.

Any serious incident that has occurred in relation to the device should be reported to the manufacturer and the competent authority of the Member State in which the user and/or patient is established.

12.1 Warnings

- Do not use the Needle unit if the sterile packaging is unintentionally opened or damaged.
- Do not re-use the Needle unit or the Chamber Unit. Both are single use consumables and shall be discarded after use to prevent contamination.
- Do not attempt to use a different vacuum/pressure system as the safety of the device cannot be guaranteed with external parts.
- Do not use any other Power supply than provided by Ascilion AB (XP Power AKM36US24CA).
- Avoid repetitive sampling on same body site. Excessive sampling may lead to skin hardening or lipohypertrophy.

12.2 Cautions

- Remove device if uncomfortable or painful. It is always possible to easily remove the device, even with negative pressure applied.
- Sampling should not be performed for a longer period than 60 minutes as this may lead to redness and or bruising of skin.
- The user needs to evaluate the need for protection in the IT environment the PELSA system, including PELSA GUI software, is used. No patient information is stored by default by the PELSA GUI software.
- Do not touch or let the patient touch the Control Unit during a sampling session.

12.3 Residual risks

- Using the PELSA system requires handling of bodily fluids. The PELSA system is intended to be used by healthcare professionals or scientists with knowledge in sampling of biological material from subjects. The user shall be trained in the PELSA system by Ascilion representative before usage.
- Redness of the skin at the sampling site that disappears within 1-3 days may occur.

13 Appendix 1. EMC information

13.1 Compliance with emissions standards

Emission	Emissions test levels
Conducted and radiated RF EMISSIONS	CISPR 11, Class A
Harmonic distortion	According to IEC 61000-3-2
Voltage fluctuations and flicker	According to IEC 61000-3-2

13.2 Compliance with immunity standards

Phenomenon	Basic EMC standard	Immunity test levels
Electrostatic discharge	IEC 61000-4-2	± 8 kV contact ± 2 kV, ± 4 kV, ± 8 kV, ± 15 kV
Radiated RF EM fields	IEC 61000-4-3	3 V/m 80 MHz – 2.7 GHz 80% AM at 1 kHz
Proximity fields from RF wireless communication equipment	IEC 61000-4-3	See table 9 in IEC 60601-1-2
Rated power frequency magnetic fields	IEC 61000-4-8	30 A/m (50 Hz)
Electrical fast transients / bursts	IEC 61000-4-4	± 2 kV 100 kHz repetition frequency
Surges line-to-line	IEC 61000-4-5	± 0.5 kV, ± 1 kV
Surges line-to-ground	IEC 61000-4-5	± 0.5 kV, ± 1 kV, ± 2 kV
Voltage dips	IEC 61000-4-11	0 % U_T; 0.5 cycle At 0°, 45°, 90°, 135°, 180°, 225°, 270° and 315°
		0 % U_T; 1 cycle and 70 % U_T; 25/30 cycles Single phase: at 0°
Voltage interruptions	IEC 61000-4-11	0 % U_T; 250/300 cycles
Conducted disturbances induces by RF fields	IEC 61000-4-6	3 V/m 0.15 MHz – 80 MHz 6 V in ISM bands between 0.15 MHz and 80 MHz 80 % AM at 1 kHz

Ascilion AB
Isafjordsgatan 22
164 40 Kista
Sweden

E-mail: info@ascilion.com
www.ascilion.com

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