CONFIDENTIAL

STATISTICAL ANALYSIS PLAN FOR PROTOCOL 207451

A Proof of Concept (POC) Clinical Study to Investigate the Effects of a Developmental Cosmetic Moisturising Cream on the Barrier Function of Human Skin on the Face and Forearm

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Table of Contents

Glossary

CI	Confidence Interval
ITT	Intent-to-Treat
PP	Per Protocol
AE	Adverse Event
MedDRA	Medical Dictionary for Regulatory Activities
TE	Treatment Emergent
TEWL	Transepidermal Water Loss

1 Introduction

This document describes the statistical methods and data presentations to be used in the summary and analysis of the final data from Protocol 207451.

2 Objectives

Objectives	Endpoints
Primary	
To assess skin barrier function on the forearm after 4 weeks of using the test product compared to no treatment.	Change from baseline in TEWL measurements on Day 29 of test product treated site vs untreated site on the forearm (Area 1 and 3).
Secondary	
To assess skin barrier function on the face after 4 weeks of using the test product compared to no treatment.	Change from baseline in TEWL measurements on Day 29 of test product treated site vs untreated site on the face (Area 6 and 8).
To assess changes in skin moisturisation and barrier function of the forearm and face during 4 weeks of using the test product compared to no treatment.	Change from baseline in corneometry and TEWL measurements at Area 1 and 3 of test product treated site vs untreated site on the forearm and Area 6 and 8 of the face at Day 1 (30 minutes after application and 6 hours after application - corneometry only), Day 2, 15, and 29. As well as Standardised Area Under Curves (AUCs) calculated using change from baseline in TEWL and corneometry over treatment period (Days 1, 2, 15, and 29)
To assess the impact on skin barrier function after physical challenge following 4 weeks of using the test product on the forearm.	Change from pre-challenge TEWL measurements of D-Squame discs following 4, 8 and 12 adhesive discs removal from skin of both test product treated and untreated sites on the forearm on Day 29 at Area 2 and 4
To assess the impact on skin barrier function after physical challenge following 4 weeks of using the test product on the face.	Change from pre-challenge TEWL measurements of D-Squame discs following 3, 6 and 9 adhesive discs removal from skin of both test product treated and untreated sites on the face on Day 29 at Area 5 and 7.
To assess the levels of protein present on D-Squame discs following 4 weeks of using the test product from sites on the	Protein analysis (SquameScan) of D-Squame discs following removal of 4, 8 and 12 adhesive discs from skin of both test product

forearm.	treated and untreated sites on the forearm on Day 29.
To assess the levels of protein present on D-Squame discs following 4 weeks of using the test product from sites on the face.	Protein analysis of D-Squame discs following removal of 3, 6 and 9 adhesive discs from skin of both test product treated and untreated sites on the face on Day 29.
To assess skin barrier function on the forearm and face through a Regression Period following 4 weeks of test product use.	Change from baseline and from Day 29 in TEWL measurements on Days 30, 31, 32, 33 and 34 in test product treated site vs untreated sites on the forearm and face. As well as standardised AUCs calculated using change from baseline and from Day 29 in TEWL over regression period (Days 30, 31, 32, 33 and 34) at Area 1 and 3 (Forearm) and Area 6 and 8 (Face).
To assess moisturisation levels on the forearm and face through a Regression Period following 4 weeks of test product use.	Change from baseline and from Day 29 in Corneometry measurements on Days 30, 31, 32, 33 and 34 in test product treated site vs untreated site on the forearm and face. As well as Standardised AUCs calculated using change from baseline and from Day 29 in corneometry over regression period (Days 30, 31, 32, 33 and 34) at Area 1 and 3 (Forearm) and Area 6 and 8 (Face).
Validation of trial with respect to skin barrier function on the forearm after 4 weeks via comparison of the positive control to no treatment.	Change from baseline in TEWL measurements on Day 29 of test product treated site vs untreated site on the forearm (Area 1 and 3).
To evaluate the local tolerance.	Frequency and severity of Adverse Events.

3 Study Design

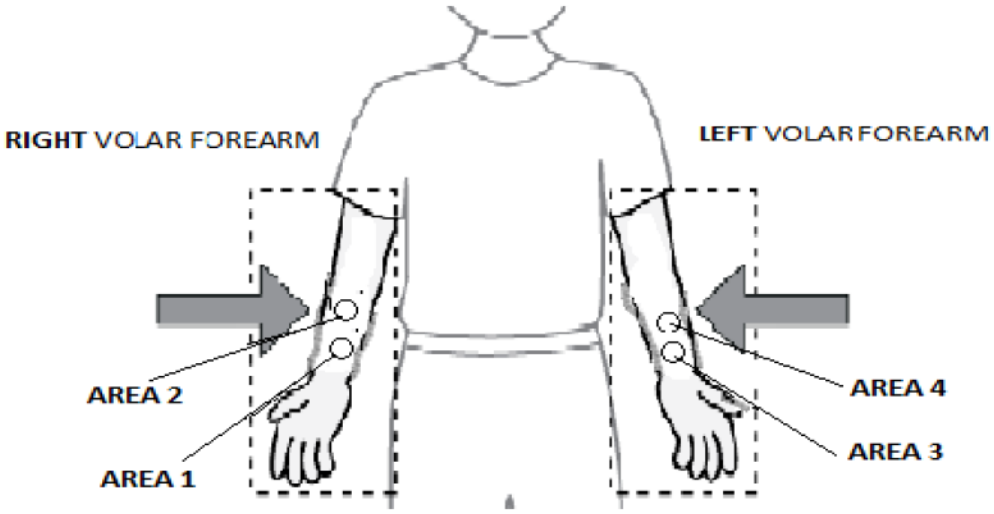
- Evaluator-blind
- Single study center
- Split-body
- Healthy female subjects aged 18 to 65 with self-reported dry, sensitive skin on their face and body

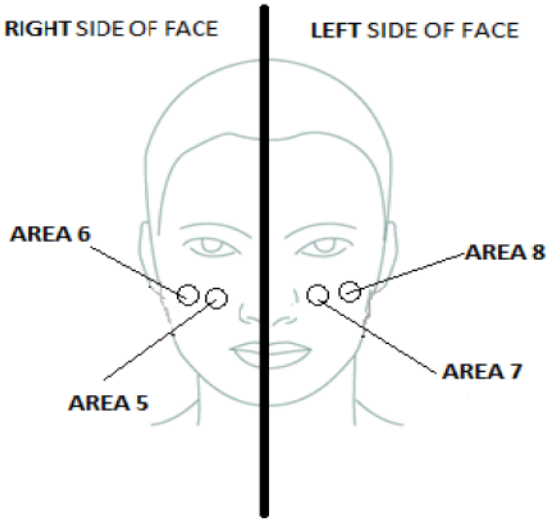
- Positive- (Olay ProX Wrinkle Smoothing Cream) and Untreated-control

- Randomization was to two of the 3 treatment arms (test product, positive control, no treatment) with treatment arm assignment further randomized to either the right side or left side of the body. Therefore, a subject was randomized to one of the 6 possible treatment groups:

Right Side of Body	**Left Side of Body**
Test Product	Positive Control
Test Product	No Treatment
Positive Control	No Treatment
Positive Control	Test Product
No Treatment	Test Product
No Treatment	Positive Control

- 8 areas for assessments were marked on the face and forearms as shown below:





4 Sample Size Determination

The primary evaluation will be the change from baseline in TEWL measurements on Day 29 of test product versus no treatment on the forearm. The only previous data available in a 29-day model is a study (GSKCH Clinical Study: PPD which assessed TEWL on the leg. In that study, the change from baseline in TEWL following 4 weeks of treatment with a similar product on the leg was not normally distributed. Using the data from that study and applying a Wilcoxon sample size adjustment to the paired t-test, 40 subjects treated with test product would be required to detect a difference of 1.5 points in change from baseline in TEWL at alpha=0.05 with at least 90% power assuming a standard deviation of 2.7 points.

With this study design, 66 subjects would need to be randomised to ensure at least 40 subjects are treated with each of the 3 treatments (test product, positive control, no treatment).

5 Data Considerations

5.1 Analysis Populations

- The 'Intent to treat' (ITT) population includes all subjects who are randomised into the study and have at least one post-baseline measurement available. All efficacy analyses will be based on the ITT population.

- The Safety population will include all subjects who applied any of the study products. All safety analyses will be performed using the Safety population.

- The Per Protocol (PP) population will consist of the subset of ITT subjects which excludes those subjects with significant protocol deviations. Confirmatory analyses of the primary efficacy endpoint (change from Baseline in TEWL of the forearm) as well as the change from Baseline in TEWL of the face and changes from baseline in corneometry of the forearm and face will be performed on the PP population.

  Subjects with a protocol violation that is deemed to affect assessments of either the forearm or face after a specific timepoint will be part of the PP population, but will have their data excluded from the relevant assessment at which the protocol violation occurred.

  Violations that may lead to the exclusion of data for PP analysis include, but are not limited to, the following:

  - Violation of inclusion or exclusion criteria at screening or baseline that may affect either the forearm or face assessments.
  - Non-compliance with assigned treatment regimen.
  - Use of prohibited treatment or medication before or during the study, which it is felt will affect forearm or face assessments.

  Violations will be documented in the Population Definitions document. The content of this document will be agreed upon between the Biostatistician and Clinical Development Director or designee prior to database lock and breaking of the study blind.

  A PP analysis will be performed on forearm and face TEWL and corneometry assessments if there is more than 10% difference in the number of subjects evaluable in any of the treatment groups for the ITT and PP populations.

5.2 Subgroups/Stratification

There was no stratification in this study.

5.3 Time Windows

All data will be accepted for analysis. Deviations from the scheduled nominal visit days are not expected. Any deviations will be noted in the deviation log and visits

may be considered for exclusion from the Per Protocol population.

5.4 Missing Data Handling

Missing data will not be imputed. Dropouts will be included in analyses up to the point of discontinuation.

6 Demographics and Baseline Characteristics

6.1 Subject Disposition

The number of subjects screened, enrolled, randomized and completing the study will be presented by treatment arm (i.e. test product, positive control, no treatment) and overall as well as in a separate summary, by treatment group (i.e. each of the 6 right side of body/left side of body treatment combinations to which subjects were randomized) and overall using frequency counts and percentages.

6.2 Demographics

Age and baseline forearm and face overall dryness scores, including each of the individual dryness parameters (dull appearance, roughness, scaling, feeling of tightness) as well as the total overall dryness score, will be summarised descriptively by treatment arm (test product, positive control, no treatment) using means, medians and standard deviations. Race and Fitzpatrick Skin Type and the 4 individual overall dryness parameters described above will be summarised using frequency counts and percentages.

7 Treatment Compliance and Concomitant Medications

7.1 Treatment Compliance

All deviations associated with the application of either the test product or positive control or the preservation of the lack of treatment, if so assigned, to the relevant side of the body (forearm and face) will be listed.

7.2 Concomitant Medications

Any concomitant medication use will be listed.

8 Analysis

The primary objective will be to assess the skin barrier function of the test product

formulation based on change from baseline in TEWL after 4 weeks of twice daily product application on the forearm (Area 1 and 3). Given this proof of concept study, the study will be considered a success if at least a trend in favor of the test product in change from baseline at Day 29 in TEWL (Area 1 and 3) is found compared to the untreated forearm. There will be no adjustment to the critical alpha level of 0.05 to account for inflation due to multiplicity. P-values resulting from inferential testing will be considered primarily as summary statistics.

Further, since the positive control (Olay® ProX Cream) has been included in the study to support validation of the clinical model, the only comparisons involving it in analyses will be to the no treatment arm. There will be no comparison of the test product to the positive control.

8.1 Primary Analysis

Change from baseline in TEWL for each subject at Day 29 (Area 1 and 3 on the forearms and Area 6 and 8 on the face) will be summarised for each of the three treatment arms (test product, positive control and no treatment) of both the forearm (primary assessment area) and face using descriptive statistics (means, medians, standard deviations, 95% confidence intervals). Changes from baseline for each treatment group will be compared to zero using t-test and the p-values and 95% confidence intervals for these within-group changes will be presented.

Test product versus no treatment and positive control versus no treatment will be compared for the change from baseline at Day 29 of both the forearms (Area 1 and 3) (primary assessment area) and face (Area 6 and 8) using analysis of covariance (ANCOVA) with subject as a random effect, treatment arm (test product, positive control and no treatment) and side of body (right, left) as main effects and baseline value as covariate. This approach allows for the inclusion of data from all subjects treated with a given treatment arm (test product, positive control or no treatment) regardless of the treatment group (test product/no treatment, test product/positive control, positive control/no treatment) to which they were randomized to derive estimates of treatment effect. Least square means from the ANCOVA model for the change from baseline will be presented for each treatment arm and for the difference between each pair of the active treatment arms and no treatment together with p-values and 95% confidence intervals.

If the assumption of normality is rejected, an appropriate transformation to the data will be performed to facilitate the above method of analysis. In the absence of an appropriate data transformation, non-parametric analyses will be performed. In the

case of a non-parametric analysis, median differences will be presented, together with 95% confidence intervals based on the Hodges-Lehmann method.

8.2 Secondary Analysis

Change from baseline in TEWL and Corneometry for each subject at Days 1 (30 minutes and 6 hours post first application – corneometry only), 2, 15, 30, 31, 32, 33, and 34 (Area 1 and 3 on the forearms and Area 6 and 8 on the face) will be summarised for all 3 treatment arms of both the forearm and face using descriptive statistics (means, medians, standard deviations, 95% confidence intervals). Changes from baseline for each treatment group will be compared to zero using t-test and the p-values and 95% confidence intervals for these within-group changes will be presented.

Test product versus no treatment and positive control versus no treatment will be compared for the change from baseline at each time point of both the forearms (Area 1 and 3) and face (Area 6 and 8) using analysis of covariance (ANCOVA) with subject as a random effect, treatment arm (test product, positive control and no treatment) and side of body treatment applied (right, left) as main effects and baseline value as covariate. This approach allows for the inclusion of data from all subjects treated with a given treatment arm (test product, positive control or no treatment) regardless of the treatment group (test product/no treatment, test product/positive control, positive control/no treatment) to which they were randomized to derive estimates of treatment effect. Least square means from the ANCOVA model for the change from baseline will be presented for each treatment arm and for the difference between each pair of the active treatment arms and no treatment together with p-values and 95% confidence intervals.

Standardised AUCs will be calculated for each subject for change from baseline in TEWL and corneometry (Area 1 and 3 on the forearms and Area 6 and 8 on the face) over the treatment period; i.e. through Day 29 (Days 1, 2, 15, and 29) and separately over the Regression period; i.e. through Day 34 (Days 30, 31, 32, 33 and 34) using the trapezoidal rule and dividing by the number of days in the period. Each of the treatment period and regression period AUCs will be similarly summarized and compared for both the forearm and face using analysis of covariance (ANCOVA) with subject as a random effect, treatment arm (test product, positive control and no treatment) and side of body treatment applied (right, left) as main effects and baseline value as covariate. Least square means from the ANCOVA model for the change from baseline will be presented for each treatment arm and for the difference

between each pair of the active treatment arms and no treatment together with p-values and 95% confidence intervals.

Day 29 change from pre-challenge in TEWL (Area 2 and 4 of the forearms and Area 5 and 7 of the face) will be summarised for all 3 treatment arms of both the forearm and face after each set of discs (4, 8 and 12 discs for the forearm and 3, 6 and 9 discs for the face) using descriptive statistics (means, medians, standard deviations, 95% confidence intervals). Day 29 change from pre-challenge for each treatment group, for both the forearm and face, and following each set of discs will be compared to zero using t-test and the p-values and 95% confidence intervals for these within-group changes will be presented. Comparisons of the changes from pre-challenge after each set of discs between the test product and no treatment for both the forearm and face will be performed using analysis of covariance (ANCOVA) with subject as a random effect, treatment arm (test product, positive control and no treatment) and side of body treatment applied (right, left) as main effects and the pre-challenge value as covariate. Least square means from the ANCOVA model for the change from pre-challenge will be presented for each treatment arm and for the difference between each pair of the active treatment arms and no treatment together with p-values and 95% confidence intervals.

ANOVA as described above for the primary endpoint (excluding the covariate) or analysis based on transformed data or an appropriate non-parametric analysis will be used to compare the total amount of protein present collected from each of the D-Squame discs at Day 29 separately for forearms (12 discs) and face (9 discs). P-values resulting from these analyses as well as 95% confidence intervals for the differences in the protein levels between each pair of the active treatment arms and no treatment will also be provided.

Change from Day 29 to Days 30, 31, 32, 33 and 34 in TEWL and corneometry (Area 1 and 3 on the forearms and Area 6 and 8 on the face) and the standardised AUC calculated over the Regression period using the trapezoidal rule and divided by the number of days in the period will be summarised and compared between each pair of the active treatment arms and no treatment, separately for forearm and face, as described above for the changes from baseline.

If the assumption of normality is rejected for any of the above analyses, an appropriate transformation to the data will be performed to facilitate the method of analysis. In the absence of an appropriate data transformation, non-parametric analyses will be performed. In the case of a non-parametric analysis, median differences will be presented, together with 95% confidence intervals based on the

Hodges-Lehmann method.

9 Safety Analysis

Treatment emergent AEs are defined as events that start on or after the first treatment date. Events occurring following the start of treatment which were also reported before treatment began with no change in severity or causality will however not be considered treatment emergent.

As per Section 7.1.1 of the protocol, the following does not constitute an AE:

- Any localised response to the D-Squame disc application and removal on the face and forearms, unless more severe than expected in which case will be captured as an AE.

AEs will be tabulated according to the current version of the MedDRA. Frequencies and percentages will be presented by product group and overall, for each system organ class, and for each preferred term. Summaries of treatment-emergent AEs, treatment-related treatment-emergent AEs, AEs leading to discontinuation, and serious AEs will be completed. AEs relating to the forearm or face will be summarized separately.

10 Interim Analysis

Not Applicable

11 Topline Summary

The following tables will be produced for the topline summary:

Table No.	Description
9.1.1	Subject Disposition by Treatment Group – All Screened Subjects
9.1.2	Subject Disposition by Treatment Arm – All Screened Subjects
9.3.1.1.1	Summary of Forearm TEWL – ITT Population
9.3.1.1.2	Analysis of Forearm TEWL – ITT Population

9.3.1.2.1	Summary of Face TEWL – ITT Population
9.3.1.2.2	Analysis of Face TEWL – ITT Population
9.4.1	Treatment-Emergent Adverse Events – Safety Population

12 Changes to Planned Analysis

There are no changes to the protocol-planned analyses.

13 References

None

Appendix 1 Study Schedule

Procedure	Visit 1 (Screening)		Visit 2 Day 1 Baseline Visit	Visit 3 Day 2 (24±1hr*)	Visit 4 Day 15 (± 24 hrs)	Visit 5 Day 29 (± 48 hrs) D-Squame Challenge		Visit 6 Day 30	Visit 7 Day 31	Visit 8 Day 32	Visit 9 Day 33	Visit 10 Day 34
	D-7 to D-5					Pre	Post	Regression Period (no product use)				
Informed Consent	X	WASHOUT PERIOD (5-7 Days)										
Demographics	X											
Medical History	X											
Current/Concomitant Medications	X		X	X	X	X		X	X	X	X	X
Assessment of Dryness [1]	X		X									
Fitzpatrick Skin Type Grading	X											
Inclusion and Exclusion criteria[2]	X		X									
Subject Eligibility	X		X									
Continued Eligibility			X	X	X	X		X	X	X	X	X
Dispense Standard Soap and Diary Cards	X											
Randomisation			X									
TEWL - Area 6 & 8 (Face) and Area 1 & 3 (Forearm)			X[3]	X	X	X		X	X	X	X	X

Procedure	Visit 1 (Screening)	WASHOUT PERIOD (5-7 Days)	Visit 2 Day 1 Baseline Visit	Visit 3 Day 2 (24±1hr*)	Visit 4 Day 15 (± 24 hrs)	Visit 5 Day 29 (± 48 hrs) D-Squame Challenge		Visit 6 Day 30	Visit 7 Day 31	Visit 8 Day 32	Visit 9 Day 33	Visit 10 Day 34
	D-7 to D-5					Pre	Post	Regression Period (no product use)				
Corneometry - Area 6 & 8 (Face) and Area 1 & 3 (Forearm)			X[4]	X	X	X		X	X	X	X	X
Dispense Product and Diary Cards			X									
Product administration (site supervision)			X[5]	X[5]	X[5]							
TEWL - Area 5 & 7 (Face) and Area 2 & 4 (Forearm) **BEFORE** D-Squame challenge						X						
D-Squame Challenge – Area 5 & 7 (Face) and Area 2 & 4 (Forearm)						X[6]						
TEWL Area 5 & 7 (Face) and Area 2 & 4 (Forearm) **AFTER** D-Squame challenge [6] *(TEWL assessed after each set of 4 discs removed (total of 12) from the forearms and each set of 3 discs removed (total of 9) from the each side of the face)*							X					
Measure protein content from all discs from each forearm and side of the face.							X					

Procedure	Visit 1 (Screening)	WASHOUT PERIOD (5-7 Days)	Visit 2 Day 1 Baseline Visit	Visit 3 Day 2 (24±1hr*)	Visit 4 Day 15 (± 24 hrs)	Visit 5 Day 29 (± 48 hrs) D-Squame Challenge		Visit 6 Day 30	Visit 7 Day 31	Visit 8 Day 32	Visit 9 Day 33	Visit 10 Day 34
	D-7 to D-5					Pre	Post	Regression Period (no product use)				
Return Standard Soap												X
Return Study Product (s) & Diary Cards						X						
Adverse event assessment [7]	X		X	X	X	X	X	X	X	X	X	X
Study Conclusion/Subject Exit												X

All Subjects will have their Visits scheduled at approximately the same time of day for each visit for the duration of the study (Except Day 29).

* = Assessments 24 hours (hrs) ± 1hr after supervised application at Visit 2

1) Inclusion criteria 6 - Trained examiner assessments and subject response for measures of dryness on each side of the face and each forearm at Screening and Baseline visits (Appendix 2).

2) Including subject self-reported of dry, sensitive skin on the face and body at Screening.

3) Visit 2 consists of Baseline trans-epidermal water loss (TEWL) assessments at Face Area 6 (RIGHT) and Area 8 (LEFT) and Forearm Area 1 (RIGHT) and Area 3 (LEFT), prior to supervised product application.

4) Visit 2 consists of Baseline pre-application corneometry assessments at Face Area 6 (RIGHT) and Area 8 (LEFT) and Forearm Area 1 (RIGHT) and Area 3 (LEFT) prior to supervised product (s) application, as well as 30 minutes and 6 hours post supervised product application.

5) Supervised product (s) application following completion of all visit assessments and measurements.

6) D-Squame challenge on Face Area 5 (RIGHT) and Area 7 (LEFT) and Forearm Area 2 (RIGHT) and Area 4 (LEFT).

   Note: 4, 8, 12 discs removed from the right and left forearms and 3, 6, 9 discs removed from the right and left side of the face.

7) Adverse events will be reported following first use of the standard soap. The use of any concomitant medication will be reported following subject provision of informed consent until completion of the study.

Appendix 2 List of Tables, Figures & Listings

In all outputs, the treatment labels and order for presentation in tables and listings is:

1) Moisturising Cream
2) Olay ProX Cream
3) No Treatment

Table No.	Table Title (including population)	Standard	Template
9.1.1	Subject Disposition by Treatment Group – All Screened Subjects	X	
9.1.2	Subject Disposition by Treatment Arm – All Screened Subjects	X	
9.2.1.1	Demographics – ITT Population	X	
9.2.1.2	Demographics – Safety Population	X	
9.2.1.3	Demographics – PP Population *(if needed)*	X	
9.3.1.1.1	Summary of Forearm TEWL – ITT Population		Appendix 3
9.3.1.1.2	Analysis of Forearm TEWL – Changes from Baseline – ITT Population		Appendix 3
9.3.1.1.3	Summary of Forearm TEWL –PP Population *(if needed)*		Table 9.3.1.1.1
9.3.1.1.4	Analysis of Forearm TEWL – Changes from Baseline – PP Population *(if needed)*		Table 9.3.1.1.2
9.3.1.2.1	Summary of Face TEWL – ITT Population		Table 9.3.1.1.1
9.3.1.2.2	Analysis of Face TEWL – Changes from Baseline – ITT Population		Table 9.3.1.1.2
9.3.1.2.3	Summary of Face TEWL – PP Population *(if needed)*		Table 9.3.1.1.1
9.3.1.2.4	Analysis of Face TEWL – Changes from Baseline – PP Population *(if needed)*		Table 9.3.1.1.2

9.3.2.1.1	Summary of Forearam Corneometry – ITT Population		Table 9.3.1.1.1
9.3.2.1.2	Analysis of Forearm Corneometry – Changes from Baseline – ITT Population		Table 9.3.1.1.2
9.3.2.1.3	Summary of Forearm Corneometry – PP Population *(if needed)*		Table 9.3.1.1.1
9.3.2.1.4	Analysis of Forearm Corneometry – Changes from Baseline – PP Population *(if needed)*		Table 9.3.1.1.2
9.3.2.2.1	Summary of Face Corneometry – ITT Population		Table 9.3.1.1.1
9.3.2.2.2	Analysis of Face Corneometry – Changes from Baseline – ITT Population		Table 9.3.1.1.2
9.3.3.1.1	Summary of D-Squame Challenge Forearm TEWL Assessments – Changes from Pre-Challenge - ITT Population		Appendix 3
9.3.3.1.2	Analysis of D-Squame Challenge Forearm TEWL Assessments – Changes from Pre-Challenge - ITT Population		Appendix 3
9.3.3.2.1	Summary of D-Squame Challenge Face TEWL Assessments – Changes from Pre-Challenge - ITT Population		Table 9.3.3.1.1
9.3.3.2.2	Analysis of D-Squame Challenge Face TEWL Assessments – Changes from Pre-Challenge - ITT Population		Table 9.3.3.1.2
9.3.4.1	Summary of Protein Levels Post D-Squame Challenge – ITT Population		Appendix 3

9.3.4.2	Analysis of Protein Levels Post D-Squame Challenge – ITT Population		Appendix 3
9.3.5.1.1	Summary of Forearm TEWL – Changes from Day 29 – ITT Population		Table 9.3.1.1.1
9.3.5.1.2	Analysis of Forearm TEWL – Changes from Day 29 – ITT Population		Table 9.3.1.1.2
9.3.5.2.1	Summary of Face TEWL – Changes from Day 29 – ITT Population		Table 9.3.1.1.1
9.3.5.2.2	Analysis of Face TEWL – Changes from Day 29 – ITT Population		Table 9.3.1.1.2
9.4.1	Treatment-Emergent Adverse Events – Safety Population	X	
9.4.1.1	Treatment-Emergent Adverse Events of the Forearm and Face – Safety Population		Table 9.4.1
9.4.2	Treatment-Emergent Treatment-Related Adverse events – Safety Population	X	
9.4.3	Treatment-Emergent Adverse Events by Severity – Safety Population	X	
9.4.4	Treatment-Emergent Treatment-Related Adverse Events by Severity – Safety Population	X	

All listings to be generated are the standard set.

Appendix 3 Templates for Tables, Figures & Listings

Table 9.3.1.1.1
Summary of Forearm TEWL
ITT Population (N=xx)

Timepoint		Moisturising Cream	Olay ProX Cream	No Treatment
Baseline*	N	XX	XX	XX
	Missing	XX	XX	XX
	Mean	X.XX	X.XX	X.XX
	SD	X.XXX	X.XXX	X.XXX
	95%CI	X.XX, X.XX	X.XX, X.XX	X.XX, X.XX
Day 2	N	XX	XX	XX
	Missing	XX	XX	XX
	Mean	X.XX	X.XX	X.XX
	SD	X.XXX	X.XXX	X.XXX
	95%CI	X.XX, X.XX	X.XX, X.XX	X.XX, X.XX
Day 2 Change from Baseline	N	XX	XX	XX
	Missing	XX	XX	XX
	Mean	X.XX	X.XX	X.XX
	SD	X.XXX	X.XXX	X.XXX
	95%CI	X.XX, X.XX	X.XX, X.XX	X.XX, X.XX
	LSMean (±SE)#	X.XX (X.XXX)	X.XX (X.XXX)	X.XX (X.XXX)
	LSMean 95%CI	X.XX, X.XX	X.XX, X.XX	X.XX, X.XX
	P-value	X.XXX	X.XXX	X.XXX
Day 15	N	XX	XX	XX

	Missing	XX	XX	XX
	Mean	X.XX	X.XX	X.XX
	SD	X.XXX	X.XXX	X.XXX
	95%CI	X.XX, X.XX	X.XX, X.XX	X.XX, X.XX
Day 15 Change from Baseline	N	XX	XX	XX
	Missing	XX	XX	XX
	Mean	X.XX	X.XX	X.XX
	SD	X.XXX	X.XXX	X.XXX
	95%CI	X.XX, X.XX	X.XX, X.XX	X.XX, X.XX
	LSMean (±SE)[#]	X.XX (X.XXX)	X.XX (X.XXX)	X.XX (X.XXX)
	LSMean 95%CI	X.XX, X.XX	X.XX, X.XX	X.XX, X.XX
	P-value	X.XXX	X.XXX	X.XXX
Day 29	N	XX	XX	XX
	Missing	XX	XX	XX
	Mean	X.XX	X.XX	X.XX
	SD	X.XXX	X.XXX	X.XXX
	95%CI	X.XX, X.XX	X.XX, X.XX	X.XX, X.XX
Day 29 Change from Baseline	N	XX	XX	XX
	Missing	XX	XX	XX
	Mean	X.XX	X.XX	X.XX
	SD	X.XXX	X.XXX	X.XXX
	95%CI	X.XX, X.XX	X.XX, X.XX	X.XX, X.XX
	LSMean (±SE)[#]	X.XX (X.XXX)	X.XX (X.XXX)	X.XX (X.XXX)
	LSMean 95%CI	X.XX, X.XX	X.XX, X.XX	X.XX, X.XX
	P-value	X.XXX	X.XXX	X.XXX

AUC Change from Baseline Days 2 through 29	N	XX	XX	XX
	Missing	XX	XX	XX
	Mean	X.XX	X.XX	X.XX
	SD	X.XXX	X.XXX	X.XXX
	95%CI	X.XX, X.XX	X.XX, X.XX	X.XX, X.XX
	LSMean (±SE)#	X.XX (X.XXX)	X.XX (X.XXX)	X.XX (X.XXX)
	LSMean 95%CI	X.XX, X.XX	X.XX, X.XX	X.XX, X.XX
	P-value	X.XXX	X.XXX	X.XXX
Etc….				

An increase in TEWL shows damage to the skin barrier function.
* Baseline is measured prior to any test product application.
# LSMean, standard error (SE) and within-group p-value derived from ANCOVA with subject as random effect, treatment arm and side of body as fixed effects, and baseline value as covariate.

Programmers Note: Continue table for each additional timepoint (Days 30,31,32,33,34 and AUC Days 30-34).
Same format for Tables 9.3.1.1.3, 9.3.1.2.1, 9.3.2.1.1, 9.3.2.1.3, 9.3.2.2.1, 9.3.5.1.1, 9.3.5.2.1 - Change the title and timepoints (corneometry additionally has 30min and 6hr on Day 1) to match protocol.

Table 9.3.1.1.2
Analysis of Forearm TEWL Change from Baseline
ITT Population (N=xx)

Timepoint		Difference	95% Confidence Interval for the Difference	P-Value
Day 2	Moisturising Cream vs. No Treatment	X.XX	X.XX, X.XX	X.XXX
	Olay ProX Cream vs No Treatment	X.XX	X.XX, X.XX	X.XXX
Day 15	Moisturising Cream vs. No Treatment	X.XX	X.XX, X.XX	X.XXX
	Olay ProX Cream vs No Treatment	X.XX	X.XX, X.XX	X.XXX
Day 29	Moisturising Cream vs. No Treatment	X.XX	X.XX, X.XX	X.XXX
	Olay ProX Cream vs No Treatment	X.XX	X.XX, X.XX	X.XXX
AUC Days 2-29	Moisturising Cream vs. No Treatment	X.XX	X.XX, X.XX	X.XXX
	Olay ProX Cream vs No Treatment	X.XX	X.XX, X.XX	X.XXX
Day 30	Moisturising Cream vs. No Treatment	X.XX	X.XX, X.XX	X.XXX
	Olay ProX Cream vs No Treatment	X.XX	X.XX, X.XX	X.XXX
Day 31	Moisturising Cream vs. No Treatment	X.XX	X.XX, X.XX	X.XXX
	Olay ProX Cream vs No Treatment	X.XX	X.XX, X.XX	X.XXX
Day 32	Moisturising Cream vs. No Treatment	X.XX	X.XX, X.XX	X.XXX
	Olay ProX Cream vs No Treatment	X.XX	X.XX, X.XX	X.XXX
Day 33	Moisturising Cream vs. No Treatment	X.XX	X.XX, X.XX	X.XXX
	Olay ProX Cream vs No Treatment	X.XX	X.XX, X.XX	X.XXX
Day 34	Moisturising Cream vs. No Treatment	X.XX	X.XX, X.XX	X.XXX
	Olay ProX Cream vs No Treatment	X.XX	X.XX, X.XX	X.XXX
AUC Days 30-34	Moisturising Cream vs. No Treatment	X.XX	X.XX, X.XX	X.XXX

	Olay ProX Cream vs No Treatment	X.XX	X.XX, X.XX	X.XXX

Difference is the first named treatment adjusted (LS) mean change from baseline minus the second named treatment adjusted mean change from baseline.

Analysis model (ANCOVA) included subject as random effect, treatment arm and side of body as fixed effects, and baseline value as covariate.

PROGRAMMER'S NOTE: Same format for Tables 9.3.1.1.4, 9.3.1.2.2, 9.3.2.1.2, 9.3.2.1.4, 9.3.2.2.2, 9.3.5.1.2, 9.3.5.2.2 - Change the title and timepoints (corneometry additionally has 30min and 6hr on Day 1) to match protocol.

Table 9.3.3.1.1

Summary of Forearm D-Squame Challenge TEWL Assessments – Changes from Pre-Challenge
ITT Population (N=xx)

Timepoint		Moisturising Cream	Olay ProX Cream	No Treatment
Pre-Challenge Day 29	N	XX	XX	XX
	Missing	XX	XX	XX
	Mean	X.XX	X.XX	X.XX
	SD	X.XXX	X.XXX	X.XXX
	95%CI	X.XX, X.XX	X.XX, X.XX	X.XX, X.XX
Change from Pre-Challenge after 4 Discs	N	XX	XX	XX
	Missing	XX	XX	XX
	Mean	X.XX	X.XX	X.XX
	SD	X.XXX	X.XXX	X.XXX
	95%CI	X.XX, X.XX	X.XX, X.XX	X.XX, X.XX
	LSMean (±SE)[#]	X.XX (X.XXX)	X.XX (X.XXX)	X.XX (X.XXX)
	LSMean 95%CI	X.XX, X.XX	X.XX, X.XX	X.XX, X.XX
	P-value	X.XXX	X.XXX	X.XXX
Change from Pre-Challenge after 8 Discs	N	XX	XX	XX
	Missing	XX	XX	XX
	Mean	X.XX	X.XX	X.XX
	SD	X.XXX	X.XXX	X.XXX
	95%CI	X.XX, X.XX	X.XX, X.XX	X.XX, X.XX
	LSMean (±SE)[#]	X.XX (X.XXX)	X.XX (X.XXX)	X.XX (X.XXX)

	LSMean 95%CI	X.XX, X.XX	X.XX, X.XX	X.XX, X.XX
	P-value	X.XXX	X.XXX	X.XXX
Change from Pre-Challenge after 12 Discs	N	XX	XX	XX
	Missing	XX	XX	XX
	Mean	X.XX	X.XX	X.XX
	SD	X.XXX	X.XXX	X.XXX
	95%CI	X.XX, X.XX	X.XX, X.XX	X.XX, X.XX
	LSMean (±SE)[#]	X.XX (X.XXX)	X.XX (X.XXX)	X.XX (X.XXX)
	LSMean 95%CI	X.XX, X.XX	X.XX, X.XX	X.XX, X.XX
	P-value	X.XXX	X.XXX	X.XXX

An increase in TEWL shows damage to the skin barrier function.
LSMean and standard error (SE) derived from ANCOVA with subject as random effect, treatment arm and side of body as fixed effects, and pre-challenge value as covariate.

PROGRAMMER'S NOTE: Same format for Tables 9.3.3.3.2.1 - Change the title and timepoint descriptors to match protocol.

Table 9.3.3.1.2
Analysis of Forearm D-Squame Challenge TEWL Assessments – Changes from Pre-Challenge
ITT Population (N=xx)

Timepoint		Difference	95% Confidence Interval for the Difference	P-Value
Pre-Challenge Day 29	Moisturising Cream vs. No Treatment	X.XX	X.XX, X.XX	X.XXX
	Olay ProX Cream vs No Treatment	X.XX	X.XX, X.XX	X.XXX
Change from Pre-Challenge after 4 Discs	Moisturising Cream vs. No Treatment	X.XX	X.XX, X.XX	X.XXX
	Olay ProX Cream vs No Treatment	X.XX	X.XX, X.XX	X.XXX
Change from Pre-Challenge after 8 Discs	Moisturising Cream vs. No Treatment	X.XX	X.XX, X.XX	X.XXX
	Olay ProX Cream vs No Treatment	X.XX	X.XX, X.XX	X.XXX
Change from Pre-Challenge after 12 Discs	Moisturising Cream vs. No Treatment	X.XX	X.XX, X.XX	X.XXX
	Olay ProX Cream vs No Treatment	X.XX	X.XX, X.XX	X.XXX

Difference is the first named treatment adjusted (LS) mean change from baseline minus the second named treatment adjusted mean change from baseline.
Analysis model (ANCOVA) included subject as random effect, treatment arm and side of body as fixed effects, and pre-challenge value as covariate.

PROGRAMMER'S NOTE: Same format for Tables 9.3.3.3.2.2 - Change the title and timepoint descriptors to match protocol.

Table 9.3.4.1

Summary of D-Squame Challenge Protein Levels
ITT Population (N=xx)

Timepoint		Moisturising Cream	Olay ProX Cream	No Treatment
Forearm (Total of 12 discs)	N	XX	XX	XX
	Missing	XX	XX	XX
	Mean	X.XX	X.XX	X.XX
	SD	X.XXX	X.XXX	X.XXX
	95%CI	X.XX, X.XX	X.XX, X.XX	X.XX, X.XX
	LSMean (±SE)	X.XX (X.XXX)	X.XX (X.XXX)	X.XX (X.XXX)
Face (Total of 9 Discs)	N	XX	XX	XX
	Missing	XX	XX	XX
	Mean	X.XX	X.XX	X.XX
	SD	X.XXX	X.XXX	X.XXX
	95%CI	X.XX, X.XX	X.XX, X.XX	X.XX, X.XX
	LSMean (±SE)	X.XX (X.XXX)	X.XX (X.XXX)	X.XX (X.XXX)

LSMean and standard error (SE) derived from ANOVA with subject as random effect, treatment arm and side of body as fixed effects.

Table 9.3.4.2
Analysis of D-Squame Challenge Protein Levels
ITT Population (N=xx)

Timepoint		Difference	95% Confidence Interval for the Difference	P-Value
Forearm (Total of 12 Discs)	Moisturising Cream vs. No Treatment	X.XX	X.XX, X.XX	X.XXX
	Olay ProX Cream vs No Treatment	X.XX	X.XX, X.XX	X.XXX
Face (Total of 9 Discs)	Moisturising Cream vs. No Treatment	X.XX	X.XX, X.XX	X.XXX
	Olay ProX Cream vs No Treatment	X.XX	X.XX, X.XX	X.XXX

Difference is the first named treatment adjusted (LS) mean change from baseline minus the second named treatment adjusted mean change from baseline.

Analysis model (ANOVA) included subject as random effect, treatment arm and side of body as fixed effects.

SIGNATURE PAGE

Statistical Analysis Plan.docx

Date	Signed By
17-Mar-2017 07:07:59	PPD
Justification	Approved

Date	Signed By
21-Mar-2017 07:35:37	PPD
Justification	Biostatistics Approval

Date	Signed By
Justification	

Date	Signed By
Justification	

Date	Signed By
Justification	

Date	Signed By
Justification	

CONFIDENTIAL

AMENDMENT TO STATISTICAL ANALYSIS PLAN FOR PROTOCOL 207451

A Proof of Concept (POC) Clinical Study to Investigate the Effects of a Developmental Cosmetic Moisturising Cream on the Barrier Function of Human Skin on the Face and Forearm

Biostatistics Department
GlaxoSmithKline Consumer Healthcare
St George's Avenue, Weybridge, Surrey, KT13 0DE United Kingdom

PPD (Statistician)

Timing of Amendment: X☐ Before unblinding ☐ After unblinding

Guidance: Green text is protocol copied. Other text is SAP specific text and bolded (added) or strikethrough (removed) text is an amendment.

Section: 5.1 Analysis Populations

Reason for amendment: Removal of 10% requirement for inclusion of PP analyses. This was not a protocol requirement.

Original text:

- The 'Intent to treat' (ITT) population includes all subjects who are randomised into the study and have at least one post-baseline measurement available. All efficacy analyses will be based on the ITT population.
- The Safety population will include all subjects who applied any of the study products. All safety analyses will be performed using the Safety population.

- The Per Protocol (PP) population will consist of the subset of ITT subjects which excludes those subjects with significant protocol deviations. Confirmatory analyses of the primary efficacy endpoint (change from Baseline in TEWL of the forearm) as well as the change from Baseline in TEWL of the face and changes from baseline in corneometry of the forearm and face will be performed on the PP population.

  Subjects with a protocol violation that is deemed to affect assessments of either the forearm or face after a specific timepoint will be part of the PP population, but will have their data excluded from the relevant assessment at which the protocol violation occurred.

  Violations that may lead to the exclusion of data for PP analysis include, but are not limited to, the following:

  - Violation of inclusion or exclusion criteria at screening or baseline that may affect either the forearm or face assessments.
  - Non-compliance with assigned treatment regimen.
  - Use of prohibited treatment or medication before or during the study, which it is felt will affect forearm or face assessments.

Violations will be documented in the Population Definitions document. The content of this document will be agreed upon between the Biostatistician and Clinical Development Director or designee prior to database lock and breaking of the study blind.

A PP analysis will be performed on forearm and face TEWL and corneometry assessments if there is more than 10% difference in the number of subjects evaluable in any of the treatment groups for the ITT and PP populations.

Amendment:

- The 'Intent to treat' (ITT) population includes all subjects who are randomised into the study and have at least one post-baseline measurement available. All efficacy analyses will be based on the ITT population.
- The Safety population will include all subjects who applied any of the study products. All safety analyses will be performed using the Safety population.

- The Per Protocol (PP) population will consist of the subset of ITT subjects which excludes those subjects with significant protocol deviations. Confirmatory analyses of the primary efficacy endpoint (change from Baseline in TEWL of the forearm) as well as the change from Baseline in TEWL of the face and changes from baseline in corneometry of the forearm and face will be performed on the PP population.

Subjects with a protocol violation that is deemed to affect assessments of either the forearm or face after a specific timepoint will be part of the PP population, but will have their data excluded from the relevant assessment at which the protocol violation occurred.

Violations that may lead to the exclusion of data for PP analysis include, but are not limited to, the following:

- Violation of inclusion or exclusion criteria at screening or baseline that may affect either the forearm or face assessments.
- Non-compliance with assigned treatment regimen.
- Use of prohibited treatment or medication before or during the study, which it is felt will affect forearm or face assessments.

Violations will be documented in the Population Definitions document. The content of this document will be agreed upon between the Biostatistician and

Clinical Development Director or designee prior to database lock and breaking of the study blind.

A PP analysis will be performed on forearm and face TEWL and corneometry assessments **if there is any difference between the** ~~more than 10% difference in the number of subjects evaluable in any of the treatment groups for the~~ ITT and PP populations.

Section: 5.4 Handling of Missing data

Reason for amendment: Clarification of calculation of AUC variables in the presence of missing data.

Original text: Missing data will not be imputed. Dropouts will be included in analyses up to the point of discontinuation.

Amendment:

Missing data will not be imputed. Dropouts will be included in analyses up to the point of discontinuation. **AUC variables with 1 or more contributing values missing will not be calculated, instead a missing value will be calculated in the derived datasets.**

Section: 6.1 Subject Disposition

Reason for amendment: Clarification of the extent of reporting.

Original text:. The number of subjects screened, enrolled, randomized and completing the study will be presented by treatment arm (i.e. test product, positive control, no treatment) and overall as well as in a separate summary, by treatment group (i.e. each of the 6 right side of body/left side of body treatment combinations to which subjects were randomized) and overall using frequency counts and percentages.

Amendment: The number of subjects screened, enrolled, randomized and completing the study will be presented by treatment **combination** arm (i.e **the pair of treatments received**~~. test product, positive control, no treatment~~) **with no account being made for side specific combinations – hence 3 columns**. ~~and overall as well as in a separate summary, by treatment group (i.e. each of the 6 right side of body/left side of body treatment combinations to which subjects were randomized) and overall using frequency counts and percentages.~~

Section: 6.2 Demographics

Reason for amendment: Clarification of clinical baseline value calculations

Original text: Age and baseline forearm and face overall dryness scores, including each of the individual dryness parameters (dull appearance, roughness, scaling, feeling of tightness) as well as the total overall dryness score, will be summarised descriptively by treatment arm (test product, positive control, no treatment) using means, medians and standard deviations. Race and Fitzpatrick Skin Type and the 4 individual overall dryness parameters described above will be summarised using frequency counts and percentages.

Amendment:

Follow on text: As the 4 overall dryness data fields are recorded for both forearms and sides of face, only the side (left/right) associated with the subsequent treatment for that side will be combined, and will therefore be a combination of right and left arm individual and overall dryness scores whichever side is the treatment indicated.

Section: 8.1 Primary Analysis

Reason for amendment: Clarification of within group analyses methods.

Original text: Change from baseline in TEWL for each subject at Day 29 (Area 1 and 3 on the forearms and Area 6 and 8 on the face) will be summarised for each of the three treatment arms (test product, positive control and no treatment) of both the forearm (primary assessment area) and face using descriptive statistics (means, medians, standard deviations, 95% confidence intervals). Changes from baseline for each treatment group will be compared to zero using t-test and the p-values and 95% confidence intervals for these within-group changes will be presented.

Test product versus no treatment and positive control versus no treatment will be compared for the change from baseline at Day 29 of both the forearms (Area 1 and 3) (primary assessment area) and face (Area 6 and 8) using analysis of covariance (ANCOVA) with subject as a random effect, treatment arm (test product, positive control and no treatment) and side of body (right, left) as main effects and baseline value as covariate. This approach allows for the inclusion of data from all subjects treated with a given treatment arm (test product, positive control or no treatment) regardless of the treatment group (test product/no treatment, test product/positive control, positive control/no treatment) to which they were randomized to derive

estimates of treatment effect. Least square means from the ANCOVA model for the change from baseline will be presented for each treatment arm and for the difference between each pair of the active treatment arms and no treatment together with p-values and 95% confidence intervals.

If the assumption of normality is rejected, an appropriate transformation to the data will be performed to facilitate the above method of analysis. In the absence of an appropriate data transformation, non-parametric analyses will be performed. In the case of a non-parametric analysis, median differences will be presented, together with 95% confidence intervals based on the Hodges-Lehmann method.

Amendment: Change from baseline in TEWL for each subject at Day 29 (Area 1 and 3 on the forearms and Area 6 and 8 on the face) will be summarised for each of the three treatment arms (test product, positive control and no treatment) of both the forearm (primary assessment area) and face using descriptive statistics (means, medians, standard deviations, 95% confidence intervals). ~~Changes from baseline for each treatment group will be compared to zero using t-test and the p-values and 95% confidence intervals for these within-group changes will be presented~~

Test product versus no treatment and positive control versus no treatment will be compared for the change from baseline at Day 29 of both the forearms (Area 1 and 3) (primary assessment area) and face (Area 6 and 8) using analysis of covariance (ANCOVA) **with change from baseline as response and with** subject as a random effect, treatment arm (test product, positive control and no treatment) and side of body (right, left) as main effects and baseline value as covariate. This approach allows for the inclusion of data from all subjects treated with a given treatment arm (test product, positive control or no treatment) regardless of the treatment group (test product/no treatment, test product/positive control, positive control/no treatment) to which they were randomized to derive estimates of treatment effect. Least square means from the ANCOVA model for the change from baseline will be presented for each treatment arm and for the difference between each pair of the active treatment arms and no treatment together with p-values and 95% confidence intervals.

If the assumption of normality is rejected, an appropriate transformation to the data will be performed to facilitate the above method of analysis. In the absence of an appropriate data transformation, non-parametric analyses will be performed. In the case of a non-parametric analysis, median differences will be presented, together with 95% confidence intervals based on the Hodges-Lehmann method.

Section: 8.2 Secondary Analysis

Reason for amendment: Clarification of within group analyses methods

Original text: Change from baseline in TEWL and Corneometry for each subject at Days 1 (30 minutes and 6 hours post first application – corneometry only), 2, 15, 30, 31, 32, 33, and 34 (Area 1 and 3 on the forearms and Area 6 and 8 on the face) will be summarised for all 3 treatment arms of both the forearm and face using descriptive statistics (means, medians, standard deviations, 95% confidence intervals). Changes from baseline for each treatment group will be compared to zero using t-test and the p-values and 95% confidence intervals for these within-group changes will be presented.

Test product versus no treatment and positive control versus no treatment will be compared for the change from baseline at each time point of both the forearms (Area 1 and 3) and face (Area 6 and 8) using analysis of covariance (ANCOVA) subject as a random effect, treatment arm (test product, positive control and no treatment) and side of body treatment applied (right, left) as main effects and baseline value as covariate. This approach allows for the inclusion of data from all subjects treated with a given treatment arm (test product, positive control or no treatment) regardless of the treatment group (test product/no treatment, test product/positive control, positive control/no treatment) to which they were randomized to derive estimates of treatment effect. Least square means from the ANCOVA model for the change from baseline will be presented for each treatment arm and for the difference between each pair of the active treatment arms and no treatment together with p-values and 95% confidence intervals.

Standardised AUCs will be calculated for each subject for change from baseline in TEWL and corneometry (Area 1 and 3 on the forearms and Area 6 and 8 on the face) over the treatment period; i.e. through Day 29 (Days 1, 2, 15, and 29) and separately over the Regression period; i.e. through Day 34 (Days 30, 31, 32, 33 and 34) using the trapezoidal rule and dividing by the number of days in the period. Each of the treatment period and regression period AUCs will be similarly summarized and compared for both the forearm and face using analysis of covariance (ANCOVA) with subject as a random effect, treatment arm (test product, positive control and no treatment) and side of body treatment applied (right, left) as main effects and baseline value as covariate. Least square means from the ANCOVA model for the change from baseline will be presented for each treatment arm and for the difference between each pair of the active treatment arms and no treatment together with p-values and 95% confidence intervals.

Day 29 change from pre-challenge in TEWL (Area 2 and 4 of the forearms and Area 5 and 7 of the face) will be summarised for all 3 treatment arms of both the forearm and face after each set of discs (4, 8 and 12 discs for the forearm and 3, 6 and 9 discs for the face) using descriptive statistics (means, medians, standard deviations, 95% confidence intervals). Day 29 change from pre-challenge for each treatment group, for both the forearm and face, and following each set of discs will be compared to zero using t-test and the p-values and 95% confidence intervals for these within-group changes will be presented. Comparisons of the changes from pre-challenge after each set of discs between the test product and no treatment for both the forearm and face will be performed using analysis of covariance (ANCOVA) with subject as a random effect, treatment arm (test product, positive control and no treatment) and side of body treatment applied (right, left) as main effects and the pre-challenge value as covariate. Least square means from the ANCOVA model for the change from pre-challenge will be presented for each treatment arm and for the difference between each pair of the active treatment arms and no treatment together with p-values and 95% confidence intervals.

ANOVA as described above for the primary endpoint (excluding the covariate) or analysis based on transformed data or an appropriate non-parametric analysis will be used to compare the total amount of protein present collected from each of the D-Squame discs at Day 29 separately for forearms (12 discs) and face (9 discs). P-values resulting from these analyses as well as 95% confidence intervals for the differences in the protein levels between each pair of the active treatment arms and no treatment will also be provided.

Change from Day 29 to Days 30, 31, 32, 33 and 34 in TEWL and corneometry (Area 1 and 3 on the forearms and Area 6 and 8 on the face) and the standardised AUC calculated over the Regression period using the trapezoidal rule and divided by the number of days in the period will be summarised and compared between each pair of the active treatment arms and no treatment, separately for forearm and face, as described above for the changes from baseline.

If the assumption of normality is rejected for any of the above analyses, an appropriate transformation to the data will be performed to facilitate the method of analysis. In the absence of an appropriate data transformation, non-parametric analyses will be performed. In the case of a non-parametric analysis, median differences will be presented, together with 95% confidence intervals based on the Hodges-Lehmann method.

Amendment:

Change from baseline in TEWL and Corneometry for each subject at Days 1 (30 minutes and 6 hours post first application – corneometry only), 2, 15, 30, 31, 32, 33, and 34 (Area 1 and 3 on the forearms and Area 6 and 8 on the face) will be summarised for all 3 treatment arms of both the forearm and face using descriptive statistics (means, medians, standard deviations, 95% confidence intervals). ~~Changes from baseline for each treatment group will be compared to zero using t test and the p-values and 95% confidence intervals for these within-group changes will be presented.~~

Test product versus no treatment and positive control versus no treatment will be compared for the change from baseline at each time point of both the forearms (Area 1 and 3) and face (Area 6 and 8) using analysis of covariance (ANCOVA) **with change from baseline as response and with** subject as a random effect, treatment arm (test product, positive control and no treatment) and side of body treatment applied (right, left) as main effects and baseline value as covariate. This approach allows for the inclusion of data from all subjects treated with a given treatment arm (test product, positive control or no treatment) regardless of the treatment group (test product/no treatment, test product/positive control, positive control/no treatment) to which they were randomized to derive estimates of treatment effect. Least square means from the ANCOVA model for the change from baseline will be presented for each treatment arm and for the difference between each pair of the active treatment arms and no treatment together with p-values and 95% confidence intervals.

Standardised AUCs will be calculated for each subject for change from baseline in TEWL and corneometry (Area 1 and 3 on the forearms and Area 6 and 8 on the face) over the treatment period; i.e. through Day 29 (Days 1, 2, 15, and 29) and separately over the Regression period; i.e. through Day 34 (Days 30, 31, 32, 33 and 34) using the trapezoidal rule and dividing by the number of days in the period. Each of the treatment period and regression period AUCs will be similarly summarized and compared for both the forearm and face using analysis of covariance (ANCOVA) with subject as a random effect, treatment arm (test product, positive control and no treatment) and side of body treatment applied (right, left) as main effects and baseline value as covariate. Least square means from the ANCOVA model for the change from baseline will be presented for each treatment arm and for the difference between each pair of the active treatment arms and no treatment together with p-values and 95% confidence intervals. **In case of any individual subject missing**

values between Day 1 and Day 29 the AUC(1-29) will not be calculated for those subjects, and if any of Day29 to Day 34 are missing the AUC(29-34) will not be calculated for those subjects.

Day 29 change from pre-challenge in TEWL (Area 2 and 4 of the forearms and Area 5 and 7 of the face) will be summarised for all 3 treatment arms of both the forearm and face after each set of discs (4, 8 and 12 discs for the forearm and 3, 6 and 9 discs for the face) using descriptive statistics (means, medians, standard deviations, 95% confidence intervals). ~~Day 29 change from pre-challenge for each treatment group, for both the forearm and face, and following each set of discs will be compared to zero using t-test and the p-values and 95% confidence intervals for these within-group changes will be presented.~~ Comparisons of the changes from pre-challenge after each set of discs between the test product and no treatment for both the forearm and face will be performed using analysis of covariance (ANCOVA) **with change from pre-challenge as response and with** subject as a random effect, treatment arm (test product, positive control and no treatment) and side of body treatment applied (right, left) as main effects and the pre-challenge value as covariate. Least square means from the ANCOVA model for the change from pre-challenge will be presented for each treatment arm and for the difference between each pair of the active treatment arms and no treatment together with p-values and 95% confidence intervals.

ANOVA as described above for the primary endpoint (excluding the covariate) or analysis based on transformed data or an appropriate non-parametric analysis will be used to compare the total amount of protein present collected from each of the D-Squame discs at Day 29 separately for forearms (12 discs) and face (9 discs). P-values resulting from these analyses as well as 95% confidence intervals for the differences in the protein levels between each pair of the active treatment arms and no treatment will also be provided.

Change from Day 29 to Days 30, 31, 32, 33 and 34 in TEWL and corneometry (Area 1 and 3 on the forearms and Area 6 and 8 on the face) and the standardised AUC calculated over the Regression period using the trapezoidal rule and divided by the number of days in the period will be summarised and compared between each pair of the active treatment arms and no treatment, separately for forearm and face, as described above for the changes from baseline. **The rules described for non calculation of AUC in missing value situations will similarly be applied.**

If the assumption of normality is rejected for any of the above analyses, an appropriate transformation to the data will be performed to facilitate the method of analysis. In the absence of an appropriate data transformation, non-parametric

analyses will be performed. In the case of a non-parametric analysis, median differences will be presented, together with 95% confidence intervals based on the Hodges-Lehmann method.

Calculated AUC's are found by using values at time T= t_1=0, t_2, …, t_k (real dates not nominal days) and changes from baseline values denoted by δ_1 =0, δ_2, … ,δ_k ; as the sum of, the area of each trapezium (above the horizontal line indicating the baseline value) and calculated as the width of the trapezium (t_{j+1} - t_j) multipled by the average applicable δ's (δ_{j+1}, δ_j) with the first δ, δ_1 =0. These are in fact areas over the baseline value (AOB). The AOB will be standardized by dividing by the overall width t_k – t_1 using real dates rather than nominal days.

Section: 9 Safety Analysis

Reason for amendment: Reference to additional pre-unblind step of assigning application site(s) AE's to treatment at that site(s). A textual field is recorded indicating such.

Original text: Treatment emergent AEs are defined as events that start on or after the first treatment date. Events occurring following the start of treatment which were also reported before treatment began with no change in severity or causality will however not be considered treatment emergent.

As per Section 7.1.1 of the protocol, the following does not constitute an AE:

- Any localised response to the D-Squame disc application and removal on the face and forearms, unless more severe than expected in which case will be captured as an AE.

AEs will be tabulated according to the current version of the MedDRA. Frequencies and percentages will be presented by product group and overall, for each system organ class, and for each preferred term. Summaries of treatment-emergent AEs, treatment-related treatment-emergent AEs, AEs leading to discontinuation, and serious AEs will be completed. AEs relating to the forearm or face will be summarized separately.

Amendment: Treatment emergent AEs are defined as events that start on or after the first treatment date. Events occurring following the start of treatment which were also reported before treatment began with no change in severity or causality will however not be considered treatment emergent.

As per Section 7.1.1 of the protocol, the following does not constitute an AE:

- Any localised response to the D-Squame disc application and removal on the face and forearms, unless more severe than expected in which case will be captured as an AE.

AEs will be tabulated according to the current version of the MedDRA. Frequencies and percentages will be presented by product group and overall, for each system organ class, and for each preferred term. Summaries of treatment-emergent AEs, treatment-related treatment-emergent AEs, AEs leading to discontinuation, and serious AEs will be completed. AEs relating to the forearm or face will be summarized separately.

A further step will take place pre-unblind to flag the AEs by application site if appropriate so that attribution to applied treatment is preserved. This will involve review of all application site AEs (including textual information) and addition of a flag to the clinical database to indicate which site(s) it is applicable to. Other whole body events will be attributed to both treatments received.

SIGNATURE PAGE

SAP Amendment1

Date	Signed By
13-Jun-2017 06:00:28	PPD
Justification	Approved

Date	Signed By
13-Jun-2017 06:57:49	PPD
Justification	Biostatistics Approval

Date	Signed By
Justification	

Date	Signed By
Justification	

Date	Signed By
Justification	

Date	Signed By
Justification	